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DESCRIPTION

Technical field of the invention

[0001] The present invention generally relates to the microbiological industry, and specifically to the production of L-serine using genetically modified bacteria. The present invention provides a genetically modified bacterium belonging to the *Enterobacteriaceae* family, wherein the genes *sdaA*, *sdaB*, *tdcG* and *glyA* have been inactivated, which makes it particularly suitable for the production of L-serine at higher yield. The present invention also provides means by which the bacterium can be made tolerant towards higher concentrations of serine. The present invention also provides methods for the production of L-serine or L-serine derivative using such genetically modified bacterium.

Background of the invention

[0002] L-serine is an amino acid that currently is used in the cosmetics, pharmaceutical and medical industry. The estimated annual production of serine is between 300-1000 tons (Leuchtenberger et al., 2005). The compound has also been identified as one of the top 30 most interesting biochemicals because of its potential use as a building block biochemical. The current production is based on conversion of glycine and methanol using resting cells (Hagishita et al., 1996), where methylotrophs convert methanol to formaldehyde and transfer the CH₂-OH unit of the molecule to glycine using serine hydroxymethyltransferase (*glyA*). This fermentation process is time consuming and glycine is an expensive starting material. Developing a method for producing serine at low cost directly from glucose is therefore attractive.

[0003] Serine has the potential to be made from glucose by fermentation with a very high theoretical yield (Burgard and Maranas, 2001). However, several challenges need to be addressed in order to increase the yield, the most crucial one being degradation of serine in the production organism. Serine has two key degradation pathways in *E. coli*. Serine to pyruvate catabolism is in *E. coli* catalyzed by three deaminases namely *sdaA*, *sdaB* and *tdcG*, while *C. glutamicum* only has one deaminase (*sdaA*) with activity towards serine. In both organisms, the conversion of serine to glycine is encoded by *glyA*. Serine production by knocking out only deaminases has been attempted in *E. coli* (Li et al., 2012) and *C. glutamicum* (Peters-Wendisch et al., 2005). In *E. coli* transient accumulation of 3.8mg/L from 1 g/L glucose was observed when only one of the pathway gene (*serA*) was overexpressed. Deletion of the deaminase on *C. glutamicum* lead to marginal and transient increase in the serine titer. In recent studies, *E. coli* was engineered to enhance the flux of 3-phosphoglycerate by perturbing the TCA-cycle and glyoxylate shunt (Gu et al., 2014). The resulting strain, where only one deaminase, was removed (*sdaA*) was reported to produce 8.45 g/L serine from 75 g/L glucose (11.2 % yield).

[0004] Down regulation of *glyA* (Peters-Wendisch et al., 2005) in *C. glutamicum* resulted in the production of 9 g/L serine from 40 g/L glucose but lead to an unstable strain. *glyA* is an important enzyme that converts serine to glycine and in this step transfers one carbon unit to tetra hydrofolate (THF), which is used as cofactor. Removal of the folic acid pathway and supplementation of folic acid lead to a stable *C. glutamicum*, and a production of 36 g/L serine, however with a relatively low yield (Stolz et al, 2007).

[0005] Deletion of both of the major serine degradation pathways (serine to pyruvate and serine to glycine) has not been previously been achieved. It is furthermore known that serine becomes toxic even at low concentrations in strains that lack the pyruvate degradation pathway (Zhang and Newman, 2008). It is expected that serine may inhibit the production of branched amino acids in *E. coli* Hama et al., 1990), and the conversion of serine to hydroxypyruvate and acrylates, which is toxic to the cell (de Lorenzo, 2014). For efficient production of L-serine or a derivative thereof, it is therefore desirable to both remove the serine degradation pathways and address the problems associated with toxicity of serine.

Summary of the invention

[0006] The object of the present invention is to provide means allowing a more efficient production of L-serine. More particularly, it is an object of the present invention to provide means allowing the production of L-serine at higher nominal yield and improved mass yield.

[0007] This is achieved by the finding that the production of L-serine can be enhanced by inactivation of the genes *sdaA*, *sdaB*, *tdcG* and *glyA*.

[0008] The present invention thus provides in a first aspect a bacterium belonging to the Enterobacteriaceae family, especially a bacterium having an ability to produce L-serine, wherein said bacterium has been modified to inactivate the genes *sdaA*, *sdaB*, *tdcG* and *glyA*.

[0009] The present invention provides in a second aspect a method for producing L-serine comprising: cultivating the bacterium as described above in a medium.

Brief description of the figures

[0010]

Figure 1: Deletion of the main genes involved in serine degradation, *sdaA*, *sdaB*, *tdcG* and *glyA* in *E. coli*. The removal of the four genes was demonstrated using PCR with primers specific to the relevant genes.

Figure 2: Vector maps of the constructs (Example 2)

Figure 3: Serine production during batch fermentation in shake flasks.

Figure 4: Increased tolerance towards serine can be achieved by overexpression of *ydeD*, a potential serine transporter. The figure shows the growth of the cells in the presence of various concentrations of serine.

Figure 5: Increased tolerance towards serine can be achieved by random mutagenesis. Growth rates of the parent strain (Q1) and evolved strains are shown at different serine concentrations.

Figure 6: Adaptive Laboratory Evolution (ALE) experiment for improving tolerance towards serine. (A) Growth rates during the evolution experiment. (B) Improved growth of evolved strains in the presence of high concentrations of serine.

Figure 7: The effect of mutations in *thrA* on tolerance towards serine. Three specific mutations of *thrA* (Y356C, S357R, S359R) were introduced into the Q1 background and the growth of the clones was compared to the growth of the Q1 strain in the presence of 6.25 g/L of serine.

Figure 8: Identification of mutations that cause increased tolerance towards serine. Amplicon sequencing analysis was used to analyze the effect of ALE mutations after introduction into the Q1 (DE3) strain by MAGE.

Figure 9: (A) Serine production and cell density (OD 600 nm) measured at different time points during fed batch fermentation of the Q1(DE3) and Q3(DE3) strains. (B) Production of serine from the glucose fed to the fermentor. The slope of the curve indicates the mass yield during the fermentation.

Figure 10: (A) Cell density and serine titer of ALE 8-8 (DE3) strain. (B) Mass yield from glucose.

Figure 11: Growth rate of mutant *E. coli* strains having different amino acid substitutions observed at positions 356 (11A), 357 (11B) and 359 (11C) of ThrA, respectively (the amino acid substitution is denoted by the respective one-letter code). The growth rate of *E. coli* carrying the wild type *thrA* gene is denoted "wt".

[0011] The present invention is now described in more detail below.

Detailed description of the invention

[0012] Unless specifically defined herein, all technical and scientific terms used have the same meaning as commonly understood by a skilled artisan in the fields of biochemistry, genetics, and microbiology.

[0013] All methods and materials similar or equivalent to those described herein can be used in the practice or testing of the present invention, with suitable methods and materials being described herein. In case of conflict, the present specification, including definitions, will prevail. Further, the materials, methods, and examples are illustrative only and are not intended to be limiting, unless otherwise specified.

[0014] The practice of the present invention will employ, unless otherwise indicated, conventional techniques of cell biology, cell culture, molecular biology, transgenic biology, microbiology, and recombinant DNA, which are within the skill of the art. Such techniques are explained fully in the literature. See, for example, Current Protocols in Molecular Biology (Frederick M. AUSUBEL, 2000, Wiley and son Inc, Library of Congress, USA); Molecular Cloning: A Laboratory Manual, Third Edition, (Sambrook et al, 2001, Cold Spring Harbor, New York: Cold Spring Harbor Laboratory Press); Oligonucleotide Synthesis (M. J. Gait ed., 1984); Mullis et al. U.S. Pat. No. 4,683,195; Nucleic Acid Hybridization (B. D. Harries & S. J. Higgins eds. 1984); Transcription And Translation (B. D. Hames & S. J. Higgins eds. 1984); Culture Of Animal Cells (R. I. Freshney, Alan R. Liss, Inc., 1987); Immobilized Cells And Enzymes (IRL Press, 1986); B. Perbal, A Practical Guide To Molecular Cloning (1984); the series, Methods In ENZYMOLOGY (J. Abelson and M. Simon, eds.-in-chief, Academic Press, Inc., New York), specifically, Vols.154 and 155 (Wu et al. eds.) and Vol. 185, "Gene Expression Technology" (D. Goeddel, ed.); Gene Transfer Vectors For Mammalian Cells (J. H. Miller and M. P. Calos eds., 1987, Cold Spring Harbor Laboratory); Immunochemical Methods In Cell And Molecular Biology (Mayer and Walker, eds., Academic Press, London, 1987); Handbook Of Experimental Immunology, Volumes I-IV (D. M. Weir and C. C. Blackwell, eds., 1986); and Manipulating the Mouse Embryo, (Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y., 1986).

Bacterium of the invention

[0015] As indicated above, the present invention is inter alia based on the finding that the production of L-serine can be enhanced by inactivation of the genes *sdaA*, *sdaB*, *tdcG* and *glyA*.

[0016] Accordingly, the present invention provides a bacterium belonging to the Enterobacteriaceae family, especially a bacterium having an ability to produce L-serine, wherein said bacterium has been modified to inactivate the genes *sdaA*, *sdaB*, *tdcG* and *glyA*.

[0017] The genes *sdaA*, *sdaB* and *tdcG* encode L-serine deaminase I (SdaA), L-serine deaminase II (SdaB) and L-serine deaminase III (TdcG), respectively, which are the three enzymes carrying out the sole step in the pathway of L-serine degradation, converting serine into the basic cellular building block, pyruvate. Further information regarding *sdaA*, *sdaB* and *tdcG* of, e.g., *Escherichia coli* is available at EcoCyc (www.biocyc.org) under Accession numbers EG10930, EG11623 and G7624, respectively. Representative nucleotide sequences of *sdaA*, *sdaB* and *tdcG* are set forth in SEQ ID NOs: 1 to 3, respectively.

[0018] The gene *glyA* encodes serine hydroxymethyltransferase (GlyA) which converts serine to glycine, transferring a methyl group to tetrahydrofolate, thus forming 5,10-methylene-tetrahydrofolate (5,10-mTHF). 5,10-mTHF is the major source of C1 units in the cell, making GlyA a key enzyme in the biosynthesis of purines, thymidine, methionine, choline and lipids. Further information regarding *glyA* of, e.g., *Escherichia coli* is available at EcoCyc (www.biocyc.org) under Accession number EG10408. A representative nucleotide sequence of *glyA* is set forth in SEQ ID NO: 4.

[0019] The lambda-red mediated gene replacement (Datsenko and Wanner, 2000) is a particularly suitable method to inactivate one or more genes as described herein. Hence, according to particular embodiments, genes are inactivated using lambda-red mediated gene replacement.

[0020] As shown in Figure 3, the inactivation of all four genes (*sdaA*, *sdaB*, *tdcG* and *glyA*) involved in serine degradation results in the highest specific productivity and the highest yield from glucose compared to inactivation of

only the three genes involved in L-serine degradation via the serine to pyruvate pathway (*sdaA*, *sdaB*, and *tdcG*).

[0021] Serine is produced from glyceraldehyde-3-phosphate using three enzymes encoded by the genes *serA* (encoding a 3-phosphoglycerate dehydrogenase), *serB* (encoding a phosphoserine phosphatase) and *serC* (encoding a phosphoserine aminotransferase). In order to increase production of L-serine, these genes may be overexpressed. Relevant information regarding *serA*, *serB* and *serC* of, e.g., *Escherichia coli* is available at EcoCyc (www.biocyc.org) under Accession numbers EG10944, EG10945 and EG10946, respectively.

[0022] Therefore, according to certain embodiments, the bacterium has been modified to overexpress a 3-phosphoglycerate dehydrogenase, a phosphoserine phosphatase and a phosphoserine aminotransferase. More particularly, the bacterium has been further modified to overexpress the genes *serA*, *serB* and *serC*. This may be achieved by introducing into the bacterium one or more (such as two or three) exogenous nucleic acid molecules, such as one or more vectors, comprising a nucleotide sequence encoding a 3-phosphoglycerate dehydrogenase, a nucleotide sequence encoding a phosphoserine phosphatase and/or a nucleotide sequence encoding a phosphoserine aminotransferase.

[0023] The 3-phosphoglycerate dehydrogenase may be derived from the same species as the bacterium in which it is overexpressed or may be derived from a species different to the one in which it is overexpressed (i.e. it is heterologous). According to certain embodiments, the 3-phosphoglycerate dehydrogenase is derived from the same species as the bacterium in which it is overexpressed. According to certain other embodiments, the 3-phosphoglycerate dehydrogenase is derived from a species different to the one in which it is overexpressed (i.e. it is heterologous).

[0024] According to certain embodiments, the bacterium comprises an exogenous nucleic acid molecule comprising a nucleotide sequence encoding a 3-phosphoglycerate dehydrogenase. The exogenous nucleic acid molecule may comprise a nucleotide sequence encoding a polypeptide comprising an amino acid sequence set forth in SEQ ID NO: 5. The exogenous nucleic acid molecule may comprise a nucleotide sequence encoding a polypeptide comprising an amino acid sequence set which has at least about 90%, at least about 93%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, or at least about 99%, sequence identity to the amino acid sequence set forth in SEQ ID NO: 5. Preferably, the polypeptide has 3-phosphoglycerate dehydrogenase activity. More preferably, the polypeptide has 3-phosphoglycerate dehydrogenase activity similar to that of the polypeptide comprising an amino acid sequence set forth in SEQ ID NO: 5. The exogenous nucleic acid molecule may comprise a nucleotide sequence encoding a polypeptide comprising an amino acid sequence set which has at least about 95%, such as at least about 96%, at least about 97%, at least about 98%, or at least about 99%, sequence identity to the amino acid sequence set forth in SEQ ID NO: 5. Preferably, the polypeptide has 3-phosphoglycerate dehydrogenase activity. More preferably, the polypeptide has 3-phosphoglycerate dehydrogenase activity similar to that of the polypeptide comprising an amino acid sequence set forth in SEQ ID NO: 5. The exogenous nucleic acid molecule may comprise a nucleotide sequence encoding a polypeptide comprising an amino acid sequence set forth in SEQ ID NO: 5, wherein 1 or more, such as about 1 to about 50, about 1 to about 40, about 1 to about 35, about 1 to about 30, about 1 to about 25, about 1 to about 20, about 1 to about 15, about 1 to about 10, about 1 to about 5, or about 1 to about 3, amino acid residues are substituted, deleted, and/or inserted. Preferably, the polypeptide has 3-phosphoglycerate dehydrogenase activity. More preferably, the polypeptide has 3-phosphoglycerate dehydrogenase activity similar to that of the polypeptide comprising an amino acid sequence set forth in SEQ ID NO: 5. The exogenous nucleic acid molecule may comprise a nucleotide sequence encoding a polypeptide comprising an amino acid sequence set forth in SEQ ID NO: 5, wherein about 1 to about 5, such as about 1 to about 3, amino acid residues are substituted, deleted, and/or inserted. Preferably, the polypeptide has 3-phosphoglycerate dehydrogenase activity. More preferably, the polypeptide has 3-phosphoglycerate dehydrogenase activity similar to that of the polypeptide comprising an amino acid sequence set forth in SEQ ID NO: 5.

[0025] It is further beneficial to overexpress a mutant *serA* gene which encodes a 3-phosphoglycerate dehydrogenase being resistant towards feedback inhibition of serine. This may, for example, be achieved by deleting the last four C-terminal residues of the wild type 3-phosphoglycerate dehydrogenase (SerA). Alternatively, feedback inhibition of SerA can be removed by mutating the three residues H344, N346 and N364 to alanine. A representative amino acid sequence of such 3-phosphoglycerate dehydrogenase mutant is set forth in SEQ ID NO: 6. Therefore, according to particular embodiments, the bacterium comprises an exogenous nucleic acid molecule comprising a nucleotide

sequence encoding a 3-phosphoglycerate dehydrogenase being resistant towards feedback inhibition of serine. The exogenous nucleic acid molecule may comprise a nucleotide sequence encoding a polypeptide comprising an amino acid sequence set forth in SEQ ID NO: 6. The exogenous nucleic acid molecule may comprise a nucleotide sequence encoding a polypeptide comprising an amino acid sequence set which has at least about 90%, at least about 93%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, or at least about 99%, sequence identity to the amino acid sequence set forth in SEQ ID NO: 6. Preferably, the polypeptide has 3-phosphoglycerate dehydrogenase activity. More preferably, the polypeptide has 3-phosphoglycerate dehydrogenase activity similar to that of the polypeptide comprising an amino acid sequence set forth in SEQ ID NO: 6. The exogenous nucleic acid molecule may comprise a nucleotide sequence encoding a polypeptide comprising an amino acid sequence set which has at least about 95%, such as at least about 96%, at least about 97%, at least about 98%, or at least about 99%, sequence identity to the amino acid sequence set forth in SEQ ID NO: 6. Preferably, the polypeptide has 3-phosphoglycerate dehydrogenase activity. More preferably, the polypeptide has 3-phosphoglycerate dehydrogenase activity similar to that of the polypeptide comprising an amino acid sequence set forth in SEQ ID NO: 6. The exogenous nucleic acid molecule may comprise a nucleotide sequence encoding a polypeptide comprising an amino acid sequence set forth in SEQ ID NO: 6, wherein 1 or more, such as about 1 to about 50, about 1 to about 40, about 1 to about 35, about 1 to about 30, about 1 to about 25, about 1 to about 20, about 1 to about 15, about 1 to about 10, about 1 to about 5, or about 1 to about 3, amino acid residues are substituted, deleted, and/or inserted. Preferably, the polypeptide has 3-phosphoglycerate dehydrogenase activity. More preferably, the polypeptide has 3-phosphoglycerate dehydrogenase activity similar to that of the polypeptide comprising an amino acid sequence set forth in SEQ ID NO: 6. The exogenous nucleic acid molecule may comprise a nucleotide sequence encoding a polypeptide comprising an amino acid sequence set forth in SEQ ID NO: 6, wherein about 1 to about 5, such as about 1 to about 3, amino acid residues are substituted, deleted, and/or inserted. Preferably, the polypeptide has 3-phosphoglycerate dehydrogenase activity. More preferably, the polypeptide has 3-phosphoglycerate dehydrogenase activity similar to that of the polypeptide comprising an amino acid sequence set forth in SEQ ID NO: 6.

[0026] The phosphoserine phosphatase may be derived from the same species as the bacterium in which it is overexpressed or may be derived from a species different to the one in which it is overexpressed (i.e. it is heterologous). According to certain embodiments, the phosphoserine phosphatase is derived from the same species as the bacterium in which it is overexpressed. According to certain other embodiments, the phosphoserine phosphatase is derived from a species different to the one in which it is overexpressed (i.e. it is heterologous).

[0027] According to certain embodiments, the bacterium comprises an exogenous nucleic acid molecule comprising a nucleotide sequence encoding a phosphoserine phosphatase. The exogenous nucleic acid molecule may comprise a nucleotide sequence encoding a polypeptide comprising an amino acid sequence set forth in SEQ ID NO: 7. The exogenous nucleic acid molecule may comprise a nucleotide sequence encoding a polypeptide comprising an amino acid sequence set which has at least about 90%, at least about 93%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, or at least about 99%, sequence identity to the amino acid sequence set forth in SEQ ID NO: 7. Preferably, the polypeptide has phosphoserine phosphatase activity. More preferably, the polypeptide has phosphoserine phosphatase activity similar to that of the polypeptide comprising an amino acid sequence set forth in SEQ ID NO: 7. The exogenous nucleic acid molecule may comprise a nucleotide sequence encoding a polypeptide comprising an amino acid sequence set which has at least about 95%, such as at least about 96%, at least about 97%, at least about 98%, or at least about 99%, sequence identity to the amino acid sequence set forth in SEQ ID NO: 7. Preferably, the polypeptide has phosphoserine phosphatase activity. More preferably, the polypeptide has phosphoserine phosphatase activity similar to that of the polypeptide comprising an amino acid sequence set forth in SEQ ID NO: 7. The exogenous nucleic acid molecule may comprise a nucleotide sequence encoding a polypeptide comprising an amino acid sequence set forth in SEQ ID NO: 7, wherein 1 or more, such as about 1 to about 50, about 1 to about 40, about 1 to about 35, about 1 to about 30, about 1 to about 25, about 1 to about 20, about 1 to about 15, about 1 to about 10, about 1 to about 5, or about 1 to about 3, amino acid residues are substituted, deleted, and/or inserted. Preferably, the polypeptide has phosphoserine phosphatase activity. More preferably, the polypeptide has phosphoserine phosphatase activity similar to that of the polypeptide comprising an amino acid sequence set forth in SEQ ID NO: 7. The exogenous nucleic acid molecule may comprise a nucleotide sequence encoding a polypeptide comprising an amino acid sequence set forth in SEQ ID NO: 7, wherein about 1 to about 5, such as about 1 to about 3, amino acid residues are substituted, deleted, and/or inserted. Preferably, the polypeptide has phosphoserine phosphatase activity. More preferably, the polypeptide has phosphoserine phosphatase activity similar to that of the polypeptide comprising an amino acid sequence set forth in SEQ ID NO: 7.

[0028] The phosphoserine aminotransferase may be derived from the same species as the bacterium in which it is overexpressed or may be derived from a species different to the one in which it is overexpressed (i.e. it is heterologous). According to certain embodiments, the phosphoserine aminotransferase is derived from the same species as the bacterium in which it is overexpressed.

[0029] According to certain other embodiments, the phosphoserine aminotransferase is derived from a species different to the one in which it is overexpressed (i.e. it is heterologous).

[0030] According to certain embodiments, the bacterium comprises an exogenous nucleic acid molecule comprising a nucleotide sequence encoding a phosphoserine aminotransferase. The exogenous nucleic acid molecule may comprise a nucleotide sequence encoding a polypeptide comprising an amino acid sequence set forth in SEQ ID NO: 8. The exogenous nucleic acid molecule may comprise a nucleotide sequence encoding a polypeptide comprising an amino acid sequence set which has at least about 90%, at least about 93%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, or at least about 99%, sequence identity to the amino acid sequence set forth in SEQ ID NO: 8. Preferably the polypeptide has phosphoserine aminotransferase activity. More preferably, the polypeptide has phosphoserine aminotransferase activity similar to that of the polypeptide comprising an amino acid sequence set forth in SEQ ID NO: 8. The exogenous nucleic acid molecule may comprise a nucleotide sequence encoding a polypeptide comprising an amino acid sequence set which has at least about 95%, such as at least about 96%, at least about 97%, at least about 98%, or at least about 99%, sequence identity to the amino acid sequence set forth in SEQ ID NO: 8. Preferably, the polypeptide has phosphoserine aminotransferase activity. More preferably, the polypeptide has phosphoserine aminotransferase activity similar to that of the polypeptide comprising an amino acid sequence set forth in SEQ ID NO: 8. The exogenous nucleic acid molecule may comprise a nucleotide sequence encoding a polypeptide comprising an amino acid sequence set forth in SEQ ID NO: 8, wherein 1 or more, such as about 1 to about 50, about 1 to about 40, about 1 to about 35, about 1 to about 30, about 1 to about 25, about 1 to about 20, about 1 to about 15, about 1 to about 10, about 1 to about 5, or about 1 to about 3, amino acid residues are substituted, deleted, and/or inserted. Preferably, the polypeptide has phosphoserine aminotransferase activity. More preferably, the polypeptide has phosphoserine aminotransferase activity similar to that of the polypeptide comprising an amino acid sequence set forth in SEQ ID NO: 8. The exogenous nucleic acid molecule may comprise a nucleotide sequence encoding a polypeptide comprising an amino acid sequence set forth in SEQ ID NO: 8, wherein about 1 to about 5, such as about 1 to about 3, amino acid residues are substituted, deleted, and/or inserted. Preferably, the polypeptide has phosphoserine aminotransferase activity. More preferably, the polypeptide has phosphoserine aminotransferase activity similar to that of the polypeptide comprising an amino acid sequence set forth in SEQ ID NO: 8.

[0031] A bacterium, such as *Escherichia coli*, which has been modified to inactivate the genes *sdaA*, *sdaB*, *tdcG* and *glyA*, may show a low tolerance towards serine. Therefore, it would be desirable to provide a bacterium which shows increased tolerance towards serine.

[0032] In this respect, the present inventors have found that product toxicity can be reduced by overexpression of novel exporters, by evolving bacterial strains by random mutagenesis, and by adaptive evolution. As a result, bacteria having increased tolerance towards serine are provided. "Increased tolerance" as used herein means that a bacterium is capable of growing in a minimal culture medium (such as M9 minimal medium) comprising L-serine at a concentration of at least about 6.25 g/L.

[0033] According to certain embodiments, a bacterium of the present invention is capable of growing in a minimal culture medium (such as M9 minimal medium) comprising L-serine at a concentration of at least about 6.25 g/L (such as at least about 12.5 g/L). According to particular embodiments, the bacterium is capable of growing in a minimal culture medium (such as M9 minimal medium) comprising L-serine at a concentration of at least about 12.5 g/L (such as at least about 25 g/L). According to particular embodiments, the bacterium is capable of growing in a minimal culture medium (such as M9 minimal medium) comprising L-serine at a concentration of at least about 25 g/L (such as at least about 40 g/L). According to particular embodiments, the bacterium is capable of growing in a minimal culture medium (such as M9 minimal medium) comprising L-serine at a concentration of at least about 40 g/L (such as at least about 50 g/L). According to particular embodiments, the bacterium is capable of growing in a minimal culture medium (such as M9 minimal medium) comprising L-serine at a concentration of at least about 50 g/L (such as at

least about 75 g/L). According to particular embodiments, the bacterium is capable of growing in a minimal culture medium (such as M9 minimal medium) comprising L-serine at a concentration of at least about 75 g/L (such as at least about 100 g/L). According to particular embodiments, the bacterium is capable of growing in a minimal culture medium (such as M9 minimal medium) comprising L-serine at a concentration of at least about 100 g/L.

[0034] Preferably, the minimal culture medium, such as M9 minimal medium, is supplemented with 2mM glycine and 2g/L glucose. The bacterium is generally cultivated using adequate aeration at about 37°C for a period of about 24 to about 40 hours.

[0035] According to certain embodiments, a bacterium of the present invention is capable of growing in a minimal culture medium (such as M9 minimal medium) comprising L-serine at a concentration of at least about 6.25 g/L (such as at least about 12.5 g/L) at a growth rate of at least about 0.1 hr⁻¹ during exponential growth. According to particular embodiments, a bacterium of the invention is capable of growing in a minimal culture medium (such as M9 minimal medium) comprising L-serine at a concentration of at least about 12.5 g/L (such as at least about 25 g/L) at a growth rate of at least about 0.1 hr⁻¹ during exponential growth. According to particular embodiments, a bacterium of the invention is capable of growing in a minimal culture medium (such as M9 minimal medium) comprising L-serine at a concentration of at least about 25 g/L (such as at least about 50 g/L) at a growth rate of at least about 0.1 hr⁻¹ during exponential growth.

[0036] Preferably, the minimal culture medium, such as M9 minimal medium, is supplemented with 2mM glycine and 2g/L glucose. The bacterium is generally cultivated using adequate aeration at about 37°C for a period of about 24 to about 40 hours.

[0037] One novel exporter which when overexpressed in a bacterium improved tolerance towards serine is the O-acetylserine/cysteine export protein encoded by the gene *ydeD*. Further information regarding *ydeD* of, e.g., *Escherichia coli* is available at EcoCyc (www.biocyc.org) under Accession numbers EG11639. A representative amino acid sequence of such exporter protein is set forth in SEQ ID NO: 9. Therefore, the present invention provides a bacterium which has been modified to overexpress the gene *ydeD*.

[0038] According to certain embodiments, a bacterium of the invention comprises an exogenous nucleic acid molecule, such as an expression vector, comprising a nucleotide sequence encoding the protein product of the gene *ydeD*. According to particular embodiments, the bacterium comprises an exogenous nucleic acid molecule comprising a nucleotide sequence encoding a polypeptide comprising an amino acid sequence set forth in SEQ ID NO: 9. The exogenous nucleic acid molecule may comprise a nucleotide sequence encoding a polypeptide comprising an amino acid sequence set which has at least about 90%, at least about 93%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, or at least about 99%, sequence identity to the amino acid sequence set forth in SEQ ID NO: 9. Preferably, the polypeptide has O-acetylserine and/or cysteine transporter activity. More preferably, the polypeptide has O-acetylserine and/or cysteine transporter activity similar to that of the polypeptide comprising an amino acid sequence set forth in SEQ ID NO: 9. The exogenous nucleic acid molecule may comprise a nucleotide sequence encoding a polypeptide comprising an amino acid sequence set which has at least about 95%, such as at least about 96%, at least about 97%, at least about 98%, or at least about 99%, sequence identity to the amino acid sequence set forth in SEQ ID NO: 9. Preferably, the polypeptide has O-acetylserine and/or cysteine transporter activity. More preferably, the polypeptide has O-acetylserine and/or cysteine transporter activity similar to that of the polypeptide comprising an amino acid sequence set forth in SEQ ID NO: 9. The exogenous nucleic acid molecule may comprise a nucleotide sequence encoding a polypeptide comprising an amino acid sequence set forth in SEQ ID NO: 9, wherein 1 or more, such as about 1 to about 50, about 1 to about 40, about 1 to about 35, about 1 to about 30, about 1 to about 25, about 1 to about 20, about 1 to about 15, about 1 to about 10, about 1 to about 5, or about 1 to about 3, amino acid residues are substituted, deleted, and/or inserted. Preferably, the polypeptide has O-acetylserine and/or cysteine transporter activity. More preferably, the polypeptide has O-acetylserine and/or cysteine transporter activity similar to that of the polypeptide comprising an amino acid sequence set forth in SEQ ID NO: 9. The exogenous nucleic acid molecule may comprise a nucleotide sequence encoding a polypeptide comprising an amino acid sequence set forth in SEQ ID NO: 9, wherein about 1 to about 5, such as about 1 to about 3, amino acid residues are substituted, deleted, and/or inserted. Preferably, the polypeptide has O-acetylserine and/or cysteine transporter activity. More preferably, the polypeptide has O-acetylserine and/or cysteine transporter activity similar to that of the

polypeptide comprising an amino acid sequence set forth in SEQ ID NO: 9.

[0039] A modified YdeD polypeptide containing an additional stretch of 6 histidine residues at the C-terminus is set forth in SEQ ID NO: 10. According to particular embodiments, the bacterium comprises an exogenous nucleic acid molecule comprising a nucleotide sequence encoding a polypeptide comprising an amino acid sequence set forth in SEQ ID NO: 10. The exogenous nucleic acid molecule may comprise a nucleotide sequence encoding a polypeptide comprising an amino acid sequence set which has at least about 90%, at least about 93%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, or at least about 99%, sequence identity to the amino acid sequence set forth in SEQ ID NO: 10. Preferably, the polypeptide has O-acetylserine and/or cysteine transporter activity. More preferably, the polypeptide has O-acetylserine and/or cysteine transporter activity similar to that of the polypeptide comprising an amino acid sequence set forth in SEQ ID NO: 10. The exogenous nucleic acid molecule may comprise a nucleotide sequence encoding a polypeptide comprising an amino acid sequence set which has at least about 95%, such as at least about 96%, at least about 97%, at least about 98%, or at least about 99%, sequence identity to the amino acid sequence set forth in SEQ ID NO: 10. Preferably, the polypeptide has O-acetylserine and/or cysteine transporter activity. More preferably, the polypeptide has O-acetylserine and/or cysteine transporter activity similar to that of the polypeptide comprising an amino acid sequence set forth in SEQ ID NO: 10. The exogenous nucleic acid molecule may comprise a nucleotide sequence encoding a polypeptide comprising an amino acid sequence set forth in SEQ ID NO: 10, wherein 1 or more, such as about 1 to about 50, about 1 to about 40, about 1 to about 35, about 1 to about 30, about 1 to about 25, about 1 to about 20, about 1 to about 15, about 1 to about 10, about 1 to about 5, or about 1 to about 3, amino acid residues are substituted, deleted, and/or inserted. Preferably, the polypeptide has O-acetylserine and/or cysteine transporter activity. More preferably, the polypeptide has O-acetylserine and/or cysteine transporter activity similar to that of the polypeptide comprising an amino acid sequence set forth in SEQ ID NO: 10. The exogenous nucleic acid molecule may comprise a nucleotide sequence encoding a polypeptide comprising an amino acid sequence set forth in SEQ ID NO: 10, wherein about 1 to about 5, such as about 1 to about 3, amino acid residues are substituted, deleted, and/or inserted. Preferably, the polypeptide has O-acetylserine and/or cysteine transporter activity. More preferably, the polypeptide has O-acetylserine and/or cysteine transporter activity similar to that of the polypeptide comprising an amino acid sequence set forth in SEQ ID NO: 10.

[0040] As shown in figure 4, growth of a bacterium, such as *E. coli*, lacking the main serine degradation pathways is severely growth inhibited in the presence of even low concentrations of serine. Upon overexpression of *ydeD*, the tolerance towards serine is increased substantially, suggesting that YdeD may potentially transport serine out of the cell.

[0041] A bacterium of the invention having improved tolerance towards serine, such as one capable of growing in a minimal culture medium comprising L-serine at a concentration of at least about 6.25 g/L as mentioned above, can be obtained by random mutagenesis or by adaptive evolution. Respective details are provided in Examples 4 and 5, respectively.

[0042] Adaptive evolution may, for example, be achieved by carrying out the following method: Prior to the start of the experiment, suitable tubes are filled with 25 ml of culture media which are kept at 37 °C in a heat block. Controlled aeration is obtained using magnetic tumble stirrers placed inside the tubes and spinning at 1,800 rpm. At the start of the experiment, a single colony (of the starter strain) is grown overnight in one of the tubes, and 100 µL aliquots are used to inoculate a new tube containing 25 ml of fresh culture media. As the bacteria grow, multiple OD measurements at 600 nm are performed. Growth rates are calculated by taking the slope of a least-square linear regression line fit to the logarithm of the OD measurements. Once reaching a target OD of 0.4, 100 µl of culture are used to inoculate a new tube containing 25 ml of culture media. This way, cultures are serially passed (2-3 times per day) to tubes with fresh media after reaching the targeted cell density such that stationary phase is never reached. The experiment is initiated with an L-serine concentration of 3 g/L serine, followed by an increase to 6 g/L of L-Serine after the desired growth rate has been reached. Once the populations achieved a stable phenotype (i.e. growth rate), the L-Serine concentration is increased to 12 g/L. This process is repeated iteratively using 24, 50, 75, and 100 g/L of L-serine. The final population may then be plated on the LB-agar for further cultivation and selection of an L-serine tolerant strain. The foregoing method may be performed manually or by using an automated system enable the propagation of evolving populations over the course of many days while monitoring their growth rates.

[0043] As further demonstrate herein, the present inventors have identified beneficial mutations in a number of genes

which confer tolerance towards L-serine. Respective genes and mutations are depicted in Table S5.

[0044] One such gene is *thrA* which encodes an aspartate kinase I / homoserine dehydrogenase I (ThrA). Further information regarding *thrA* of, e.g., *Escherichia coli* is available at EcoCyc (www.biocyc.org) under Accession numbers EG10998. A representative amino acid sequence of a wild type Aspartate kinase I / homoserine dehydrogenase I (ThrA) is set forth in SEQ ID NO: 11. As demonstrated in Example 6 and 10, introducing certain mutation within the amino acid sequence of the aspartate kinase I / homoserine dehydrogenase results in a very significant increase in tolerance towards serine (Figures 7 and 11). Particularly, the following mutations have been shown to be beneficial: Y356C, S357R and S359R.

[0045] According to certain embodiments, the present invention provides a bacterium which expresses an aspartate kinase I / homoserine dehydrogenase I (ThrA) mutant which is not inhibited by L-serine.

[0046] According to certain embodiments, the present invention provides a bacterium which comprises within the *thrA* gene one or more nucleotide substitutions resulting in one or more amino acid substitutions which increase tolerance towards L-serine. More particularly, a bacterium is provided which comprises within the *thrA* gene one or more nucleotide substitutions resulting in one or more amino acid substitutions in the encoded polypeptide at a position selected from the group consisting of Y356, S357 and S359. A bacterium of the invention may thus express an aspartate kinase I / homoserine dehydrogenase I (ThrA) having one or more amino acid substitutions at a position selected from the group consisting of Y356, S357 and S359. More particularly, a bacterium of the invention may express a polypeptide having the amino acid sequence set forth in SEQ ID NO: 11 which comprises one or more amino acid substitutions at a position selected from the group consisting of Y356, S357 and S359. Preferably, the one or more amino acid substitutions are non-conservative substitutions.

[0047] According to certain embodiments, the present invention provides a bacterium which comprises within the *thrA* gene one or more nucleotide substitutions resulting in an amino acid substitution in the encoded polypeptide at position Y356. A bacterium of the invention may thus express a polypeptide encoded by the *thrA* gene, wherein said polypeptide comprises an amino acid substitution at position Y356. According to particular embodiments, the amino acid substitution is selected from the group consisting of Y356C, Y356T, Y356V, Y356S, Y356W, Y356Q, Y356G, Y356N, Y356D, Y356E, Y356F, Y356A, Y356I, Y356P, Y356H, Y356R, and Y356L. According to other particular embodiments, the amino acid substitution is selected from the group consisting of Y356C, Y356T, Y356V, Y356W, Y356Q, Y356G, Y356E, Y356F, Y356A, Y356I, Y356P, Y356H, Y356R, and Y356L. According to more particular embodiments, the amino acid substitution is selected from the group consisting of Y356C, Y356T, Y356W, Y356F, Y356A, Y356I, Y356P, Y356H, Y356R, and Y356L. According to other more particular embodiments, the amino acid substitution is selected from the group consisting of Y356C, Y356W, Y356F, Y356I, Y356P, Y356R, and Y356L.

[0048] According to certain embodiments, the bacterium comprises within the *thrA* gene one or more nucleotide substitutions resulting in a Y356C substitution in the encoded polypeptide. A bacterium of the invention may thus express a polypeptide encoded by the *thrA* gene, wherein said polypeptide comprises a Y356C substitution. According to certain embodiments, the bacterium comprises within the *thrA* gene one or more nucleotide substitutions resulting in a Y356T substitution in the encoded polypeptide. A bacterium of the invention may thus express a polypeptide encoded by the *thrA* gene, wherein said polypeptide comprises a Y356T substitution. According to certain embodiments, the bacterium comprises within the *thrA* gene one or more nucleotide substitutions resulting in a Y356V substitution in the encoded polypeptide. A bacterium of the invention may thus express a polypeptide encoded by the *thrA* gene, wherein said polypeptide comprises a Y356V substitution. According to certain embodiments, the bacterium comprises within the *thrA* gene one or more nucleotide substitutions resulting in a Y356S substitution in the encoded polypeptide. A bacterium of the invention may thus express a polypeptide encoded by the *thrA* gene, wherein said polypeptide comprises a Y356S substitution. According to certain embodiments, the bacterium comprises within the *thrA* gene one or more nucleotide substitutions resulting in a Y356W substitution in the encoded polypeptide. A bacterium of the invention may thus express a polypeptide encoded by the *thrA* gene, wherein said polypeptide comprises a Y356W substitution. According to certain embodiments, the bacterium comprises within the *thrA* gene one or more nucleotide substitutions resulting in a Y356G substitution in the encoded polypeptide. A bacterium of the invention may thus express a polypeptide encoded by the *thrA* gene, wherein said polypeptide comprises a Y356G substitution. According to certain embodiments, the bacterium comprises within the *thrA* gene one or more nucleotide substitutions resulting in a Y356N substitution in the encoded polypeptide. A bacterium of the invention may thus

[0057] According to certain embodiments, the present invention provides a bacterium which comprises within the *thrA* gene one or more nucleotide substitutions resulting in an amino acid substitution in the encoded polypeptide at position Y356, one or more nucleotide substitutions resulting in an amino acid substitution in the encoded polypeptide at position S357 and one or more nucleotide substitutions resulting in an amino acid substitution in the encoded polypeptide at position S359; wherein the substitution at position Y356 is selected from the group consisting of Y356C, Y356T, Y356V, Y356S, Y356W, Y356Q, Y356G, Y356N, Y356D, Y356E, Y356F, Y356A, Y356I, Y356P, Y356H, Y356R and Y356L; the substitution at position S357 is selected from the group consisting of S357R, S357V, S357P, S357G, S357L, S357Y, S357A, S357N, S357F, S357H, S357K, S357I and S357M; and the substitution at position S359 is selected from the group consisting of S359R, S359G, S359M, S359F, S359T, S359P, S359V, S359Q, S359A, S359C, S359K, S359E and S359L.

[0058] According to certain embodiments, the present invention provides a bacterium which comprises within the *thrA* gene one or more nucleotide substitutions resulting in an amino acid substitution in the encoded polypeptide at position Y356 and one or more nucleotide substitutions resulting in an amino acid substitution in the encoded polypeptide at position S357; wherein the substitution at position Y356 is selected from the group consisting of Y356C, Y356T, Y356V, Y356S, Y356W, Y356Q, Y356G, Y356N, Y356D, Y356E, Y356F, Y356A, Y356I, Y356P, Y356H, Y356R and Y356L; and the substitution at position S357 is selected from the group consisting of S357R, S357V, S357P, S357G, S357L, S357Y, S357A, S357N, S357F, S357H, S357K, S357I and S357M.

[0059] According to certain embodiments, the present invention provides a bacterium which comprises within the *thrA* gene one or more nucleotide substitutions resulting in an amino acid substitution in the encoded polypeptide at position Y356 and one or more nucleotide substitutions resulting in an amino acid substitution in the encoded polypeptide at position S359; wherein the substitution at position Y356 is selected from the group consisting of Y356C, Y356T, Y356V, Y356S, Y356W, Y356Q, Y356G, Y356N, Y356D, Y356E, Y356F and Y356A; and the substitution at position S359 is selected from the group consisting of S359R, S359G, S359M, S359F, S359T, S359P, S359V, S359Q, S359A, S359C, S359K, S359E and S359L.

[0060] According to certain embodiments, the present invention provides a bacterium which comprises within the *thrA* gene one or more nucleotide substitutions resulting in an amino acid substitution in the encoded polypeptide at position S357 and one or more nucleotide substitutions resulting in an amino acid substitution in the encoded polypeptide at position S359; wherein the substitution at position S357 is selected from the group consisting of S357R, S357V, S357P, S357G, S357L, S357Y, S357A, S357N, S357F, S357H, S357K, S357I and S357M; and the substitution at position S359 is selected from the group consisting of S359R, S359G, S359M, S359F, S359T, S359P, S359V, S359Q, S359A, S359C, S359K, S359E and S359L.

[0061] According to certain embodiments, a bacterium of the invention express a polypeptide having the amino acid sequence set forth in SEQ ID NO: 11 which comprises an amino acid substitution at position Y356, S357 and/or S359, wherein the substitution at position Y356 is selected from the group consisting of Y356C, Y356T, Y356V, Y356S, Y356W, Y356Q, Y356G, Y356N, Y356D, Y356E, Y356F, Y356A, Y356I, Y356P, Y356H, Y356R and Y356L; the substitution at position S357 is selected from the group consisting of S357R, S357V, S357P, S357G, S357L, S357Y, S357A, S357N, S357F, S357H, S357K, S357I and S357M; and the substitution at position S359 is selected from the group consisting of S359R, S359G, S359M, S359F, S359T, S359P, S359V, S359Q, S359A, S359C, S359K, S359E and S359L.

[0062] According to certain embodiments, a bacterium of the invention express a polypeptide having the amino acid sequence set forth in SEQ ID NO: 11 which comprises an amino acid substitution at position Y356 and/or S357, wherein the substitution at position Y356 is selected from the group consisting of Y356C, Y356T, Y356V, Y356S, Y356W, Y356Q, Y356G, Y356N, Y356D, Y356E, Y356F, Y356A, Y356I, Y356P, Y356H, Y356R and Y356L; and the substitution at position S357 is selected from the group consisting of S357R, S357V, S357P, S357G, S357L, S357Y, S357A, S357N, S357F, S357H, S357K, S357I and S357M.

[0063] According to certain embodiments, a bacterium of the invention express a polypeptide having the amino acid sequence set forth in SEQ ID NO: 11 which comprises an amino acid substitution at position Y356 and/or S359, wherein the substitution at position Y356 is selected from the group consisting of Y356C, Y356T, Y356V, Y356S,

Y356W, Y356Q, Y356G, Y356N, Y356D, Y356E, Y356F, Y356A, Y356I, Y356P, Y356H, Y356R and Y356L; and the substitution at position S359 is selected from the group consisting of S359R, S359G, S359M, S359F, S359T, S359P, S359V, S359Q, S359A, S359C, S359K, S359E and S359L.

[0064] According to certain embodiments, a bacterium of the invention express a polypeptide having the amino acid sequence set forth in SEQ ID NO: 11 which comprises an amino acid substitution at position S357 and/or S359, wherein the substitution at position S357 is selected from the group consisting of S357R, S357V, S357P, S357G, S357L, S357Y, S357A, S357N, S357F, S357H, S357K, S357I and S357M; and the substitution at position S359 is selected from the group consisting of S359R, S359G, S359M, S359F, S359T, S359P, S359V, S359Q, S359A, S359C, S359K, S359E and S359L.

[0065] According to certain embodiments, a bacterium of the invention express a polypeptide having the amino acid sequence set forth in SEQ ID NO: 11 which comprises an amino acid substitution at position Y356, wherein the substitution at position Y356 is selected from the group consisting of Y356C, Y356T, Y356V, Y356S, Y356W, Y356Q, Y356G, Y356N, Y356D, Y356E, Y356F, Y356A, Y356I, Y356P, Y356H, Y356R and Y356L.

[0066] According to certain embodiments, a bacterium of the invention express a polypeptide having the amino acid sequence set forth in SEQ ID NO: 11 which comprises an amino acid substitution at position S357, wherein the substitution at position S357 is selected from the group consisting of S357R, S357V, S357P, S357G, S357L, S357Y, S357A, S357N, S357F, S357H, S357K, S357I and S357M; and the substitution at position S359 is selected from the group consisting of S359R, S359G, S359M, S359F, S359T, S359P, S359V, S359Q, S359A, S359C, S359K, S359E and S359L.

[0067] According to certain embodiments, a bacterium of the invention express a polypeptide having the amino acid sequence set forth in SEQ ID NO: 11 which comprises an amino acid substitution at position S359, wherein the substitution at position S359 is selected from the group consisting of S359R, S359G, S359M, S359F, S359T, S359P, S359V, S359Q, S359A, S359C, S359K, S359E and S359L.

[0068] According to certain embodiments, a bacterium of the invention express a polypeptide having the amino acid sequence set forth in SEQ ID NO: 11 which comprises an amino acid substitution at position Y356 and S357, wherein the substitution at position Y356 is selected from the group consisting of Y356C, Y356T, Y356V, Y356S, Y356W, Y356Q, Y356G, Y356N, Y356D, Y356E, Y356F and Y356A; and the substitution at position S357 is selected from the group consisting of S357R, S357V, S357P, S357G, S357L, S357Y, S357A, S357N, S357F, S357H, S357K, S357I and S357M.

[0069] According to certain embodiments, a bacterium of the invention express a polypeptide having the amino acid sequence set forth in SEQ ID NO: 11 which comprises an amino acid substitution at position Y356 and S359, wherein the substitution at position Y356 is selected from the group consisting of Y356C, Y356T, Y356V, Y356S, Y356W, Y356Q, Y356G, Y356N, Y356D, Y356E, Y356F, Y356A, Y356I, Y356P, Y356H, Y356R and Y356L; and the substitution at position S359 is selected from the group consisting of S359R, S359G, S359M, S359F, S359T, S359P, S359V, S359Q, S359A, S359C, S359K, S359E and S359L.

[0070] According to certain embodiments, a bacterium of the invention express a polypeptide having the amino acid sequence set forth in SEQ ID NO: 11 which comprises an amino acid substitution at position S357 and S359, wherein the substitution at position S357 is selected from the group consisting of S357R, S357V, S357P, S357G, S357L, S357Y, S357A, S357N, S357F, S357H, S357K, S357I and S357M; and the substitution at position S359 is selected from the group consisting of S359R, S359G, S359M, S359F, S359T, S359P, S359V, S359Q, S359A, S359C, S359K, S359E and S359L.

[0071] According to certain embodiments, a bacterium of the invention express a polypeptide having the amino acid sequence set forth in SEQ ID NO: 11 which comprises an amino acid substitution at position Y356, S357 and S359, wherein the substitution at position Y356 is selected from the group consisting of Y356C, Y356T, Y356V, Y356S, Y356W, Y356Q, Y356G, Y356N, Y356D, Y356E, Y356F, Y356A, Y356I, Y356P, Y356H, Y356R and Y356L; the substitution at position S357 is selected from the group consisting of S357R, S357V, S357P, S357G, S357L, S357Y, S357A, S357N, S357F, S357H, S357K, S357I and S357M; and the substitution at position S359 is selected from the

group consisting of S359R, S359G, S359M, S359F, S359T, S359P, S359V, S359Q, S359A, S359C, S359K, S359E and S359L.

[illegible]

[0073] According to certain embodiments, a bacterium of the invention expresses a polypeptide having the amino acid sequence set forth in SEQ ID NO: 11, wherein at position 357 serine is replaced by arginine. According to certain embodiments, a bacterium of the invention expresses a polypeptide having the amino acid sequence set forth in SEQ ID NO: 11, wherein at position 357 serine is replaced by valine. According to certain embodiments, a bacterium of the invention expresses a polypeptide having the amino acid sequence set forth in SEQ ID NO: 11, wherein at position 357 serine is replaced by proline. According to certain embodiments, a bacterium of the invention expresses a polypeptide having the amino acid sequence set forth in SEQ ID NO: 11, wherein at position 357 serine is replaced by glycine. According to certain embodiments, a bacterium of the invention expresses a polypeptide having the amino acid sequence set forth in SEQ ID NO: 11, wherein at position 357 serine is replaced by leucine. According to certain embodiments, a bacterium of the invention expresses a polypeptide having the amino acid sequence set forth in SEQ ID NO: 11, wherein at position 357 serine is replaced by tyrosine. According to certain embodiments, a bacterium of the invention expresses a polypeptide having the amino acid sequence set forth in SEQ ID NO: 11, wherein at position 357 serine is replaced by alanine. According to certain embodiments, a bacterium of the invention expresses a polypeptide having the amino acid sequence set forth in SEQ ID NO: 11, wherein at position 357 serine is replaced by asparagine. According to certain embodiments, a bacterium of the invention expresses a polypeptide having the amino acid sequence set forth in SEQ ID NO: 11, wherein at position 357 serine is replaced by phenylalanine. According to certain embodiments, a bacterium of the invention expresses a polypeptide having the amino acid sequence set forth in SEQ ID NO: 11, wherein at position 357 serine is replaced by histidine.

[0074] According to certain embodiments, a bacterium of the invention expresses a polypeptide having the amino acid

sequence set forth in SEQ ID NO: 11, wherein at position 357 serine is replaced by lysine. According to certain embodiments, a bacterium of the invention expresses a polypeptide having the amino acid sequence set forth in SEQ ID NO: 11, wherein at position 357 serine is replaced by isoleucine. According to certain embodiments, a bacterium of the invention expresses a polypeptide having the amino acid sequence set forth in SEQ ID NO: 11, wherein at position 357 serine is replaced by methionine.

[0075] According to certain embodiments, a bacterium of the invention expresses a polypeptide having the amino acid sequence set forth in SEQ ID NO: 11, wherein at position 359 serine is replaced by arginine. According to certain embodiments, a bacterium of the invention expresses a polypeptide having the amino acid sequence set forth in SEQ ID NO: 11, wherein at position 359 serine is replaced by glycine. According to certain embodiments, a bacterium of the invention expresses a polypeptide having the amino acid sequence set forth in SEQ ID NO: 11, wherein at position 359 serine is replaced by methionine. According to certain embodiments, a bacterium of the invention expresses a polypeptide having the amino acid sequence set forth in SEQ ID NO: 11, wherein at position 359 serine is replaced by phenylalanine. According to certain embodiments, a bacterium of the invention expresses a polypeptide having the amino acid sequence set forth in SEQ ID NO: 11, wherein at position 359 serine is replaced by threonine. According to certain embodiments, a bacterium of the invention expresses a polypeptide having the amino acid sequence set forth in SEQ ID NO: 11, wherein at position 359 serine is replaced by proline. According to certain embodiments, a bacterium of the invention expresses a polypeptide having the amino acid sequence set forth in SEQ ID NO: 11, wherein at position 359 serine is replaced by valine. According to certain embodiments, a bacterium of the invention expresses a polypeptide having the amino acid sequence set forth in SEQ ID NO: 11, wherein at position 359 serine is replaced by glutamine. According to certain embodiments, a bacterium of the invention expresses a polypeptide having the amino acid sequence set forth in SEQ ID NO: 11, wherein at position 359 serine is replaced by alanine. According to certain embodiments, a bacterium of the invention expresses a polypeptide having the amino acid sequence set forth in SEQ ID NO: 11, wherein at position 359 serine is replaced by cysteine. According to certain embodiments, a bacterium of the invention expresses a polypeptide having the amino acid sequence set forth in SEQ ID NO: 11, wherein at position 359 serine is replaced by lysine. According to certain embodiments, a bacterium of the invention expresses a polypeptide having the amino acid sequence set forth in SEQ ID NO: 11, wherein at position 359 serine is replaced by glutamic acid. According to certain embodiments, a bacterium of the invention expresses a polypeptide having the amino acid sequence set forth in SEQ ID NO: 11, wherein at position 359 serine is replaced by leucine.

[0076] According to certain embodiments, a bacterium of the invention expresses a polypeptide having an amino acid sequence which has at least about 90%, such as at least about 93%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, or at least about 99%, sequence identity to the amino acid sequence set forth in SEQ ID NO: 11 which comprises an amino acid substitution at position Y356, S357 and/or S359, wherein the substitution at position Y356 is selected from the group consisting of Y356C, Y356T, Y356V, Y356S, Y356W, Y356Q, Y356G, Y356N, Y356D, Y356E, Y356F, Y356A, Y356I, Y356P, Y356H, Y356R and Y356L; the substitution at position S357 is selected from the group consisting of S357R, S357V, S357P, S357G, S357L, S357Y, S357A, S357N, S357F, S357H, S357K, S357I and S357M; and the substitution at position S359 is selected from the group consisting of S359R, S359G, S359M, S359F, S359T, S359P, S359V, S359Q, S359A, S359C, S359K, S359E and S359L.

[0077] According to certain embodiments, a bacterium of the invention expresses a polypeptide having an amino acid sequence which has at least about 90%, such as at least about 93%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, or at least about 99%, sequence identity to the amino acid sequence set forth in SEQ ID NO: 11 which comprises an amino acid substitution at position Y356 and/or S357, wherein the substitution at position Y356 is selected from the group consisting of Y356C, Y356T, Y356V, Y356S, Y356W, Y356Q, Y356G, Y356N, Y356D, Y356E, Y356F, Y356A, Y356I, Y356P, Y356H, Y356R and Y356L; and the substitution at position S357 is selected from the group consisting of S357R, S357V, S357P, S357G, S357L, S357Y, S357A, S357N, S357F, S357H, S357K, S357I and S357M.

[0078] According to certain embodiments, a bacterium of the invention expresses a polypeptide having an amino acid sequence which has at least about 90%, such as at least about 93%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, or at least about 99%, sequence identity to the amino acid sequence set forth in SEQ ID NO: 11 which comprises an amino acid substitution at position Y356 and/or S359, wherein the substitution at position Y356 is selected from the group consisting of Y356C, Y356T, Y356V, Y356S, Y356W, Y356Q, Y356G, Y356N,

Y356D, Y356E, Y356F, Y356A, Y356I, Y356P, Y356H, Y356R and Y356L; and the substitution at position S359 is selected from the group consisting of S359R, S359G, S359M, S359F, S359T, S359P, S359V, S359Q, S359A, S359C, S359K, S359E and S359L.

[0079] According to certain embodiments, a bacterium of the invention expresses a polypeptide having an amino acid sequence which has at least about 90%, such as at least about 93%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, or at least about 99%, sequence identity to the amino acid sequence set forth in SEQ ID NO: 11 which comprises an amino acid substitution at position S357 and/or S359, wherein the substitution at position S357 is selected from the group consisting of S357R, S357V, S357P, S357G, S357L, S357Y, S357A, S357N, S357F, S357H, S357K, S357I and S357M; and the substitution at position S359 is selected from the group consisting of S359R, S359G, S359M, S359F, S359T, S359P, S359V, S359Q, S359A, S359C, S359K, S359E and S359L.

[0080] According to certain embodiments, a bacterium of the invention expresses a polypeptide having an amino acid sequence which has at least about 90%, such as at least about 93%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, or at least about 99%, sequence identity to the amino acid sequence set forth in SEQ ID NO: 11 which comprises an amino acid substitution at position Y356, wherein the substitution at position Y356 is selected from the group consisting of Y356C, Y356T, Y356V, Y356S, Y356W, Y356Q, Y356G, Y356N, Y356D, Y356E, Y356F, Y356A, Y356I, Y356P, Y356H, Y356R and Y356L.

[0081] According to certain embodiments, a bacterium of the invention expresses a polypeptide having an amino acid sequence which has at least about 90%, such as at least about 93%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, or at least about 99%, sequence identity to the amino acid sequence set forth in SEQ ID NO: 11 which comprises an amino acid substitution at position S357, wherein the substitution at position S357 is selected from the group consisting of S357R, S357V, S357P, S357G, S357L, S357Y, S357A, S357N, S357F, S357H, S357K, S357I and S357M.

[0082] According to certain embodiments, a bacterium of the invention expresses a polypeptide having an amino acid sequence which has at least about 90%, such as at least about 93%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, or at least about 99%, sequence identity to the amino acid sequence set forth in SEQ ID NO: 11 which comprises an amino acid substitution at position S359, wherein the substitution at position S359 is selected from the group consisting of S359R, S359G, S359M, S359F, S359T, S359P, S359V, S359Q, S359A, S359C, S359K, S359E and S359L.

[0083] According to certain embodiments, a bacterium of the invention expresses a polypeptide having an amino acid sequence which has at least about 93%, such as at least about 95%, at least about 96%, at least about 97%, at least about 98%, or at least about 99%, sequence identity to the amino acid sequence set forth in SEQ ID NO: 11 which comprises an amino acid substitution at position Y356, wherein the substitution at position Y356 is selected from the group consisting of Y356C, Y356T, Y356V, Y356S, Y356W, Y356Q, Y356G, Y356N, Y356D, Y356E, Y356F, Y356A, Y356I, Y356P, Y356H, Y356R and Y356L.

[0084] According to certain embodiments, a bacterium of the invention expresses a polypeptide having an amino acid sequence which has at least about 93%, such as at least about 95%, at least about 96%, at least about 97%, at least about 98%, or at least about 99%, sequence identity to the amino acid sequence set forth in SEQ ID NO: 11 which comprises an amino acid substitution at position S357, wherein the substitution at position S357 is selected from the group consisting of S357R, S357V, S357P, S357G, S357L, S357Y, S357A, S357N, S357F, S357H, S357K, S357I and S357M.

[0085] According to certain embodiments, a bacterium of the invention expresses a polypeptide having an amino acid sequence which has at least about 93%, such as at least about 95%, at least about 96%, at least about 97%, at least about 98%, or at least about 99%, sequence identity to the amino acid sequence set forth in SEQ ID NO: 11 which comprises an amino acid substitution at position S359, wherein the substitution at position S359 is selected from the group consisting of S359R, S359G, S359M, S359F, S359T, S359P, S359V, S359Q, S359A, S359C, S359K, S359E and S359L.

[0086] According to certain embodiments, a bacterium of the invention expresses a polypeptide having an amino acid

sequence which has at least about 95%, such as at least about 96%, at least about 97%, at least about 98%, or at least about 99%, sequence identity to the amino acid sequence set forth in SEQ ID NO: 11 which comprises an amino acid substitution at position Y356, wherein the substitution at position Y356 is selected from the group consisting of Y356C, Y356T, Y356V, Y356S, Y356W, Y356Q, Y356G, Y356N, Y356D, Y356E, Y356F, Y356A, Y356I, Y356P, Y356H, Y356R and Y356L.

[0087] According to certain embodiments, a bacterium of the invention expresses a polypeptide having an amino acid sequence which has at least about 95%, such as at least about 96%, at least about 97%, at least about 98%, or at least about 99%, sequence identity to the amino acid sequence set forth in SEQ ID NO: 11 which comprises an amino acid substitution at position S357, wherein the substitution at position S357 is selected from the group consisting of S357R, S357V, S357P, S357G, S357L, S357Y, S357A, S357N, S357F, S357H, S357K, S357I and S357M.

[0088] According to certain embodiments, a bacterium of the invention expresses a polypeptide having an amino acid sequence which has at least about 95%, such as at least about 96%, at least about 97%, at least about 98%, or at least about 99%, sequence identity to the amino acid sequence set forth in SEQ ID NO: 11 which comprises an amino acid substitution at position S359, wherein the substitution at position S359 is selected from the group consisting of S359R, S359G, S359M, S359F, S359T, S359P, S359V, S359Q, S359A, S359C, S359K, S359E and S359L.

[0089] According to certain embodiments, a bacterium of the invention expresses a polypeptide having an amino acid sequence which has at least about 90%, such as at least about 93%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, or at least about 99%, sequence identity to the amino acid sequence set forth in SEQ ID NO: 11 which comprises an amino acid substitution at position Y356, S357 and S359, wherein the substitution at position Y356 is selected from the group consisting of Y356C, Y356T, Y356V, Y356S, Y356W, Y356Q, Y356G, Y356N, Y356D, Y356E, Y356F, Y356A, Y356I, Y356P, Y356H, Y356R and Y356L; the substitution at position S357 is selected from the group consisting of S357R, S357V, S357P, S357G, S357L, S357Y, S357A, S357N, S357F, S357H, S357K, S357I and S357M; and the substitution at position S359 is selected from the group consisting of S359R, S359G, S359M, S359F, S359T, S359P, S359V, S359Q, S359A, S359C, S359K, S359E and S359L.

[0090] According to certain embodiments, a bacterium of the invention expresses a polypeptide having an amino acid sequence which has at least about 90%, such as at least about 93%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, or at least about 99%, sequence identity to the amino acid sequence set forth in SEQ ID NO: 11 which comprises an amino acid substitution at position Y356 and S357, wherein the substitution at position Y356 is selected from the group consisting of Y356C, Y356T, Y356V, Y356S, Y356W, Y356Q, Y356G, Y356N, Y356D, Y356E, Y356F, Y356A, Y356I, Y356P, Y356H, Y356R and Y356L; and the substitution at position S357 is selected from the group consisting of S357R, S357V, S357P, S357G, S357L, S357Y, S357A, S357N, S357F, S357H, S357K, S357I and S357M.

[0091] According to certain embodiments, a bacterium of the invention expresses a polypeptide having an amino acid sequence which has at least about 90%, such as at least about 93%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, or at least about 99%, sequence identity to the amino acid sequence set forth in SEQ ID NO: 11 which comprises an amino acid substitution at position Y356 and S359, wherein the substitution at position Y356 is selected from the group consisting of Y356C, Y356T, Y356V, Y356S, Y356W, Y356Q, Y356G, Y356N, Y356D, Y356E, Y356F, Y356A, Y356I, Y356P, Y356H, Y356R and Y356L; and the substitution at position S359 is selected from the group consisting of S359R, S359G, S359M, S359F, S359T, S359P, S359V, S359Q, S359A, S359C, S359K, S359E and S359L.

[0092] According to certain embodiments, a bacterium of the invention expresses a polypeptide having an amino acid sequence which has at least about 90%, such as at least about 93%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, or at least about 99%, sequence identity to the amino acid sequence set forth in SEQ ID NO: 11 which comprises an amino acid substitution at position S357 and S359, wherein the substitution at position Y356 is selected from the group consisting of Y356C, Y356T, Y356V, Y356S, Y356W, Y356Q, Y356G, Y356N, Y356D, Y356E, Y356F, Y356A, Y356I, Y356P, Y356H, Y356R and Y356L; the substitution at position S357 is selected from the group consisting of S357R, S357V, S357P, S357G, S357L, S357Y, S357A, S357N, S357F, S357H, S357K, S357I and S357M; and the substitution at position S359 is selected from the group consisting of S359R, S359G, S359M, S359F, S359T, S359P, S359V, S359Q, S359A, S359C, S359K, S359E and S359L.

[0093] According to certain embodiments, a bacterium of the invention expresses a polypeptide having an amino acid sequence set forth in SEQ ID NO: 11 which comprises an amino acid substitution at position Y356, S357 and/or S359, wherein the substitution at position Y356 is selected from the group consisting of Y356C, Y356T, Y356V, Y356S, Y356W, Y356Q, Y356G, Y356N, Y356D, Y356E, Y356F, Y356A, Y356I, Y356P, Y356H, Y356R and Y356L; the substitution at position S357 is selected from the group consisting of S357R, S357V, S357P, S357G, S357L, S357Y, S357A, S357N, S357F, S357H, S357K, S357I and S357M; and the substitution at position S359 is selected from the group consisting of S359R, S359G, S359M, S359F, S359T, S359P, S359V, S359Q, S359A, S359C, S359K, S359E and S359L; and wherein 1 or more, such as about 1 to about 50, about 1 to about 40, about 1 to about 35, about 1 to about 30, about 1 to about 25, about 1 to about 20, about 1 to about 15, about 1 to about 10, about 1 to about 5, or about 1 to about 3, further amino acid residues are substituted, deleted, and/or inserted.

[0094] The bacterium may express a polypeptide having an amino acid sequence set forth in SEQ ID NO: 11 which comprises an amino acid substitution at position Y356, S357 and/or S359, wherein the substitution at position Y356 is selected from the group consisting of Y356C, Y356T, Y356V, Y356S, Y356W, Y356Q, Y356G, Y356N, Y356D, Y356E, Y356F, Y356A, Y356I, Y356P, Y356H, Y356R and Y356L; the substitution at position S357 is selected from the group consisting of S357R, S357V, S357P, S357G, S357L, S357Y, S357A, S357N, S357F, S357H, S357K, S357I and S357M; and the substitution at position S359 is selected from the group consisting of S359R, S359G, S359M, S359F, S359T, S359P, S359V, S359Q, S359A, S359C, S359K, S359E and S359L; and wherein about 1 to about 5, such as about 1 to about 3, further amino acid residues are substituted, deleted, and/or inserted.

[0095] According to certain embodiments, the present invention provides a bacterium which comprises within the *thrA* gene one or more nucleotide substitutions resulting in one or more amino acid substitutions in the encoded polypeptide selected from the group consisting of Y356C, S357R and S359R. A bacterium of the invention may thus express a polypeptide encoded by the *thrA* gene, wherein said polypeptide comprises one or more (such as two or three) amino acid substitutions selected from the group consisting of Y356C, S357R and S359R. According to certain embodiments, a bacterium of the invention comprises within the *thrA* gene one or more nucleotide substitutions resulting in one or more (such as two) amino acid substitutions in the encoded polypeptide selected from the group consisting of Y356C and S357R. A bacterium of the invention may thus express a polypeptide encoded by the *thrA* gene, wherein said polypeptide comprises one or more (such as two) amino acid substitutions in the encoded polypeptide selected from the group consisting of Y356C and S357R. According to certain embodiments, a bacterium of the invention comprises within the *thrA* gene one or more nucleotide substitutions resulting in one or more (such as two) amino acid substitutions in the encoded polypeptide selected from the group consisting of Y356C and S359R. A bacterium of the invention may thus express a polypeptide encoded by the *thrA* gene, wherein said polypeptide comprises one or more (such as two) amino acid substitutions in the encoded polypeptide selected from the group consisting of Y356C and S359R. According to certain embodiments, a bacterium of the invention comprises within the *thrA* gene one or more nucleotide substitutions resulting in one or more (such as two) amino acid substitutions in the encoded polypeptide selected from the group consisting of S357R and S359R. A bacterium of the invention may thus express a polypeptide encoded by the *thrA* gene, wherein said polypeptide comprises one or more (such as two) amino acid substitutions in the encoded polypeptide selected from the group consisting of S357R and S359R.

[0096] According to the certain embodiments, the bacterium comprises within the *thrA* gene one or more nucleotide substitutions resulting in a Y356C substitution in the encoded polypeptide. A bacterium of the invention may thus express a polypeptide encoded by the *thrA* gene, wherein said polypeptide comprises a Y356C substitution. According to the certain embodiments, the bacterium comprises within the *thrA* gene one or more nucleotide substitutions resulting in a S357R substitution in the encoded polypeptide. A bacterium of the invention may thus express a polypeptide encoded by the *thrA* gene, wherein said polypeptide comprises a S357R substitution. According to the certain embodiments, the bacterium comprises within the *thrA* gene one or more nucleotide substitutions resulting in a S359R substitution in the encoded polypeptide. A bacterium of the invention may thus express a polypeptide encoded by the *thrA* gene, wherein said polypeptide comprises a S359R substitution.

[0097] A bacterium of the invention may thus express an aspartate kinase I / homoserine dehydrogenase I (ThrA) having one or more amino acid substitutions selected from the group consisting of Y356C, S357R and S359R. More particularly, a bacterium of the invention may express a polypeptide having the amino acid sequence set forth in SEQ ID NO: 11 which comprises one or more amino acid substitutions selected from the group consisting of Y356C, S357R

and S359R. According to certain embodiments, the bacterium expresses a polypeptide having the amino acid sequence set forth in SEQ ID NO: 11, wherein at position 356 tyrosine is replaced by cysteine. According to certain embodiments, the bacterium expresses a polypeptide having the amino acid sequence set forth in SEQ ID NO: 11, wherein at position 357 serine is replaced by arginine. According to certain embodiments, the bacterium expresses a polypeptide having the amino acid sequence set forth in SEQ ID NO: 11, wherein at position 359 serine is replaced by arginine.

[0098] According to certain embodiments, the bacterium expresses a polypeptide having an amino acid sequence set forth which has at least about 90%, at least about 93%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, or at least about 99%, sequence identity to the amino acid sequence set forth in SEQ ID NO: 11 which comprises one or more amino acid substitutions selected from the group consisting of Y356C, S357R and S359R.

[0099] According to certain embodiments, the bacterium expresses a polypeptide having an amino acid sequence set forth which has at least about 90%, at least about 93%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, or at least about 99%, sequence identity to the amino acid sequence set forth in SEQ ID NO: 11, wherein at position 356 tyrosine is replaced by cysteine.

[0100] According to particular embodiments, the bacterium expresses a polypeptide having an amino acid sequence set forth which has at least about 95%, such as at least about 96%, at least about 97%, at least about 98%, or at least about 99%, sequence identity to the amino acid sequence set forth in SEQ ID NO: 11, wherein at position 356 tyrosine is replaced by cysteine.

[0101] According to certain embodiments, the bacterium expresses a polypeptide having the amino acid sequence set forth which has at least about 90%, at least about 93%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, or at least about 99%, sequence identity to the amino acid sequence set forth in SEQ ID NO: 11, wherein at position 357 serine is replaced by arginine. According to particular embodiments, the bacterium expresses a polypeptide having the amino acid sequence set forth which has at least about 95%, such as at least about 96%, at least about 97%, at least about 98%, or at least about 99%, sequence identity to the amino acid sequence set forth in SEQ ID NO: 11, wherein at position 357 serine is replaced by arginine.

[0102] According to certain embodiments, the bacterium expresses a polypeptide having the amino acid sequence set forth which has at least about 90%, at least about 93%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, or at least about 99%, sequence identity to the amino acid sequence set forth in SEQ ID NO: 11, wherein at position 359 serine is replaced by arginine. According to particular embodiments, the bacterium expresses a polypeptide having the amino acid sequence set forth which has at least about 95%, at least about 96%, at least about 97%, at least about 98%, or at least about 99%, sequence identity to the amino acid sequence set forth in SEQ ID NO: 11, wherein at position 359 serine is replaced by arginine.

[0103] According to certain embodiments, the bacterium expresses a polypeptide having an amino acid sequence set forth in SEQ ID NO: 11 which comprises one or more amino acid substitutions selected from the group consisting of Y356C, S357R and S359R, wherein 1 or more, such as about 1 to about 50, about 1 to about 40, about 1 to about 35, about 1 to about 30, about 1 to about 25, about 1 to about 20, about 1 to about 15, about 1 to about 10, about 1 to about 5, or about 1 to about 3, further amino acid residues are substituted, deleted, and/or inserted. The bacterium may express a polypeptide having an amino acid sequence set forth in SEQ ID NO: 11 which comprises one or more amino acid substitutions selected from the group consisting of Y356C, S357R and S359R, wherein about 1 to about 5, or about 1 to about 3, further amino acid residues are substituted, deleted, and/or inserted.

[0104] The ThrA polypeptide mutant(s) described above may be (over-)expressed by the bacterium by way of an exogenous nucleic acid molecule, such as an expression vector, which has been introduced into the bacterium. Therefore, according to certain embodiments, the bacterium of the invention comprises an exogenous nucleic acid molecule comprising a nucleotide sequence encoding a aspartate kinase I / homoserine dehydrogenase I (ThrA) polypeptide mutant as described above.

[0105] For example, a bacterium of the invention may comprise an exogenous nucleic acid molecule, such as an expression vector, comprising a nucleotide sequence encoding a polypeptide having the amino acid sequence set

forth in SEQ ID NO: 11 which comprises an amino acid substitution at position Y356, S357 and/or S359. According to certain embodiments, a bacterium of the invention thus comprises an exogenous nucleic acid molecule comprising a nucleotide sequence encoding a polypeptide having the amino acid sequence set forth in SEQ ID NO: 11 which comprises an amino acid substitution at position Y356, S357 and/or S359, wherein the substitution at position Y356 is selected from the group consisting of Y356C, Y356T, Y356V, Y356S, Y356W, Y356Q, Y356G, Y356N, Y356D, Y356E, Y356F, Y356A, Y356I, Y356P, Y356H, Y356R and Y356L; the substitution at position S357 is selected from the group consisting of S357R, S357V, S357P, S357G, S357L, S357Y, S357A, S357N, S357F, S357H, S357K, S357I and S357M; and the substitution at position S359 is selected from the group consisting of S359R, S359G, S359M, S359F, S359T, S359P, S359V, S359Q, S359A, S359C, S359K, S359E and S359L.

[0106] According to certain embodiments, a bacterium of the invention comprises an exogenous nucleic acid molecule comprising a nucleotide sequence encoding a polypeptide having an amino acid sequence which has at least about 90%, such as at least about 93%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, or at least about 99%, sequence identity to the amino acid sequence set forth in SEQ ID NO: 11 which comprises an amino acid substitution at position Y356, S357 and/or S359. According to certain embodiments, a bacterium of the invention comprises an exogenous nucleic acid molecule comprising a nucleotide sequence encoding a polypeptide having an amino acid sequence which has at least about 90%, such as at least about 93%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, or at least about 99%, sequence identity to the amino acid sequence set forth in SEQ ID NO: 11 which comprises an amino acid substitution at position Y356, S357 and/or S359, wherein the substitution at position Y356 is selected from the group consisting of Y356C, Y356T, Y356V, Y356S, Y356W, Y356Q, Y356G, Y356N, Y356D, Y356E, Y356F, Y356A, Y356I, Y356P, Y356H, Y356R and Y356L; the substitution at position S357 is selected from the group consisting of S357R, S357V, S357P, S357G, S357L, S357Y, S357A, S357N, S357F, S357H, S357K, S357I and S357M; and the substitution at position S359 is selected from the group consisting of S359R, S359G, S359M, S359F, S359T, S359P, S359V, S359Q, S359A, S359C, S359K, S359E and S359L.

[0107] According to certain embodiments, a bacterium of the invention comprises an exogenous nucleic acid molecule comprising a nucleotide sequence encoding a polypeptide having an amino acid sequence which has at least about 93%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, or at least about 99%, sequence identity to the amino acid sequence set forth in SEQ ID NO: 11 which comprises an amino acid substitution at position Y356, S357 and/or S359. According to certain embodiments, a bacterium of the invention comprises an exogenous nucleic acid molecule comprising a nucleotide sequence encoding a polypeptide having an amino acid sequence which has at least about 93%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, or at least about 99%, sequence identity to the amino acid sequence set forth in SEQ ID NO: 11 which comprises an amino acid substitution at position Y356, S357 and/or S359, wherein the substitution at position Y356 is selected from the group consisting of Y356C, Y356T, Y356V, Y356S, Y356W, Y356Q, Y356G, Y356N, Y356D, Y356E, Y356F, Y356A, Y356I, Y356P, Y356H, Y356R and Y356L; the substitution at position S357 is selected from the group consisting of S357R, S357V, S357P, S357G, S357L, S357Y, S357A, S357N, S357F, S357H, S357K, S357I and S357M; and the substitution at position S359 is selected from the group consisting of S359R, S359G, S359M, S359F, S359T, S359P, S359V, S359Q, S359A, S359C, S359K, S359E and S359L.

[0108] According to certain embodiments, a bacterium of the invention comprises an exogenous nucleic acid molecule comprising a nucleotide sequence encoding a polypeptide having an amino acid sequence which has at least about 95%, at least about 96%, at least about 97%, at least about 98%, or at least about 99%, sequence identity to the amino acid sequence set forth in SEQ ID NO: 11 which comprises an amino acid substitution at position Y356, S357 and/or S359. According to certain embodiments, a bacterium of the invention comprises an exogenous nucleic acid molecule comprising a nucleotide sequence encoding a polypeptide having an amino acid sequence which has at least about 95%, at least about 96%, at least about 97%, at least about 98%, or at least about 99%, sequence identity to the amino acid sequence set forth in SEQ ID NO: 11 which comprises an amino acid substitution at position Y356, S357 and/or S359, wherein the substitution at position Y356 is selected from the group consisting of Y356C, Y356T, Y356V, Y356S, Y356W, Y356Q, Y356G, Y356N, Y356D, Y356E, Y356F, Y356A, Y356I, Y356P, Y356H, Y356R and Y356L; the substitution at position S357 is selected from the group consisting of S357R, S357V, S357P, S357G, S357L, S357Y, S357A, S357N, S357F, S357H, S357K, S357I and S357M; and the substitution at position S359 is selected from the group consisting of S359R, S359G, S359M, S359F, S359T, S359P, S359V, S359Q, S359A, S359C, S359K, S359E and S359L.

[0109] In this connection, the present invention further provides a (isolated) nucleic acid molecule, such an expression vector, comprising a nucleotide sequence encoding a ThrA mutant as described above. Such nucleic acid may be introduced into the bacterium of the invention. According to certain embodiments, the nucleic acid molecule comprises a nucleotide sequence encoding a polypeptide having an amino acid sequence which has at least about 90%, such as at least about 93%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, or at least about 99%, sequence identity to the amino acid sequence set forth in SEQ ID NO: 11 which comprises an amino acid substitution at position Y356, S357 and/or S359. According to certain embodiments, the nucleic acid molecule comprises a nucleotide sequence encoding a polypeptide having an amino acid sequence which has at least about 90%, such as at least about 93%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, or at least about 99%, sequence identity to the amino acid sequence set forth in SEQ ID NO: 11 which comprises an amino acid substitution at position Y356, S357 and/or S359; wherein the substitution at position Y356 is selected from the group consisting of Y356C, Y356T, Y356V, Y356S, Y356W, Y356Q, Y356G, Y356N, Y356D, Y356E, Y356F, Y356A, Y356I, Y356P, Y356H, Y356R and Y356L; the substitution at position S357 is selected from the group consisting of S357R, S357V, S357P, S357G, S357L, S357Y, S357A, S357N, S357F, S357H, S357K, S357I and S357M; and the substitution at position S359 is selected from the group consisting of S359R, S359G, S359M, S359F, S359T, S359P, S359V, S359Q, S359A, S359C, S359K, S359E and S359L.

[0110] According to certain embodiments, the nucleic acid molecule comprising a nucleotide sequence encoding a polypeptide having the amino acid sequence set forth in SEQ ID NO: 11 which comprises an amino acid substitution at position Y356, S357 and/or S359. According to certain embodiments, the nucleic acid molecule comprising a nucleotide sequence encoding a polypeptide having the amino acid sequence set forth in SEQ ID NO: 11 which comprises an amino acid substitution at position Y356, S357 and/or S359, wherein the substitution at position Y356 is selected from the group consisting of Y356C, Y356T, Y356V, Y356S, Y356W, Y356Q, Y356G, Y356N, Y356D, Y356E, Y356F, Y356A, Y356I, Y356P, Y356H, Y356R and Y356L; the substitution at position S357 is selected from the group consisting of S357R, S357V, S357P, S357G, S357L, S357Y, S357A, S357N, S357F, S357H, S357K, S357I and S357M; and the substitution at position S359 is selected from the group consisting of S359R, S359G, S359M, S359F, S359T, S359P, S359V, S359Q, S359A, S359C, S359K, S359E and S359L.

[0111] The (isolated) nucleic acid molecule may be the exogenous nucleic acid as detailed above. The (isolated) nucleic acid molecule may comprise suitable regulatory elements such as a promoter that is functional in the bacterial cell to cause the production of an mRNA molecule and that is operably linked to the nucleotide sequence encoding said polypeptide. Further details on suitable regulatory elements are provided below with respect to an "exogenous" nucleic acid molecule, and apply *mutatis mutandis*.

[0112] The present invention also provides a bacterium which comprises within the *lrp* gene one or more nucleotide substitutions resulting in the amino acid substitution D143G in the encoded polypeptide. Further information regarding *lrp* of, e.g., *Escherichia coli* is available at EcoCyc (www.biocyc.org) under Accession number EG10547. According to certain embodiments, a bacterium of the invention expresses a polypeptide encoded by the *lrp* gene, wherein in said polypeptide at position 143 D is replaced by G. A representative amino acid sequence of a polypeptide encoded by the *lrp* gene is set forth in SEQ ID NO: 12. According to particular embodiments, a bacterium of the invention expresses a polypeptide having the amino acid sequence set forth in SEQ ID NO: 12 or a polypeptide having the amino acid sequence which has at least about 90%, at least about 93%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, or at least about 99%, sequence identity to the amino acid sequence set forth in SEQ ID NO: 12, wherein in said amino acid sequence at position 143 D is replaced by G.

[0113] According to certain embodiments, the present invention provides a bacterium which comprises within the *lrp* gene one or more nucleotide substitutions resulting in one or more amino acid substitutions which increase tolerance towards L-serine. More particularly, a bacterium is provided which comprises within the *lrp* gene one or more nucleotide substitutions resulting in an amino acid substitution in the encoded polypeptide at position D143. According to particular embodiments, a bacterium of the invention expresses a polypeptide having the amino acid sequence set forth in SEQ ID NO: 12 or a polypeptide having the amino acid sequence which has at least about 90%, at least about 93%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, or at least about 99%, sequence identity to the amino acid sequence set forth in SEQ ID NO: 12, wherein in said amino acid sequence at position 143 D is replaced by another amino acid. Preferably, the one or more amino acid substitutions are non-conservative substitutions.

[0114] The present invention also provides a bacterium which comprises within the *rho* gene one or more nucleotide substitutions resulting in the amino acid substitution R87L in the encoded polypeptide. Further information regarding *rho* of, e.g., *Escherichia coli* such as nucleotide sequence of the gene or amino acid sequence of the encoded polypeptide is available at EcoCyc (www.biocyc.org) under Accession number EG10845. According to certain embodiments, a bacterium of the invention expresses a polypeptide encoded by the *rho* gene, wherein in said polypeptide at position 87 R is replaced by L. A representative amino acid sequence of a polypeptide encoded by the *rho* gene is set forth in SEQ ID NO: 13. According to particular embodiments, a bacterium of the invention expresses a polypeptide having the amino acid sequence set forth in SEQ ID NO: 13 or a polypeptide having the amino acid sequence which has at least about 90%, at least about 93%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, or at least about 99%, sequence identity to the amino acid sequence set forth in SEQ ID NO: 13, wherein in said amino acid sequence at position 87 R is replaced by L.

[0115] According to certain embodiments, the present invention provides a bacterium which comprises within the *rho* gene one or more nucleotide substitutions resulting in one or more amino acid substitutions which increase tolerance towards L-serine. More particularly, a bacterium is provided which comprises within the *rho* gene one or more nucleotide substitutions resulting in an amino acid substitution in the encoded polypeptide at position R87. According to particular embodiments, a bacterium of the invention expresses a polypeptide having the amino acid sequence set forth in SEQ ID NO: 13 or a polypeptide having the amino acid sequence which has at least about 90%, at least about 93%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, or at least about 99%, sequence identity to the amino acid sequence set forth in SEQ ID NO: 13, wherein in said amino acid sequence at position 87 R is replaced by another amino acid. Preferably, the one or more amino acid substitutions are non-conservative substitutions.

[0116] The present invention also provides a bacterium which comprises within the *eno* gene one or more nucleotide substitutions resulting in the amino acid substitution V164L in the encoded polypeptide. Further information regarding *eno* of, e.g., *Escherichia coli* is available at EcoCyc (www.biocyc.org) under Accession number EG10258. According to certain embodiments, a bacterium of the invention expresses a polypeptide encoded by the *eno* gene, wherein in said polypeptide at position 164 V is replaced by L. A representative amino acid sequence of a polypeptide encoded by the *eno* gene is set forth in SEQ ID NO: 14. According to particular embodiments, a bacterium of the invention may thus express a polypeptide having the amino acid sequence set forth in SEQ ID NO: 14 or a polypeptide having the amino acid sequence which has at least about 90%, at least about 93%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, or at least about 99%, sequence identity to the amino acid sequence set forth in SEQ ID NO: 14, wherein in said amino acid sequence at position at position 164 V is replaced by L.

[0117] According to certain embodiments, the present invention provides a bacterium which comprises within the *eno* gene one or more nucleotide substitutions resulting in one or more amino acid substitutions which increase tolerance towards L-serine. More particularly, a bacterium is provided which comprises within the *eno* gene one or more nucleotide substitutions resulting in an amino acid substitution in the encoded polypeptide at position V164. According to particular embodiments, a bacterium of the invention expresses a polypeptide having the amino acid sequence set forth in SEQ ID NO: 14 or a polypeptide having the amino acid sequence which has at least about 90%, at least about 93%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, or at least about 99%, sequence identity to the amino acid sequence set forth in SEQ ID NO: 14, wherein in said amino acid sequence at position 164 V is replaced by another amino acid. Preferably, the one or more amino acid substitutions are non-conservative substitutions.

[0118] The present invention also provides a bacterium which comprises within the *argP* gene one or more nucleotide substitutions resulting in the amino acid substitution V164L in the encoded polypeptide. Further information regarding *argP* of, e.g., *Escherichia coli* is available at EcoCyc (www.biocyc.org) under Accession number EG10490. According to certain embodiments, a bacterium of the invention expresses a polypeptide encoded by the *argP* gene, wherein in said polypeptide at position 132 Q is replaced by K. A representative amino acid sequence of a polypeptide encoded by the *argP* gene is set forth in SEQ ID NO: 15. According to particular embodiments, a bacterium of the invention expresses a polypeptide having the amino acid sequence set forth in SEQ ID NO: 15 or a polypeptide having the amino acid sequence which has at least about 90%, at least about 93%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, or at least about 99%, sequence identity to the amino acid sequence set forth in

SEQ ID NO: 15, wherein in said amino acid sequence at position at position 132 Q is replaced by K.

[0119] According to certain embodiments, the present invention provides a bacterium which comprises within the *argP* gene one or more nucleotide substitutions resulting in one or more amino acid substitutions which increase tolerance towards L-serine. More particularly, a bacterium is provided which comprises within the *argP* gene one or more nucleotide substitutions resulting in an amino acid substitution in the encoded polypeptide at position Q132. According to particular embodiments, a bacterium of the invention expresses a polypeptide having the amino acid sequence set forth in SEQ ID NO: 15 or a polypeptide having the amino acid sequence which has at least about 90%, at least about 93%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, or at least about 99%, sequence identity to the amino acid sequence set forth in SEQ ID NO: 15, wherein in said amino acid sequence at position 132 Q is replaced by another amino acid. Preferably, the one or more amino acid substitutions are non-conservative substitutions.

[0120] The present invention also provides a bacterium which comprises within the *tufA* gene one or more nucleotide substitutions resulting in the amino acid substitution G19V in the encoded polypeptide. Further information regarding *tufA* of, e.g., *Escherichia coli* is available at EcoCyc (www.biocyc.org) under Accession number EG11036. According to certain embodiments, a bacterium of the invention expresses a polypeptide encoded by the *tufA* gene, wherein in said polypeptide at position 19 G is replaced by V. A representative amino acid sequence of a polypeptide encoded by the *tufA* gene is set forth in SEQ ID NO: 16. According to particular embodiments, a bacterium of the invention expresses a polypeptide having the amino acid sequence set forth in SEQ ID NO: 16 or a polypeptide having the amino acid sequence which has at least about 90%, at least about 93%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, or at least about 99%, sequence identity to the amino acid sequence set forth in SEQ ID NO: 16, wherein in said amino acid sequence at position at position 19 G is replaced by V.

[0121] According to certain embodiments, the present invention provides a bacterium which comprises within the *tufA* gene one or more nucleotide substitutions resulting in one or more amino acid substitutions which increase tolerance towards L-serine. More particularly, a bacterium is provided which comprises within the *tufA* gene one or more nucleotide substitutions resulting in an amino acid substitution in the encoded polypeptide at position G19. According to particular embodiments, a bacterium of the invention expresses a polypeptide having the amino acid sequence set forth in SEQ ID NO: 16 or a polypeptide having the amino acid sequence which has at least about 90%, at least about 93%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, or at least about 99%, sequence identity to the amino acid sequence set forth in SEQ ID NO: 16, wherein in said amino acid sequence at position 19 G is replaced by another amino acid. Preferably, the one or more amino acid substitutions are non-conservative substitutions.

[0122] The present invention also provides a bacterium which comprises within the *cycA* gene one or more nucleotide substitutions resulting in the amino acid substitution I220V in the encoded polypeptide. Further information regarding *cycA* of, e.g., *Escherichia coli* is available at EcoCyc (www.biocyc.org) under Accession numbers EG12504. According to certain embodiments, a bacterium of the invention expresses a polypeptide encoded by the *cycA* gene, wherein in said polypeptide at position 220 I is replaced by V. A representative amino acid sequence of a polypeptide encoded by the *cycA* gene is set forth in SEQ ID NO: 17. According to particular embodiments, a bacterium of the invention expresses a polypeptide having the amino acid sequence set forth in SEQ ID NO: 17 or a polypeptide having the amino acid sequence which has at least about 90%, at least about 93%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, or at least about 99%, sequence identity to the amino acid sequence set forth in SEQ ID NO: 17, wherein in said amino acid sequence at position at position 220 I is replaced by V.

[0123] According to certain embodiments, the present invention provides a bacterium which comprises within the *cycA* gene one or more nucleotide substitutions resulting in one or more amino acid substitutions which increase tolerance towards L-serine. More particularly, a bacterium is provided which comprises within the *cycA* gene one or more nucleotide substitutions resulting in an amino acid substitution in the encoded polypeptide at position 1220. According to particular embodiments, a bacterium of the invention expresses a polypeptide having the amino acid sequence set forth in SEQ ID NO: 17 or a polypeptide having the amino acid sequence which has at least about 90%, at least about 93%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, or at least about 99%, sequence identity to the amino acid sequence set forth in SEQ ID NO: 17, wherein in said amino acid sequence at position 220 I is replaced by another amino acid. Preferably, the one or more amino acid substitutions are non-conservative

substitutions.

[0124] The present invention also provides a bacterium which comprises within the *rpe* gene one or more nucleotide substitutions resulting in the amino acid substitution I202T in the encoded polypeptide. Further information regarding *rpe* of, e.g., *Escherichia coli* is available at EcoCyc (www.biocyc.org) under Accession numbers M004. According to certain embodiments, a bacterium of the invention expresses a polypeptide encoded by the *rpe* gene, wherein in said polypeptide at position 202 I is replaced by T. A representative amino acid sequence of a polypeptide encoded by the *rpe* gene is set forth in SEQ ID NO: 18. According to particular embodiments, a bacterium of the invention expresses a polypeptide having the amino acid sequence set forth in SEQ ID NO: 18 or a polypeptide having the amino acid sequence which has at least about 90%, at least about 93%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, or at least about 99%, sequence identity to the amino acid sequence set forth in SEQ ID NO: 18, wherein in said amino acid sequence at position at position 202 I is replaced by T.

[0125] According to certain embodiments, the present invention provides a bacterium which comprises within the *rpe* gene one or more nucleotide substitutions resulting in one or more amino acid substitutions which increase tolerance towards L-serine. More particularly, a bacterium is provided which comprises within the *rpe* gene one or more nucleotide substitutions resulting in an amino acid substitution in the encoded polypeptide at position 2021. According to particular embodiments, a bacterium of the invention expresses a polypeptide having the amino acid sequence set forth in SEQ ID NO: 18 or a polypeptide having the amino acid sequence which has at least about 90%, at least about 93%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, or at least about 99%, sequence identity to the amino acid sequence set forth in SEQ ID NO: 18, wherein in said amino acid sequence at position 202 I is replaced by another amino acid. Preferably, the one or more amino acid substitutions are non-conservative substitutions.

[0126] The present invention also provides a bacterium which comprises within the *yojI* gene one or more nucleotide substitutions resulting in the amino acid substitution D334H in the encoded polypeptide. Further information regarding *yojI* of, e.g., *Escherichia coli* is available at EcoCyc (www.biocyc.org) under Accession numbers EG12070. According to certain embodiments, a bacterium of the invention expresses a polypeptide encoded by the *yojI* gene, wherein in said polypeptide at position 334 D is replaced by H. A representative amino acid sequence of a polypeptide encoded by the *yojI* gene is set forth in SEQ ID NO: 19. According to particular embodiments, a bacterium of the invention expresses a polypeptide having the amino acid sequence set forth in SEQ ID NO: 19 or a polypeptide having the amino acid sequence which has at least about 90%, at least about 93%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, or at least about 99%, sequence identity to the amino acid sequence set forth in SEQ ID NO: 19, wherein in said amino acid sequence at position at position 334 D is replaced by H.

[0127] According to certain embodiments, the present invention provides a bacterium which comprises within the *yojI* gene one or more nucleotide substitutions resulting in one or more amino acid substitutions which increase tolerance towards L-serine. More particularly, a bacterium is provided which comprises within the *yojI* gene one or more nucleotide substitutions resulting in an amino acid substitution in the encoded polypeptide at position D334. According to particular embodiments, a bacterium of the invention expresses a polypeptide having the amino acid sequence set forth in SEQ ID NO: 19 or a polypeptide having the amino acid sequence which has at least about 90%, at least about 93%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, or at least about 99%, sequence identity to the amino acid sequence set forth in SEQ ID NO: 19, wherein in said amino acid sequence at position 334 D is replaced by another amino acid. Preferably, the one or more amino acid substitutions are non-conservative substitutions.

[0128] The present invention also provides a bacterium which comprises within the *hyaF* gene one or more nucleotide substitutions resulting in the amino acid substitution V120G in the encoded polypeptide. Further information regarding *hyaF* of, e.g., *Escherichia coli* is available at EcoCyc (www.biocyc.org) under Accession numbers EG10473. According to certain embodiments, a bacterium of the invention expresses a polypeptide encoded by the *hyaF* gene, wherein in said polypeptide at position 120 V is replaced by G. A representative amino acid sequence of a polypeptide encoded by the *hyaF* gene is set forth in SEQ ID NO: 20. According to particular embodiments, a bacterium of the invention expresses a polypeptide having the amino acid sequence set forth in SEQ ID NO: 20 or a polypeptide having the amino acid sequence which has at least about 90%, at least about 93%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, or at least about 99%, sequence identity to the amino acid sequence set forth in

SEQ ID NO: 20, wherein in said amino acid sequence at position at position 120 V is replaced by G.

[0129] According to certain embodiments, the present invention provides a bacterium which comprises within the *hyaF* gene one or more nucleotide substitutions resulting in one or more amino acid substitutions which increase tolerance towards L-serine. More particularly, a bacterium is provided which comprises within the *hyaF* gene one or more nucleotide substitutions resulting in an amino acid substitution in the encoded polypeptide at position V120. According to particular embodiments, a bacterium of the invention expresses a polypeptide having the amino acid sequence set forth in SEQ ID NO: 20 or a polypeptide having the amino acid sequence which has at least about 90%, at least about 93%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, or at least about 99%, sequence identity to the amino acid sequence set forth in SEQ ID NO: 20, wherein in said amino acid sequence at position 120 V is replaced by another amino acid. Preferably, the one or more amino acid substitutions are non-conservative substitutions.

[0130] The present invention also provides a bacterium which comprises within the *pykF* gene one or more nucleotide substitutions resulting in the amino acid substitution E250* in the encoded polypeptide, where * designates a stop codon. Alternatively, the *pykF* gene may comprise one or more nucleotide substitutions resulting in the termination of the encoded polypeptide at a position upstream of position 250. Further information regarding *pykF* of, e.g., *Escherichia coli* is available at EcoCyc (www.biocyc.org) under Accession numbers EG10804. According to certain embodiments, a bacterium of the invention expresses a polypeptide encoded by the *pykF* gene, wherein said polypeptide terminates after position 249 or any position upstream thereof. A representative amino acid sequence of a polypeptide encoded by the *pykF* gene is set forth in SEQ ID NO: 21. According to particular embodiments, a bacterium of the invention expresses a polypeptide having the amino acid sequence set forth in SEQ ID NO: 22 or a polypeptide having the amino acid sequence which has at least about 90%, at least about 93%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, or at least about 99%, sequence identity to the amino acid sequence set forth in SEQ ID NO: 22.

[0131] According to other certain embodiments, a bacterium of the invention has been further modified to attenuate the expression of the *pykF* gene (e.g., by inactivation of the gene). Attenuation, and more particularly inactivation, of the gene expression can be achieved as described herein above. For example, lambda red mediated gene replacement may be used for inactivating gene expression.

[0132] The present invention also provides a bacterium which comprises within the *malT* gene one or more nucleotide substitutions resulting in the amino acid substitution Q420* in the encoded polypeptide, where * designates a stop codon. Alternatively, the *malT* gene may comprise one or more nucleotide substitutions resulting in the termination of the encoded polypeptide at a position upstream of position 420. Further information regarding *malT* of, e.g., *Escherichia coli* is available at EcoCyc (www.biocyc.org) under Accession numbers EG10562. According to certain embodiments, a bacterium of the invention expresses a polypeptide encoded by the *malT* gene, wherein said polypeptide terminates after position 419 or any position upstream thereof. A representative amino acid sequence of a polypeptide encoded by the *malT* gene is set forth in SEQ ID NO: 23. According to particular embodiments, a bacterium of the invention expresses a polypeptide having the amino acid sequence set forth in SEQ ID NO: 24 or a polypeptide having the amino acid sequence which has at least about 90%, at least about 93%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, or at least about 99%, sequence identity to the amino acid sequence set forth in SEQ ID NO: 24.

[0133] According to other certain embodiments, a bacterium of the invention has been further modified to attenuate the expression of the *malT* gene (e.g., by inactivation of the gene). Attenuation, and more particularly inactivation, of the gene expression can be achieved as described herein above. For example, lambda red mediated gene replacement may be used for inactivating gene expression.

[0134] The present invention also provides a bacterium which comprises within the *rpoB* gene one or more nucleotide substitutions resulting in the amino acid substitution P520L in the encoded polypeptide. Further information regarding *rpoB* of, e.g., *Escherichia coli* is available at EcoCyc (www.biocyc.org) under Accession numbers EG10894. According to certain embodiments, a bacterium of the invention expresses a polypeptide encoded by the *rpoB* gene, wherein in said polypeptide at position 520 P is replaced by L. A representative amino acid sequence of a polypeptide encoded by the *rpoB* gene is set forth in SEQ ID NO: 25. According to particular embodiments, a bacterium of the invention

expresses a polypeptide having the amino acid sequence set forth in SEQ ID NO: 25 or a polypeptide having the amino acid sequence which has at least about 90%, at least about 93%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, or at least about 99%, sequence identity to the amino acid sequence set forth in SEQ ID NO: 25, wherein in said amino acid sequence at position at position 520 P is replaced by L.

[0135] According to certain embodiments, the present invention provides a bacterium which comprises within the *rpoB* gene one or more nucleotide substitutions resulting in one or more amino acid substitutions which increase tolerance towards L-serine. More particularly, a bacterium is provided which comprises within the *rpoB* gene one or more nucleotide substitutions resulting in an amino acid substitution in the encoded polypeptide at position P520. According to particular embodiments, a bacterium of the invention expresses a polypeptide having the amino acid sequence set forth in SEQ ID NO: 25 or a polypeptide having the amino acid sequence which has at least about 90%, at least about 93%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, or at least about 99%, sequence identity to the amino acid sequence set forth in SEQ ID NO: 25, wherein in said amino acid sequence at position 520 P is replaced by another amino acid. Preferably, the one or more amino acid substitutions are non-conservative substitutions.

[0136] The present invention also provides a bacterium which comprises within the *fumB* gene one or more nucleotide substitutions resulting in the amino acid substitution T218P in the encoded polypeptide. Further information regarding *fumB* of, e.g., *Escherichia coli* is available at EcoCyc (www.biocyc.org) under Accession numbers EG10357. According to certain embodiments, a bacterium of the invention expresses a polypeptide encoded by the *fumB* gene, wherein in said polypeptide at position 218 T is replaced by P. A representative amino acid sequence of a polypeptide encoded by the *fumB* gene is set forth in SEQ ID NO: 26. According to particular embodiments, a bacterium of the invention expresses a polypeptide having the amino acid sequence set forth in SEQ ID NO: 26 or a polypeptide having the amino acid sequence which has at least about 90%, at least about 93%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, or at least about 99%, sequence identity to the amino acid sequence set forth in SEQ ID NO: 26, wherein in said amino acid sequence at position at position 218 T is replaced by P.

[0137] According to certain embodiments, the present invention provides a bacterium which comprises within the *fumB* gene one or more nucleotide substitutions resulting in one or more amino acid substitutions which increase tolerance towards L-serine. More particularly, a bacterium is provided which comprises within the *fumB* gene one or more nucleotide substitutions resulting in an amino acid substitution in the encoded polypeptide at position T218. According to particular embodiments, a bacterium of the invention expresses a polypeptide having the amino acid sequence set forth in SEQ ID NO: 26 or a polypeptide having the amino acid sequence which has at least about 90%, at least about 93%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, or at least about 99%, sequence identity to the amino acid sequence set forth in SEQ ID NO: 26, wherein in said amino acid sequence at position 218 T is replaced by another amino acid. Preferably, the one or more amino acid substitutions are non-conservative substitutions.

[0138] The present invention also provides a bacterium which comprises within the *gshA* gene one or more nucleotide substitutions resulting in the amino acid substitution A178V in the encoded polypeptide. Further information regarding *gshA* of, e.g., *Escherichia coli* is available at EcoCyc (www.biocyc.org) under Accession numbers EG10418. According to certain embodiments, a bacterium of the invention expresses a polypeptide encoded by the *gshA* gene, wherein in said polypeptide at position 178 A is replaced by V. A representative amino acid sequence of a polypeptide encoded by the *gshA* gene is set forth in SEQ ID NO: 27. According to particular embodiments, a bacterium of the invention expresses a polypeptide having the amino acid sequence set forth in SEQ ID NO: 27 or a polypeptide having the amino acid sequence which has at least about 90%, at least about 93%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, or at least about 99%, sequence identity to the amino acid sequence set forth in SEQ ID NO: 27, wherein in said amino acid sequence at position at position 178 A is replaced by V.

[0139] According to certain embodiments, the present invention provides a bacterium which comprises within the *gshA* gene one or more nucleotide substitutions resulting in one or more amino acid substitutions which increase tolerance towards L-serine. More particularly, a bacterium is provided which comprises within the *gshA* gene one or more nucleotide substitutions resulting in an amino acid substitution in the encoded polypeptide at position A178. According to particular embodiments, a bacterium of the invention expresses a polypeptide having the amino acid sequence set forth in SEQ ID NO: 27 or a polypeptide having the amino acid sequence which has at least about 90%, at least about

93%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, or at least about 99%, sequence identity to the amino acid sequence set forth in SEQ ID NO: 27, wherein in said amino acid sequence at position 178 A is replaced by another amino acid. Preferably, the one or more amino acid substitutions are non-conservative substitutions.

[0140] The present invention also provides a bacterium which comprises within the *lamB* gene one or more nucleotide substitutions resulting in the amino acid substitution Q112* in the encoded polypeptide, where * designates a stop codon. Alternatively, the *lamB* gene may comprise one or more nucleotide substitutions resulting in the termination of the encoded polypeptide at a position upstream of position 112. Further information regarding *lamB* of, e.g., *Escherichia coli* such as nucleotide sequence of the gene or amino acid sequence of the encoded polypeptide is available at EcoCyc (www.biocyc.org) under Accession numbers EG10528. According to certain embodiments, a bacterium of the invention expresses a polypeptide encoded by the *lamB* gene, wherein said polypeptide terminates after position 111 or any position upstream thereof. A representative amino acid sequence of a polypeptide encoded by the *lamB* gene is set forth in SEQ ID NO: 28. According to particular embodiments, a bacterium of the invention expresses a polypeptide having the amino acid sequence set forth in SEQ ID NO: 29 or a polypeptide having the amino acid sequence which has at least about 90%, at least about 93%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, or at least about 99%, sequence identity to the amino acid sequence set forth in SEQ ID NO: 29.

[0141] According to other certain embodiments, a bacterium of the invention has been further modified to attenuate the expression of the *lamB* gene (e.g., by inactivation of the gene). Attenuation, and more particularly inactivation, of the gene expression can be achieved as described herein above. For example, lambda red mediated gene replacement may be used for inactivating gene expression.

[0142] A bacterium of the present invention may comprise one or more, such as two or more, three or more, four or more, or five or more, gene mutations as mentioned.

[0143] For example, the bacterium may comprise one or more (such as two or more) gene mutations selected from the group consisting of: one or more nucleotide substitutions within the *lrp* gene resulting in an amino acid substitution at position D143 in the encoded polypeptide, one or more nucleotide substitutions within the *rho* gene resulting in an amino acid substitution at position R87 in the encoded polypeptide, one or more nucleotide substitutions within the *eno* gene resulting in an amino acid substitution at position V164 in the encoded polypeptide, and one or more nucleotide substitutions within the *argP* gene resulting in an amino acid substitution at position V164 in the encoded polypeptide.

[0144] For example, the bacterium may comprise one or more (such as two or more) gene mutations selected from the group consisting of: one or more nucleotide substitutions within the *lrp* gene resulting in the amino acid substitution D143G in the encoded polypeptide, one or more nucleotide substitutions within the *rho* gene resulting in the amino acid substitution R87L in the encoded polypeptide, one or more nucleotide substitutions within the *eno* gene resulting in the amino acid substitution V164L in the encoded polypeptide, and one or more nucleotide substitutions within the *argP* gene resulting in the amino acid substitution V164L in the encoded polypeptide.

[0145] According to certain embodiments, a bacterium of the invention comprises within the *thrA* gene one or more nucleotide substitutions resulting in an amino acid substitution in the encoded polypeptide at position Y356, said substitution being selected from the group consisting of Y356C, Y356T, Y356V, Y356S, Y356W, Y356Q, Y356G, Y356N, Y356D, Y356E, Y356F, Y356A, Y356I, Y356P, Y356H, Y356R and Y356L; one or more nucleotide substitutions resulting in an amino acid substitution in the encoded polypeptide at position S357, said substitution being selected from the group consisting of S357R, S357V, S357P, S357G, S357L, S357Y, S357A, S357N, S357F, S357H, S357K, S357I and S357M; and/or one or more nucleotide substitutions resulting in an amino acid substitution in the encoded polypeptide at position S359, said substitution being selected from the group consisting of S359R, S359G, S359M, S359F, S359T, S359P, S359V, S359Q, S359A, S359C, S359K, S359E and S359L; and one or more (such as two or more) gene mutations selected from the group consisting of: one or more nucleotide substitutions within the *lrp* gene resulting in an amino acid substitution at position D143 (such as D143G) in the encoded polypeptide, one or more nucleotide substitutions within the *rho* gene resulting in the amino acid substitution at position R87 (such as R87L) in the encoded polypeptide, one or more nucleotide substitutions within the *eno* gene resulting in the amino acid substitution at position V164 (such as V164L) in the encoded polypeptide, and one or more nucleotide substitutions

within the *argP* gene resulting in an amino acid substitution at position V164 (such as V164L) in the encoded polypeptide.

[0146] According to certain embodiments, a bacterium of the present invention comprises one or more nucleotide substitutions within the *thrA* gene resulting in one or more amino acid substitution in the encoded polypeptide selected from the group consisting of Y356C, S357R and S359R; and one or more (such as two or more) gene mutations selected from the group consisting of: one or more nucleotide substitutions within the *lrp* gene resulting in the amino acid substitution D143G in the encoded polypeptide, one or more nucleotide substitutions within the *rho* gene resulting in the amino acid substitution R87L in the encoded polypeptide, one or more nucleotide substitutions within the *eno* gene resulting in the amino acid substitution V164L in the encoded polypeptide, and one or more nucleotide substitutions within the *argP* gene resulting in the amino acid substitution V164L in the encoded polypeptide.

[0147] According to certain embodiments, a bacterium of the present invention comprises one or more nucleotide substitutions within the *thrA* gene resulting in the amino acid substitution Y356C in the encoded polypeptide; and one or more (such as two or more) gene mutations selected from the group consisting of: one or more nucleotide substitutions within the *lrp* gene resulting in the amino acid substitution D143G in the encoded polypeptide, one or more nucleotide substitutions within the *rho* gene resulting in the amino acid substitution R87L in the encoded polypeptide, one or more nucleotide substitutions within the *eno* gene resulting in the amino acid substitution V164L in the encoded polypeptide, and one or more nucleotide substitutions within the *argP* gene resulting in the amino acid substitution V164L in the encoded polypeptide.

[0148] According to certain embodiments, a bacterium of the present invention comprises one or more nucleotide substitutions within the *thrA* gene resulting in the amino acid substitution S357R in the encoded polypeptide; and one or more (such as two or more) gene mutations selected from the group consisting of: one or more nucleotide substitutions within the *lrp* gene resulting in the amino acid substitution D143G in the encoded polypeptide, one or more nucleotide substitutions within the *rho* gene resulting in the amino acid substitution R87L in the encoded polypeptide, one or more nucleotide substitutions within the *eno* gene resulting in the amino acid substitution V164L in the encoded polypeptide, and one or more nucleotide substitutions within the *argP* gene resulting in the amino acid substitution V164L in the encoded polypeptide.

[0149] According to certain embodiments, a bacterium of the present invention comprises one or more nucleotide substitutions within the *thrA* gene resulting in the substitution S359R in the encoded polypeptide; and one or more (such as two or more) gene mutations selected from the group consisting of: one or more nucleotide substitutions within the *lrp* gene resulting in the amino acid substitution D143G in the encoded polypeptide, one or more nucleotide substitutions within the *rho* gene resulting in the amino acid substitution R87L in the encoded polypeptide, one or more nucleotide substitutions within the *eno* gene resulting in the amino acid substitution V164L in the encoded polypeptide, and one or more nucleotide substitutions within the *argP* gene resulting in the amino acid substitution V164L in the encoded polypeptide.

[0150] According to certain embodiments, a bacterium of the invention comprises within its genome a deletion of the first 5 bp of gene *rhtA* gene, a complete deletion of genes *ompX* and *ybiP*, a deletion of 239 bp of sRNA *rybA* and a deletion of 77 bp of gene *mntS*. According to certain embodiments, the bacterium comprises within its genome a deletion of about 2854 bp from a location which corresponds to location 850092 in the genome sequence NC_000913. This deletion results in a deletion of the first 5 bp of gene *rhtA* gene, a complete deletion of genes *ompX* and *ybiP*, a deletion of 239 bp of sRNA *rybA* and a 77 bp deletion of gene *mntS*. Such deletion can be achieved by using the lambda-red or cam-sacB-system.

[0151] According to certain embodiments, a bacterium of the invention comprises within its genome an insertion of an insertion sequence element IS1 (e.g., having a length of about 768 bp) in the intergenic region between genes *trxA* and *rho*. According to certain embodiments, the bacterium comprises within its genome an insertion of an insertion sequence element IS1 (e.g., having a length of about 768 bp) in the lagging strand at a location which corresponds to location 3966174 in the genome sequence NC_000913. According to particular embodiments, the bacterium further comprises a duplication of around 9 bp upstream and downstream of insertion sequence.

[0152] According to certain embodiments, a bacterium of the invention comprises within its genome an insertion of

1bp in the intergenic region between genes *gcvA* and *ygdI*. According to certain embodiments, the bacterium comprises within its genome an insertion of 1bp at a location which corresponds to location 2942629 in the genome sequence NC_000913.

[0153] According to certain embodiments, a bacterium of the invention comprises within its genome an insertion of an insertion sequence element IS4 (e.g., having a length of about 1342 bp) in the intergenic region between genes *gcvA* and *ygdI*. According to certain embodiments, the bacterium comprises within its genome an insertion of an insertion sequence element IS4 (e.g., having a length of about 1342 bp) at a location which corresponds to location 2942878 in the genome sequence NC_000913. According to particular embodiments, the bacterium further comprises a duplication of about 13 bp upstream and downstream of insertion sequence.

[0154] According to certain embodiments, a bacterium of the invention comprises within its genome an insertion of 1bp in the intergenic region between genes *dapA* and *gcvR*. According to certain embodiments, the bacterium comprises within its genome an insertion of 1bp at a location which corresponds to location 2599854 in the genome sequence NC_000913.

[0155] According to certain embodiments, a bacterium of the invention comprises within its genome an insertion of an insertion sequence element IS1 (e.g., having a length of about 768 bp) which lead to a truncation of gene *frc*. According to certain embodiments, the bacterium comprises within its genome an insertion sequence element IS1 (e.g., having a length of about 768 bp) in the lagging strand at a location which corresponds to location 2492323 in the genome sequence NC_000913. According to particular embodiments, the bacterium further comprises a duplication of 9 bp upstream and downstream of insertion sequence.

[0156] According to other certain embodiments, a bacterium of the invention has been further modified to attenuate the expression of the *frc* gene (e.g., by inactivation of the gene). Attenuation, and more particularly inactivation, of the gene expression can be achieved as described herein above. For example, lambda red mediated gene replacement may be used for inactivating gene expression.

[0157] According to certain embodiments, a bacterium of the invention comprises within its genome an insertion of an insertion sequence element IS5 (e.g., having a length of about 1195 bp) which leads to deletion of the majority of gene *aroP*. According to certain embodiments, the bacterium comprises within its genome an insertion sequence element IS5 (e.g., having a length of about 1195 bp) at a location which corresponds to location 121518 in the genome sequence NC_000913. According to particular embodiments, the bacterium further comprises a duplication of 4 bp upstream and downstream of insertion sequence.

[0158] According to other certain embodiments, a bacterium of the invention has been further modified to attenuate the expression of the *aroP* gene (e.g., by inactivation of the gene). Attenuation, and more particularly inactivation, of the gene expression can be achieved as described herein above. For example, lambda red mediated gene replacement may be used for inactivating gene expression.

[0159] According to certain embodiments, a bacterium of the invention comprises within its genome an insertion of an insertion sequence element IS1 (e.g., having a length of about 768 bp) in the intergenic region between genes *mdtJ* and *tgsA*. According to certain embodiments, the bacterium comprises within its genome an insertion sequence element IS1 (e.g., having a length of about 768 bp) in the lagging strand at a location which corresponds to location 1673670 in the genome sequence NC_000913. According to particular embodiments, the bacterium further comprises a duplication of around 9 bp upstream and downstream of insertion sequence.

[0160] According to certain embodiments, a bacterium of the invention comprises within its genome a nucleotide substitution, such as a C->T substitution, within the intergenic region between genes *trxB* and *lrp*. According to certain embodiments, the bacterium comprises within its genome a nucleotide substitution, such as a C->T substitution, at a location which corresponds to location 923321 in the genome sequence NC_000913. Such mutation is 271 bp upstream of *trxB* and 274 bp upstream of *lrp*.

[0161] According to certain embodiments, a bacterium of the invention comprises within its genome a nucleotide substitution, such as a T->C substitution, within the intergenic region between genes *yftB* and *fkfB*. According to certain

embodiments, the bacterium comprises within its genome a nucleotide substitution, such as a T->C substitution, at a location which corresponds to location 4428871 in the genome sequence NC_000913. Such mutation is 154 bp upstream of *yftB* and 64 bp upstream of *fkIB*.

[0162] As further demonstrate herein, attenuating (e.g., by inactivating of the gene) the expression of a gene coding for a polypeptide having Glucose 6-phosphate-1-dehydrogenase (G6PDH) activity in a reversed engineered strain resulted in a significantly increased production and yield of L-serine from glucose as shown in Table S9 (Example 8).

[0163] Therefore, according to certain embodiments, a bacterium of the invention has been modified to attenuate expression of a gene coding for a polypeptide having glucose 6-phosphate-1-dehydrogenase (G6PDH) activity. More particularly, the present invention provides a bacterium which has been modified to attenuate the expression of the gene *zwf*. Further information regarding *zwf* of, e.g., *Escherichia coli* is available at EcoCyc (www.biocyc.org) under Accession numbers EG11221. A representative nucleotide sequence of *zwf* is set forth in SEQ ID NO: 30.

[0164] The gene expression may be attenuated by inactivation of the gene. Thus, a bacterium according to the invention can be one which has been modified to inactivate the gene coding for a polypeptide having Glucose 6-phosphate-1-dehydrogenase (G6PDH) activity (e.g, by inactivation of the gene).

[0165] Attenuation, and more particularly inactivation, of the gene expression can be achieved as described herein above. For example, lambda red mediated gene replacement may be used for inactivating gene expression.

[0166] According to certain embodiments, a bacterium of the invention expresses a polypeptide encoded by the *brnQ* gene, wherein said polypeptide terminates after position 308 or any position upstream thereof. According to other certain embodiments, a bacterium of the invention has been further modified to attenuate the expression of the *brnQ* gene (e.g., by inactivation of the gene). Attenuation, and more particularly inactivation, of the gene expression can be achieved as described herein above. For example, lambda red mediated gene replacement may be used for inactivating gene expression.

[0167] According to certain embodiments, a bacterium of the invention has been further modified to attenuate expression of a gene coding for a polypeptide having glucose 6-phosphate-1-dehydrogenase (G6PDH) activity; express a polypeptide encoded by the *thrA* gene, wherein in said polypeptide at position 357 serine is replaced by arginine; expresses a polypeptide encoded by the *rho* gene, wherein in said polypeptide at position 87 R is replaced by L; and expresses a polypeptide encoded by the *brnQ* gene, wherein said polypeptide terminates after position 308 or any position upstream thereof. Alternatively, the bacterium may be modified to attenuate the expression of the *brnQ* gene (e.g., by inactivation of the gene). Suitable methods for attenuation of gene expression are described above.

[0168] Further information regarding *brnQ* of, e.g., *Escherichia coli* is available at EcoCyc (www.biocyc.org) under Accession numbers EG12168. A representative amino acid sequence of *brnQ* is set forth in SEQ ID NO: 31.

[0169] According to particular embodiments, the bacterium has been further modified to attenuate expression of the gene *zwf* (e.g, by inactivation of the gene); expresses a polypeptide having the amino acid sequence set forth in SEQ ID NO: 11 or a polypeptide having at least about 90%, at least about 93%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, or at least about 99%, sequence identity to the amino acid sequence set forth in SEQ ID NO: 11, wherein in said amino acid sequence at position 357 serine is replaced by arginine; express a polypeptide having the amino acid sequence set forth in SEQ ID NO: 13 or a polypeptide having the amino acid sequence which has at least about 90%, at least about 93%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, or at least about 99%, sequence identity to the amino acid sequence set forth in SEQ ID NO: 13, wherein in said amino acid sequence at position 87 R is replaced by L; and expresses a polypeptide having the amino acid sequence set forth in SEQ ID NO: 32 or a polypeptide having the amino acid sequence which has at least about 90%, at least about 93%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, or at least about 99%, sequence identity to the amino acid sequence set forth in SEQ ID NO: 32.

[0170] According to particular embodiments, the bacterium has been further modified to attenuate expression of the gene *zwf* and *brnQ* (e.g, by inactivation of the genes); and expresses a polypeptide having the amino acid sequence set forth in SEQ ID NO: 11 or a polypeptide having at least about 90%, at least about 93%, at least about 95%, at least

about 96%, at least about 97%, at least about 98%, or at least about 99%, sequence identity to the amino acid sequence set forth in SEQ ID NO: 11, wherein in said amino acid sequence at position 357 serine is replaced by arginine; express a polypeptide having the amino acid sequence set forth in SEQ ID NO: 13 or a polypeptide having the amino acid sequence which has at least about 90%, at least about 93%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, or at least about 99%, sequence identity to the amino acid sequence set forth in SEQ ID NO: 13, wherein in said amino acid sequence at position 87 R is replaced by L.

[0171] As detailed above, a bacterium of the invention may have been modified to overexpress certain polypeptides as detailed herein, which means that an exogenous nucleic acid molecule, such as a DNA molecule, which comprises a nucleotide sequence encoding said polypeptide has been introduced in the bacterium. Techniques for introducing exogenous nucleic acid molecule, such as a DNA molecule, into a bacterial cells are well-known to those of skill in the art, and include transformation (e.g., heat shock or natural transformation) among others.

[0172] In order to facilitate overexpression of a polypeptide in the bacterium, the exogenous nucleic acid molecule may comprise suitable regulatory elements such as a promoter that is functional in the bacterial cell to cause the production of an mRNA molecule and that is operably linked to the nucleotide sequence encoding said polypeptide.

[0173] Promoters useful in accordance with the invention are any known promoters that are functional in a given host cell to cause the production of an mRNA molecule. Many such promoters are known to the skilled person. Such promoters include promoters normally associated with other genes, and/or promoters isolated from any bacteria. The use of promoters for protein expression is generally known to those of skilled in the art of molecular biology, for example, see Sambrook et al., *Molecular cloning: A Laboratory Manual*, Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y., 1989. The promoter employed may be inducible, such as a temperature inducible promoter (e.g., a pL or pR phage lambda promoters, each of which can be controlled by the temperature-sensitive lambda repressor c1857). The term "inducible" used in the context of a promoter means that the promoter only directs transcription of an operably linked nucleotide sequence if a stimulus is present, such as a change in temperature or the presence of a chemical substance ("chemical inducer"). As used herein, "chemical induction" according to the present invention refers to the physical application of an exogenous or endogenous substance (incl. macromolecules, e.g., proteins or nucleic acids) to a host cell. This has the effect of causing the target promoter present in the host cell to increase the rate of transcription. Alternatively, the promoter employed may be constitutive. The term "constitutive" used in the context of a promoter means that the promoter is capable of directing transcription of an operably linked nucleotide sequence in the absence of stimulus (such as heat shock, chemicals etc.).

[0174] Temperature induction systems work, for example, by employing promoters that are repressed by thermolabile repressors. These repressors are active at lower temperatures for example at 30°C, while unable to fold correctly at 37 °C and are therefore inactive. Such circuits therefore can be used to directly regulate the genes of interest (St-Pierre et al. 2013) also by genome integration of the genes along with the repressors. Examples of such as a temperature inducible expression system are based on the pL and/or pR λ phage promoters which are regulated by the thermolabile c1857 repressor. Similar to the genome integrated DE3 system, the expression of the T7 RNA polymerase gene may also be controlled using a temperature controlled promoter system (Mertens et al. 1995), while the expression of the genes of interest can be controlled using a T7 promoter.

[0175] Non-limiting examples of promoters functional in bacteria, such as *Escherichia coli*, include both constitutive and inducible promoters such as T7 promoter, the beta-lactamase and lactose promoter systems; alkaline phosphatase (phoA) promoter, a tryptophan (trp) promoter system, tetracycline promoter, lambda-phage promoter, ribosomal protein promoters; and hybrid promoters such as the tac promoter. Other bacterial and synthetic promoters are also suitable.

[0176] Besides a promoter, the exogenous nucleic acid molecule may further comprise at least one regulatory element selected from a 5' untranslated region (5'UTR) and 3' untranslated region (3' UTR). Many such 5' UTRs and 3' UTRs derived from prokaryotes and eukaryotes are well known to the skilled person. Such regulatory elements include 5' UTRs and 3' UTRs normally associated with other genes, and/or 5' UTRs and 3' UTRs isolated from any bacteria.

[0177] Usually, the 5' UTR contains a ribosome binding site (RBS), also known as the Shine Dalgarno sequence which

is usually 3-10 base pairs upstream from the initiation codon.

[0178] The exogenous nucleic acid molecule may be a vector or part of a vector, such as an expression vector. Normally, such a vector remains extrachromosomal within the bacterial cell which means that it is found outside of the nucleus or nucleoid region of the bacterium.

[0179] It is also contemplated by the present invention that the exogenous nucleic acid molecule is stably integrated into the genome of the bacterium. Means for stable integration into the genome of a host cell, e.g., by homologous recombination, are well known to the skilled person.

[0180] A bacterium in accordance with the present invention can be produced from any suitable bacterium belonging to the *Enterobacteriaceae* family.

[0181] Examples of bacteria which can be used to derive a bacterium of the invention are bacteria belonging to a genus selected from the group consisting of *Escherichia*, *Arsenophonus*, *Biostraticola*, *Brenneria*, *Buchnera*, *Budvicia*, *Buttiauxella*, *Cedecea*, *Citrobacter*, *Cosenzaea*, *Cronobacter*, *Dickeya*, *Edwardsiella*, *Enterobacter*, *Erwinia*, *Ewingella*, *Gibbsiella*, *Hafnia*, *Klebsiella*, *Leclercia*, *Leminorella*, *Lonsdalea*, *Mangrovibacter*, *Moellerella*, *Morganella*, *Obesumbacterium*, *Pantoea*, *Pectobacterium*, *Phaseolibacter*, *Photorhabdus*, *Plesiomonas*, *Proteus*, *Rahnella*, *Raoultella*, *Saccharobacter*, *Salmonella*, *Samsonia*, *Serratia*, *Shimwellia*, *Sodalis*, *Tatumella*, *Thorsellia*, *Trabulsiella*, *Wigglesworthia*, *Yersinia* and *Yokenella*.

[0182] According to particular embodiments, the bacterium belongs to the genus *Escherichia*. According to particular embodiments, the bacterium is *Escherichia coli*. Non-limiting examples of a bacterium belonging to the genus *Escherichia*, which can be used to derive a bacterium of the invention, are *Escherichia coli* K-12 (especially substrain MG1655 or W3110), BL21, W, or Crooks. According to more particular embodiments, the bacterium is *Escherichia coli* K-12.

Method of the invention

[0183] The present invention also provides methods for producing L-serine or a L-serine derivative using a bacterium according to the present invention. Particularly, the present invention provides a method for producing L-serine or a L-serine derivative, said method comprises cultivating a bacterium as detailed herein in a culture medium.

[0184] According to certain embodiments, present invention provides a method for producing L-serine. Particularly, the present invention provides a method for producing L-serine, said method comprises cultivating a bacterium as detailed herein in a culture medium. The method may further comprise collecting L-serine from the culture medium.

[0185] According to certain embodiments, present invention provides a method for producing a L-serine derivative. Particularly, the present invention provides a method for producing L-serine derivative, said method comprises cultivating a bacterium as detailed herein in a culture medium. The L-serine derivative may be selected from the group consisting of L-cysteine, L-methionine, L-glycine, O-acetylserine, L-tryptophan, thiamine, ethanolamine and ethylene glycol. The method may further comprise collecting the L-serine derivative from the culture medium.

[0186] According to certain embodiments, present invention provides a method for producing L-cysteine. Particularly, the present invention provides a method for producing L-cysteine, said method comprises cultivating a bacterium as detailed herein in a culture medium. The method may further comprise collecting L- cysteine from the culture medium.

[0187] According to certain embodiments, present invention provides a method for producing L-methionine. Particularly, the present invention provides a method for producing L-methionine; said method comprises cultivating a bacterium as detailed herein in a culture medium. The method may further comprise collecting L- methionine from the culture medium.

[0188] According to certain embodiments, present invention provides a method for producing L-glycine. Particularly, the present invention provides a method for producing L-glycine; said method comprises cultivating a bacterium as

detailed herein in a culture medium. The method may further comprise collecting L-glycine from the culture medium.

[0189] According to certain embodiments, present invention provides a method for producing O-acetylserine. Particularly, the present invention provides a method for producing O-acetylserine; said method comprises cultivating a bacterium as detailed herein in a culture medium. The method may further comprise collecting O-acetylserine from the culture medium.

[0190] According to certain embodiments, present invention provides a method for producing L-tryptophan. Particularly, the present invention provides a method for producing L-tryptophan; said method comprises cultivating a bacterium as detailed herein in a culture medium. The method may further comprise collecting L-tryptophan from the culture medium.

[0191] According to certain embodiments, present invention provides a method for producing thiamine. Particularly, the present invention provides a method for producing thiamine; said method comprises cultivating a bacterium as detailed herein in a culture medium. The method may further comprise collecting thiamine from the culture medium.

[0192] According to certain embodiments, present invention provides a method for producing ethanolamine. Particularly, the present invention provides a method for producing ethanolamine; said method comprises cultivating a bacterium as detailed herein in a culture medium. The method may further comprise collecting ethanolamine from the culture medium.

[0193] According to certain embodiments, present invention provides a method for producing ethylene glycol. Particularly, the present invention provides a method for producing ethylene glycol; said method comprises cultivating a bacterium as detailed herein in a culture medium. The method may further comprise collecting ethylene glycol from the culture medium.

[0194] The culture medium employed may be any conventional medium suitable for culturing a bacterium cell in question, and may be composed according to the principles of the prior art. The medium will usually contain all nutrients necessary for the growth and survival of the respective bacterium, such as carbon and nitrogen sources and other inorganic salts. Suitable media, e.g. minimal or complex media, are available from commercial suppliers, or may be prepared according to published receipts, e.g. the American Type Culture Collection (ATCC) Catalogue of strains. Non-limiting standard medium well known to the skilled person include Luria Bertani (LB) broth, Sabouraud Dextrose (SD) broth, MS broth, Yeast Peptone Dextrose, BMMY, GMMY, or Yeast Malt Extract (YM) broth, which are all commercially available. A non-limiting example of suitable media for culturing bacterial cells, such as E. coli cells, including minimal media and rich media such as Luria Broth (LB), M9 media, M17 media, SA media, MOPS media, Terrific Broth, YT and others.

[0195] In order to further increase the yield of L-serine or L-serine derivative, the culture medium may further be supplemented with L-threonine. The culture medium may generally contain L-threonine at a concentration from about 0.05 to about 10 g/L, such as from about 0.05 to about 5 g/L, from about 0.05 to about 2.5 g/L, from about 0.05 to about 1 g/L or from about 0.05 to about 0.5 g/L. According to certain embodiments, the culture medium contains L-threonine at a concentration from about 0.05 to about 5 g/L. According to certain other embodiments, the culture medium contains L-threonine at a concentration from about 0.05 to about 2.5 g/L. According to certain other embodiments, the culture medium contains L-threonine at a concentration from about 0.05 to about 1 g/L. According to certain other embodiments, the culture medium contains L-threonine at a concentration from about 0.05 to about 0.5 g/L. According to certain other embodiments, the culture medium contains L-threonine at a concentration from about 0.1 to about 1 g/L. According to other embodiments, the culture medium contains L-threonine at a concentration from about 0.2 to about 1 g/L.

[0196] The carbon source may be any suitable carbon substrate known in the art, and in particular any carbon substrate commonly used in the cultivation of bacteria and/ or fermentation. Non-limiting examples of suitable fermentable carbon substrates are C5 sugars (such as arabinose or xylose), C6 sugars (such as glucose), acetate, glycerol, plant oils, yeast extract, peptone, casaminoacids or mixtures thereof. A carbon source of particular interest is a C6 sugar such as glucose.

[0197] As the nitrogen source, various ammonium salts such as ammonia and ammonium sulfate, other nitrogen compounds such as amines, a natural nitrogen source such as peptone, soybean-hydrolysate, and digested fermentative microorganism can be used. As minerals, potassium monophosphate, magnesium sulfate, sodium chloride, ferrous sulfate, manganese sulfate, calcium chloride, and the like can be used.

[0198] The cultivation can be preferably performed under aerobic conditions, such as by a shaking culture, and by a stirring culture with aeration, at a temperature of about 20 to about 40 °C, such as about 30 to 38 °C, preferably about 37°C. The pH of the culture is usually from about 5 and about 9, such as from about 6.5 and 7.5. The pH of the culture can be adjusted with ammonia, calcium carbonate, various acids, various bases, and buffers. Usually, 1 to 5-day cultivation leads to accumulation of L-serine in the culture medium.

[0199] After cultivation, solids such as cells can be removed from the culture medium by centrifugation or membrane filtration. L-serine or the L-serine derivative can be collected by conventional method for isolation and purification chemical compounds from a medium. Well-known purification procedures include, but are not limited to, centrifugation or filtration, precipitation, ion exchange, chromatographic methods such as e.g. ion exchange chromatography or gel filtration chromatography, and crystallization methods.

[0200] The present invention thus provides L-serine or a L-serine derivative obtainable by a method as detailed herein.

Certain other definitions

[0201] The term "bacterium having ability to produce L-serine" as used herein means a bacterium which is able to produce and cause accumulation of L-serine in a culture medium, can mean that the bacterium is able to cause accumulation in an amount not less than 0.4 g/L, when cultured in minimal M9 media supplemented with 2 g/L glucose, 2 mM glycine and 1 mM threonine at 37°C with adequate aeration for 40 hours.

[0202] The phrase "bacterium which has been modified to attenuate expression of genes encoding polypeptides having serine deaminase activity" as used herein means that the bacterium has been modified in such a way that the modified bacterium contains a reduced amount of the polypeptides having serine deaminase activity. More particularly, the phrase means that the bacterium is unable to synthesize polypeptides having serine deaminase activity.

[0203] The phrase "bacterium which has been modified to attenuate expression of the gene encoding a polypeptide having serine hydroxymethyltransferase activity" as used herein means that the bacterium has been modified in such a way that the modified bacterium contains a reduced amount of the polypeptide having serine hydroxymethyltransferase activity. More particularly, the phrase means that the bacterium is unable to synthesize a polypeptide having serine hydroxymethyltransferase activity.

[0204] The phrase "bacterium which has been modified to attenuate expression of the gene encoding a polypeptide having glucose 6-phosphate-1-dehydrogenase (G6PDH) activity" as used herein means that the bacterium has been modified in such a way that the modified bacterium contains a reduced amount of the polypeptide having glucose 6-phosphate-1-dehydrogenase (G6PDH) activity. More particularly, the phrase means that the bacterium is unable to synthesize a polypeptide having glucose 6-phosphate-1-dehydrogenase (G6PDH) activity.

[0205] The phrase "inactivation of a gene" can mean that the modified gene encodes a completely non-functional protein. It is also possible that the modified DNA region is unable to naturally express the gene due to the deletion of a part of or the entire gene sequence, the shifting of the reading frame of the gene, the introduction of missense/nonsense mutation(s), or the modification of an adjacent region of the gene, including sequences controlling gene expression, such as a promoter, enhancer, attenuator, ribosome-binding site, etc. Preferably, a gene of interest is inactivated by deletion of a part of or the entire gene sequence, such as by gene replacement.

[0206] The presence or absence of a gene on the chromosome of a bacterium can be detected by well-known methods, including PCR, Southern blotting, and the like. In addition, the level of gene expression can be estimated by measuring the amount of mRNA transcribed from the gene using various well-known methods, including Northern

blotting, quantitative RT-PCR, and the like. The amount of the protein encoded by the gene can be measured by well-known methods, including SDS-PAGE followed by an immunoblotting assay (Western blotting analysis), and the like.

[0207] "Polypeptide" and "protein" are used interchangeably herein to denote a polymer of at least two amino acids covalently linked by an amide bond, regardless of length or post-translational modification (e.g., glycosylation, phosphorylation, lipidation, myristilation, ubiquitination, etc.). Included within this definition are D- and L-amino acids, and mixtures of D- and L-amino acids.

[0208] "Nucleic acid" or "polynucleotide" are used interchangeably herein to denote a polymer of at least two nucleic acid monomer units or bases (e.g., adenine, cytosine, guanine, thymine) covalently linked by a phosphodiester bond, regardless of length or base modification.

[0209] "Recombinant" or "non-naturally occurring" when used with reference to, e.g., a host cell, nucleic acid, or polypeptide, refers to a material, or a material corresponding to the natural or native form of the material, that has been modified in a manner that would not otherwise exist in nature, or is identical thereto but produced or derived from synthetic materials and/or by manipulation using recombinant techniques. Non-limiting examples include, among others, recombinant host cells expressing genes that are not found within the native (non-recombinant) form of the cell or express native genes that are otherwise expressed at a different level.

[0210] "Heterologous" as used herein means that a polypeptide is normally not found in or made (i.e. expressed) by the host organism, but derived from a different species.

[0211] "Substitution" or "substituted" refers to modification of the polypeptide by replacing one amino acid residue with another, for instance the replacement of an Serine residue with a Glycine or Alanine residue in a polypeptide sequence is an amino acid substitution. When used with reference to a polynucleotide, "substitution" or "substituted" refers to modification of the polynucleotide by replacing one nucleotide with another, for instance the replacement of a cytosine with a thymine in a polynucleotide sequence is a nucleotide substitution.

[0212] "Conservative substitution", when used with reference to a polypeptide, refers to a substitution of an amino acid residue with a different residue having a similar side chain, and thus typically involves substitution of the amino acid in the polypeptide with amino acids within the same or similar class of amino acids. By way of example and not limitation, an amino acid with an aliphatic side chain may be substituted with another aliphatic amino acid, e.g., alanine, valine, leucine, and isoleucine; an amino acid with hydroxyl side chain is substituted with another amino acid with a hydroxyl side chain, e.g., serine and threonine; an amino acid having an aromatic side chain is substituted with another amino acid having an aromatic side chain, e.g., phenylalanine, tyrosine, tryptophan, and histidine; an amino acid with a basic side chain is substituted with another amino acid with a basic side chain, e.g., lysine and arginine; an amino acid with an acidic side chain is substituted with another amino acid with an acidic side chain, e.g., aspartic acid or glutamic acid; and a hydrophobic or hydrophilic amino acid is replaced with another hydrophobic or hydrophilic amino acid, respectively.

[0213] "Non-conservative substitution", when used with reference to a polypeptide, refers to a substitution of an amino acid in a polypeptide with an amino acid with significantly differing side chain properties. Non-conservative substitutions may use amino acids between, rather than within, the defined groups and affects (a) the structure of the peptide backbone in the area of the substitution (e.g., serine for glycine), (b) the charge or hydrophobicity, or (c) the bulk of the side chain. By way of example and not limitation, an exemplary non-conservative substitution can be an acidic amino acid substituted with a basic or aliphatic amino acid; an aromatic amino acid substituted with a small amino acid; and a hydrophilic amino acid substituted with a hydrophobic amino acid.

[0214] "Deletion" or "deleted", when used with reference to a polypeptide, refers to modification of the polypeptide by removal of one or more amino acids in the reference polypeptide. Deletions can comprise removal of 1 or more amino acids, 2 or more amino acids, 5 or more amino acids, 10 or more amino acids, 15 or more amino acids, or 20 or more amino acids, up to 10% of the total number of amino acids, or up to 20% of the total number of amino acids making up the polypeptide while retaining enzymatic activity and/or retaining the improved properties of an engineered enzyme. Deletions can be directed to the internal portions and/or terminal portions of the polypeptide, in various embodiments, the deletion can comprise a continuous segment or can be discontinuous.

[0215] "Insertion" or "inserted", when used with reference to a polypeptide, refers to modification of the polypeptide by addition of one or more amino acids to the reference polypeptide. Insertions can comprise addition of 1 or more amino acids, 2 or more amino acids, 5 or more amino acids, 10 or more amino acids, 15 or more amino acids, or 20 or more amino acids. Insertions can be in the internal portions of the polypeptide, or to the carboxy or amino terminus. The insertion can be a contiguous segment of amino acids or separated by one or more of the amino acids in the reference polypeptide.

[0216] "Expression" includes any step involved in the production of a polypeptide (e.g., encoded enzyme) including, but not limited to, transcription, post-transcriptional modification, translation, post-translational modification, and secretion.

[0217] As used herein, "vector" refers to a nucleic acid molecule capable of transporting another nucleic acid molecule to which it has been linked. One type of vector is a "plasmid", which refers to a circular double stranded nucleic acid loop into which additional nucleic acid segments can be ligated. Certain vectors are capable of directing the expression of genes to which they are operatively linked. Such vectors are referred to herein as "expression vectors". Certain other vectors are capable of facilitating the insertion of an exogenous nucleic acid molecule into a genome of a bacterium. Such vectors are referred to herein as "transformation vectors". In general, vectors of utility in recombinant nucleic acid techniques are often in the form of plasmids. In the present specification, "plasmid" and "vector" can be used interchangeably as the plasmid is the most commonly used form of a vector. Large numbers of suitable vectors are known to those of skill in the art and commercially available.

[0218] As used herein, "promoter" refers to a sequence of DNA, usually upstream (5') of the coding region of a structural gene, which controls the expression of the coding region by providing recognition and binding sites for RNA polymerase and other factors which may be required for initiation of transcription. The selection of the promoter will depend upon the nucleic acid sequence of interest. A suitable "promoter" is generally one which is capable of supporting the initiation of transcription in a bacterium of the invention, causing the production of an mRNA molecule.

[0219] As used herein, "operably linked" refers to a juxtaposition wherein the components described are in a relationship permitting them to function in their intended manner. A control sequence "operably linked" to a coding sequence is ligated in such a way that expression of the coding sequence is achieved under conditions compatible with the control sequence. A promoter sequence is "operably-linked" to a gene when it is in sufficient proximity to the transcription start site of a gene to regulate transcription of the gene.

[0220] "Percentage of sequence identity," "% sequence identity" and "percent identity" are used herein to refer to comparisons between an amino acid sequence and a reference amino acid sequence. The "% sequence identify", as used herein, is calculated from the two amino acid sequences as follows:

[0221] The sequences are aligned using Version 9 of the Genetic Computing Group's GAP (global alignment program), using the default BLOSUM62 matrix (see below) with a gap open penalty of -12 (for the first null of a gap) and a gap extension penalty of -4 (for each additional null in the gap). After alignment, percentage identity is calculated by expressing the number of matches as a percentage of the number of amino acids in the reference amino acid sequence.

[0222] The following BLOSUM62 matrix is used:

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Trp	-3	-3	-4	-4	-2	-2	-3	-2	-2	-3	-2	-3	-1	1	-4	-3	-2	11		
Tyr	-2	-2	-2	-3	-2	-1	-2	-3	-2	-1	-1	-2	-1	3	-3	-2	-2	7		
Val	0	-3	-3	-3	-1	-2	-2	-3	-3	-3	1	-2	-1	-1	-2	-2	0	-3	-1	4
	Ala	Arg	Asn	Asp	Cys	Gln	Glu	Gly	His	Ile	Leu	Lys	Met	Phe	Pro	Ser	Thr	Trp	Tyr	Val

[0223] "Reference sequence" or "reference amino acid sequence" refers to a defined sequence to which another sequence is compared. In the context of the present invention a reference amino acid sequence may, for example, be an amino acid sequence set forth in SEQ ID NO: 5 or 6.

[0224] As used herein, "L-serine derivative" refers to a compound, such as an amino acid, resulting from reaction of L-serine at the amino group or the carboxy group, or from the replacement of any hydrogen of L-serine by a heteroatom. Non-limiting examples of a "L-serine derivative" include L-cysteine, L-methionine, L-glycine, O-acetylserine, L-tryptophan, thiamine, ethanolamine and ethylene glycol. Further examples of a "L-serine derivative" are described by Chemical Entities of Biological Interest (ChEBI) [<https://www.ebi.ac.uk/chebi/init.do>], for example, under ChEBI ID CHEBI:84135.

[0225] Where a numerical limit or range is stated herein, the endpoints are included. Also, all values and sub ranges within a numerical limit or range are specifically included as if explicitly written out.

[0226] Having generally described this invention, a further understanding can be obtained by reference to certain specific examples, which are provided herein for purposes of illustration only, and are not intended to be limiting unless otherwise specified.

Examples

[0227] For the first time, the present inventors show that a bacterium, such as *E. coli*, lacking all four serine degradation genes (*sdaA*, *sdaB*, *tdcG* and *glyA*) can be constructed (Example 1). Said strain shows higher serine production yield than single and triple deaminase knock outs, when serine pathway is upregulated (Example 2). However, the resulting strain had a low tolerance towards serine, which has also been reported in an *E. coli* triple deletion strain lacking *sdaA*, *sdaB* and *tdcG* (Zhang and Newman, 2008). The inventors furthermore demonstrate that product toxicity can be reduced by over-expression of novel exporters (Example 3), by evolving strains by random mutagenesis (Example 4), and by adaptive evolution (Example 5). The strain was furthermore reverse engineered in order to identify the causative mutations (Example 6). During fed batch fermentation, the tolerant strain shows improved serine production (12.6 g/L with a mass yield of 36.7 % from glucose) when compared to the parental quadruple deletion strain (Example 7). This is the highest serine mass yield reported so far from glucose in any production organism.

[0228] The inventors additionally demonstrate that inhibition of the pentose phosphate pathway by deletion of *zwf* in the presence of other causative mutations leads to a further increase in serine production yield (Example 8).

Example 1 - Deletion of key degradation pathways

[0229] Serine has two key degradation pathways in *E. coli*: Serine to pyruvate, which is encoded by three deaminases namely *sdaA*, *sdaB* and *tdcG*, and conversion of serine to glycine, which is encoded by *glyA*. The deletion of *glyA* renders *E. coli* auxotrophic for glycine. Deletion of *sdaA*, *sdaB* and *tdcG* was done sequentially by using the lambda red mediated gene replacement method (Datsenko and Wanner, 2000). The protocol applied for deleting these genes is similar to the protocol described by Sawitzke et al. (2013). Primers used for amplification of the kanamycin cassette are shown in Table S1. The PCR reaction contained 250 nM each of KF and KR primer of the given gene, 250 µM of dNTP mix, 4 Units of Phusion polymerase (Thermoscientific, Waltham, MA, USA), 40 µl of HF buffer and 10 ng of pKD4 plasmid. The following two-step PCR protocol was used for the PCR amplification: An initial denaturation step at 98°C for 40 seconds, followed by 5 cycles of denaturation at 98°C for 10 seconds, annealing at 55°C for 30 seconds, extension at 72°C for 90 seconds the cycle, followed by 20 cycles, where the annealing temperature was increased from 55°C to 65°C. The PCR products were column purified (Macherey Nagel, Durn, Germany) and concentration was

measured using a Nanodrop instrument (Thermoscientific, Waltham, MA, USA) and subjected to overnight DpnI digestion. *E. coli* MG1655 was used as parent strain to make sequential knock outs. The parent strain was transformed with pKD46 were grown in 2YT-amp media at 30°C and 250 rpm. The expression of exo, beta and gamma proteins were induced by addition of 20 mM arabinose at mid log phase (O.D. 0.4 to 0.5) and the cells were harvested after additional 1 h incubation. Culture was then transferred to 50 ml ice cold falcon tubes and centrifuged at 6500 rpm for 5 min at 4°C. Supernatant was discarded and cells were washed twice with ice cold 10 % glycerol. These electro-competent cells were transformed with 200 ng of kanamycin cassette, and transformants were plated on LB-kan plates. The kanamycin cassette was removed using the plasmid pcp20 encoding flippase gene. Primers for checking the loop out are shown in Table S2. Serine hydroxymethyltransferase encoded by *glyA* was deleted using the P1 phage system protocol (Thomason et al., 2007). A strain from the Keio collection (Baba et al., 2006) harboring *glyA::kan* was grown in 5 ml of LB-kan supplemented with 0.2 % Glucose, 25 mM CaCl₂ and 1 mM Glycine media. At early log phase (O.D. 0.1), culture was transduced with P1 phage lysate. The culture was incubated for three hours at 37°C and 250 rpm for cell lysis. Lysate was filter sterilized by using 0.45 µm filter. 1 ml of overnight culture of the *E. coli* strain having *sdaA*, *sdaB* and *tdcG* deleted, was resuspended in 200 µL of P1 salt solution and was incubated with 100 µL of the above lysate for 1 h. Cells were then grown overnight in 2 mL LB media supplemented with 2mM glycine and 200 mM sodium citrate. Cells were plated on LB-kan plate supplemented with 2mM glycine and 10 mM sodium citrate. The clones were restreaked onto a new plate in order to remove any phage contamination, and isolated colonies were checked for cassette insertion. The loop out was done using pcp20 plasmid. The resulting quadruple deletion strain (Figure 1) is referred to as Q1. This example demonstrates that it is possible to delete *sdaA*, *sdaB*, *tdcG* and *glyA* in *E. coli*, something that has not previously been achieved, and is thus unexpected.

Table S1: Primers used for amplification of kanamycin cassette

Primer name	Sequence
sdaA_KF	GCGCTGTTATTAGTTCGTTACTGGAAGTCCAGTCACCTGTCAGGAGTATTATCGTGGTGTAG GCTGGAGCTGCTTCG
sdaA_KR	CGCCCATCCGTTGCAGATGGGCGAGTAAGAAGTATTAGTCACACTGGACCATATGAATATCC TCCTTAGTTCC
sdaB_KF	CGCTTTCGGGCGGCGCTTCCTCCGTTTAAACGCGATGTATTTCTATGGTGTAGGCTGGAGCT GCTTCG
sdaB_KR	GGCCTCGAAAACGAGGCCTTTGGAGAGCGATTAATCGCAGGCAACCATATGAATATCCTCC TTAGTTCC
tdcG_KF	CGTTCCGCTCCACTTCACTGAACGGCAATCCGAGGGTGTGGATATGGTGTAGGCTGGAGCTG CTTCG
tdcG_KR	GTGCACCCAAGGATGAAAGCTGACAGCAATGTCAGCCGAGACCACCATATGAATATCCTCC TTAGTTCC
glyA_KF	GTTAGCTGAGTCAGGAGATGCGGATGTAAAGCGTGAAATGAACATTGCCGTGTAGGCTGG AGCTGCTTCG
glyA_KR	CAACGAGCACATTGACAGCAAATCACCGTTTCGCTTATGCGTAAACCGGCATATGAATATCCT CCTTAGTTCC

Table S2: Primers used for checking the deletion of given genes

Primer name	Sequence
sdaA_cF	GCGCTGTTATTAGTTCGTTACTGGAAGTCC

Primer name	Sequence
sdaA_cR	CGCCCATCCGTTGCAGATGGGC
sdaB_cF	CGCTTTCGGGCGGCGCTTCCTC
sdaB_cR	GGCCTCGCAAAACGAGGCCTTTGG
tdcG_cF	CGTTCCGCTCCACTTCACTGAACGG
tdcG_cR	GTGCACCCAAGGATGAAAGCTGACAGC
glyA_cF	GTTAGCTGAGTCAGGAGATGCGGATGTT
glyA_cR	CAACGAGCACATTGACAGCAAATCACCG

Example 2 - Overexpression of serine biosynthesis pathway for production of serine

[0230] Serine is produced in *E. coli* from three enzymes encoded by *serA*, *serB* and *serC*. All genes were isolated from *E. coli* MG1655 using primers with respective gene names (Table S3). The 100 µl PCR mixture contain 250 nM each of forward and reverse primer, 250 µM of dNTP, 2 U of Phusion polymerase, 1 X HF buffer, 1 µl of overnight culture. The following two-step PCR protocol was used for the PCR amplification: An initial denaturation step at 98°C for 40, followed by 5 cycles of denaturation at 98°C for 10 seconds, annealing at 55°C for 30 seconds, extension at 72°C for 90 seconds the cycle, followed by 20 cycles, where the annealing temperature was increased from 55°C to 65°C. After column purification, the gene products and plasmids were digested using Fast digest enzymes (Thermoscientific, Waltham, MA, USA). About 500 ng of PCR product or 1 µg of plasmids were subjected to digestion by 1 µl each of the restriction enzymes in 1X fast digest buffer. The reaction was incubated for 3h and then column purified again. *serA* was subjected to double digestion with NcoI and NotI while *serC* was digested with NdeI and PacI. pCDF-Duet was first digested with NcoI and NotI for cloning of *serA* leading to plasmid pCDF-Duet-*serA* and this plasmid was later used for cloning of *serC* thus making pCDF-Duet-*serA-serC*. The gene encoding *serB* was cloned in pACYC-Duet vector at NcoI and PacI site leading to pACYC-*serB*. Typical ligation reaction include 1 X T4 ligase buffer 50 ng of plasmid DNA and 100 ng of insert and 0.3 µl/10 µl of T4DNA ligase (Thermoscientific, Waltham, MA, USA).

[0231] Feedback inhibition of *serA* was removed by mutating three residues H344, N346 and N364 to alanine (Al-Rabee et al., 1996) by site directed mutagenesis (Table S4). The master mix was used as mentioned above with the only modification that the master mix was divided in two equal aliquots, after which forward and reverse primers were added to each aliquot. A total of 100 ng of pCDF-Duet-*serA-serC* plasmid was used as a template. The two step PCR program: initial denaturation at 98°C for 40 sec, denaturation at 98°C for 10 sec, annealing 60°C for 30 sec, extension 72°C for 4 min and 30 sec the cycle was repeated 5 times and then the two aliquots were mixed and redistributed for additional 15 cycles. To enable swapping of vector backbones, the NcoI site inside *serC* was removed by the same approach using the primers listed in Table S4.

[0232] Figure 2 shows the vector maps of the plasmid constructs.

Table S3: Primers used for amplification and cloning of serine production pathway

Primer name	Sequence
serA-NcoI	GGCCCATGGCAAAGGTATCGCTGGAG
serA NotI_R	ATTGCGGCCGCTTAGTACAGCAGACGGGCGCGA
serB_NcoI_F	GGCCCATGGCTAACATTACCTGGTGCG
serB_R_HindIII	GCCAAGCTT TTATTACTTCTGATTCAGGCTGCC
serB_PacI_R	GCCTTAATTAATTATTACTTCTGATTCAGGCTGCC
serC_F_NdeI	GGCCATATGATGGCTCAAATCTTCAATTTTAGTTCTGG
serC_R_PacI	GCCTTAATTAATCAT TAACCGTGACGGCGTTTGAAC
ydeD_F_NdeI	CCGCATATGTCGCGAAAAGATGGGGTG
ydeD_cHis_R-PacI	GCCTTAATTAATGATGATGATGATGACTTC CCACCT TTACCGCTT TACGCC

Primer name	Sequence
ydeD_Ncol_F1	CCGCCATGGCGCGAAAAGATGGGGTG

Table S4: Primers used for site directed mutagenesis of serine production pathway

Primer name	Sequence
serA N364A_F	CGAGCAGGGCGTCGCTATCGCCGCGCAATA
serA N364A_R	TATTGCGCGGCGATAGCGACGCCCTGCTCG
serA H344AN346A_F	CTGAT CACATCGCTGAAGCT CGTCCGGGGCGTGC
serA H344AN346A_R	GCACGCCCGGACGAGCTTCAGCGATGTGCATCAG
serC_Ncolc_F	CAAGGTATTATTCTGTCATGGCGGTGGTCGCG
serC_Ncolc_R	CGCGACCACCGCCATGACAGAATAATACCTTG

DE3 integration and serine production during batch fermentation

[0233] The serine production was checked in M9 minimal media. Glucose M9 minimal media consisted of 2 g/L glucose, 0.1 mM CaCl₂, 2.0 mM MgSO₄, 1× trace element solution, and 1× M9 salts. The 4,000x trace element stock solution consisted of 27 g/L FeCl₃*6H₂O, 2 g/L ZnCl₂*4H₂O, 2 g/L CoCl₂*6H₂O, 2 g/L NaMoO₄*2H₂O, 1 g/L CaCl₂*H₂O, 1.3 g/L CuCl₂*6H₂O, 0.5 g/L H₃BO₃, and Concentrated HCl dissolved in ddH₂O and sterile filtered. The 10× M9 salts stock solution consisted of 68 g/L Na₂HPO₄ anhydrous, 30 g/L KH₂PO₄, 5 g/L NaCl, and 10 g/L NH₄Cl dissolved in ddH₂O and autoclaved.

[0234] To use pET vectors as expression system, a DE3 cassette containing T7 polymerase was integrated into the genome of each deletion mutant using a Lambda DE3 lysogenization kit (Millipore, Damstadt Germany). Subsequently, strains (MG1655 (DE3)) carrying single (Δ *sdaA*), triple (Δ *sdaA*, Δ *tdcG*, Δ *sdaB*), and quadruple deletions (Δ *sdaA*, Δ *tdcG*, Δ *sdaB* and Δ *glyA*) were transformed with pCDF-Duet1-serAmut-serC and pACYC-serB. The resulting glycerol stocks were grown overnight in 2YT medium containing 0.1 % glucose and supplemented with required antibiotics (Spectinomycin and chloramphenicol). Overnight cultures were inoculated in triplicates into flasks containing M9 medium supplemented with 0.2 % glucose, 1mM threonine, required antibiotics (For the quadruple deletion strain, 2 mM glycine was also supplemented). Flasks were incubated at 37°C at 250 rpm. Serine production was induced by addition of 40 μ M IPTG after the cultures reached an optical density of 0.55 to 0.65. O.D. measurements and sampling were done at regular time intervals for following 60 hr. Samples were filtered and subjected to HPLC for glucose and byproduct analysis using a method described previously (Kildegaard et al., 2014) with the only exception that the column temperature was kept at 30°C. Serine concentrations were determined using LCMS. The LC-MS/MS system consisted of a CTC autosampler module, a high pressure mixing pump and a column module (Advance, Bruker, Fremont, CA, USA). The injection volume was 1 μ l. The chromatography was performed on a ZIC-cHILIC column, 150mm $\times$ 2.1mm, 3 μ m pore size, (SeQuant, Merck Millipore). In front of the separation column was a 0.5 μ depth filter and guard column, the filter (KrudKatcher Classic, phenomenex) and guard column ZIC-cHILIC, 20 $\times$ 2.1 mm (SeQuant, Merck). Eluent A: 20 mM ammonium acetate pH adjusted to 3.5 with formic acid in milliQ water. Eluent B: Acetonitrile. The total flow rate of eluent A and B was 0.4 ml/min. The isocratic elution 35%, and the total run time was 5 minutes. Retention time was 2.8 minutes for serine. The MS-MS detection was performed on a EVOQ triple quadrupole instrument (Bruker, California, USA) equipped with an atmospheric pressure ionization (API) interface. The mass spectrometer was operated with electrospray in the positive ion mode (ESI+). The spray voltage was set to 4500 V. The con gas flow was 20 l/h, and the cone temperature was set at 350 °C. The heated probe gas flow was set at 50 l/h with a temperature of 350 °C. Nebulizer flow was set at 50 l/h, and the exhaust gas was turned on. Argon was used as collision gas at a pressure of 1.5 mTorr. Detection was performed in multiple reacting monitoring (MRM) mode. The quantitative transition was 106 $\rightarrow$ 60, and the qualitative transition was 106 $\rightarrow$ 70. The collision energy was optimized to 7eV for both transitions.

[0235] Calibration standards of serine were prepared in media used for serine production and diluted 50:50 with 0.2% Formic acid in Acetonitrile. The concentration of the calibration standards was in the range from 0.001 to 0.5 g/L.

[0236] The experiment demonstrates that the deletion of all four genes involved in serine degradation in *E. coli*, referred to as Q1(DE3), results in the highest specific productivity and the highest yield from glucose as shown on Figure 3.

Example 3: Reducing product toxicity by overexpression of transporter

[0237] Deletion of the main serine degradation pathways in *E. coli* results in significant decreased tolerance towards serine. In order to increase the tolerance, a transporter with a potential specificity for serine (*ydeD*) was overexpressed and tested during growth in high concentrations of serine. *ydeD* was cloned in pCDF-1b at NcoI and PacI site by amplification with primers mentioned in Table S3 and the protocol given in Example 1.

[0238] Q1(DE3) was transformed with the pCDF-1b empty vector and the pCDF-*ydeD*-c-His plasmid (Figure 4). Transformants were selected on LB-spectinomycin plates. Overnight cultures of these transformants were inoculated in M9 media supplemented with 2mM glycine, 2 g/L glucose and spectinomycin in 1:50 ratio. Cultures were incubated at 37°C and 250 rpm at an optical density of 0.4 to 0.5, after which 800 µl was added to a 48-well biolector plate (M2P labs, Baeswieler, Germany) containing 800 µl M9 media with varying serine concentration (12.5, 25 and 50 g/L) and 2mM glycine. The expression of *ydeD* was induced with 100 µM IPTG. The growth was then monitored in the Biolector instrument (M2P labs, Baeswieler, Germany) at 37°C and 70 % humidity with continuous shaking. Gain was set to 20 % and scattering intensity was measured every 5 min for next 40 h.

[0239] The experiment demonstrates that the growth of *E. coli* lacking the main serine degradation pathways are severely growth inhibited in the presence of even low concentrations of serine. Upon overexpression of *ydeD*, the tolerance towards serine is increased substantially (Figure 4), suggesting that YdeD may potentially transport serine out of the cell.

Example 4 - Random mutagenesis for serine tolerant strain

[0240] Inactivation of the main serine degradation pathways in *E. coli* results in significant decreased tolerance towards serine. In order to increase the tolerance, the Q1 strain (obtained in Example 1) was grown in M9 media supplemented with 2mM glycine and 3 g/L serine overnight. 1 ml of culture was spread in 6 well petri plate and exposed to UV irradiation (CBS Scientific, San Diego, CA, USA) for 30 min. The culture was then added to 5 ml M9 media supplemented with 2 mM glycine, 2 g/L glucose and 50 g/L serine for enrichment of the tolerant mutants. The culture was incubated at 37°C and 250 rpm for 3 days and then plated on M9 plate supplemented with 50 g/L serine for the selection of the tolerant clones. Colonies were isolated and the growth rates and tolerance was estimated using the following method:

[0241] Cultures were grown overnight in 2xYT media. They were then inoculated in 96 well flat bottom micro titer plates with 150 µL of M9 media containing 0.2 % glucose and various concentration of Serine (in triplicates). For strains containing the *glyA* deletion, 2mM glycine was also supplemented to the media. A total of 1.5 µL of cells (1:100) was used as inoculum. If required minor changes in volume were done to ensure equal amount of cells. Plates were sealed with Microamp clear adhesive film (Applied Biosystems, Warrington, UK) and were incubated in Microtiter plate reader (Biotek, Winooski VT, USA). The reader was set at 37°C with continuous shaking, and the O.D was monitored every 5 to 10 minutes at 630 nm for 32 hours.

[0242] The experiment demonstrates that random mutagenesis can be used to increase the tolerance towards serine significantly (Figure 5). The resulting strain is referred to as Q3 below.

Example 5 - Adaptive evolution for L-serine tolerance

[0243] Apart from random mutagenesis, serine tolerance of a strain having the main serine degradation pathways inactivated was also increased by Adaptive evolution. Seven independent populations of the Q1 strain were adaptively

evolved in M9 minimal media supplemented with 2 mM glycine and increasing concentrations of the amino acid L-Serine at 37 °C along 60 days.

[0244] An Adaptive Laboratory Evoution (ALE) experiment was achieved by using an automated system, which enables the propagation of evolving populations over the course of many days while monitoring their growth rates. Prior to the start of the experiment, the system filled the required tubes with 25 ml of culture media and kept them at 37 °C in a heat block. Controlled aeration was obtained using magnetic tumble stirrers placed inside the tubes and spinning at 1,800 rpm. At the start of the experiment, a single colony was grown overnight in one of the tubes placed inside the system, and 100 µL aliquots were used by the robotics platform to inoculate seven independent flasks. As the bacteria grew, the automated system performed multiple OD measurements at 600 nm for each flask. Growth rates were automatically calculated by taking the slope of a least-square linear regression line fit to the logarithm of the OD measurements (Figure 6). Once reaching a target OD of 0.4,100 µl of culture was used to inoculate a new flask. This way, cultures were serially passed (~2-3 times per day) to flasks with fresh media after reaching a targeted cell density such that stationary phase was never reached. The populations were initially incubated with 3 g/L serine after desired growth rate was reached culture was supplemented with 6 g/L of L-Serine. When populations achieved a stable phenotype (i.e. growth rate), the L-Serine concentration was increased to 12 g/L. This process was repeated iteratively using 24, 50, 75, and 100 g/L of L-serine. The final population was plated on the LB-agar and 2 clones of each of the populations selected. The evolved strains had significantly increased tolerance when tested for growth in the presence of high concentrations of serine by using the MTP based assay described in Example 5, above. The results are shown in Figure 6.

Genomic DNA extraction, library sequencing and analysis

[0245] Two clones of each evolved cultures as well as the strain evolved by random UV-mutagenesis (Example 5) were genome sequenced. Genomic DNA was extracted from 1.5 ml of overnight cultures of *E. coli* strains using QIAamp DNA Mini Kit (QIAGEN, Germany). The genomic libraries were generated using the TruSeq ®Nano DNA LT Sample Preparation Kit (Illumina Inc., San Diego CA, USA). Briefly, 100 ng of genomic DNA diluted in 52.5 ul TE buffer was fragmented in Covaris Crimp Cap microtubes on a Covaris E220 ultrasonicator (Covaris, Brighton, UK) with 5% duty factor, 175 W peak incident power, 200 cycles/burst, and 50-s duration under frequency sweeping mode at 5.5 to 6°C (Illumina recommendations for a 350-bp average fragment size). The ends of fragmented DNA were repaired by T4 DNA polymerase, Klenow DNA polymerase, and T4 polynucleotide kinase. The Klenow exo minus enzyme was then used to add an 'A' base to the 3' end of the DNA fragments. After the ligation of the adapters to the ends of the DNA fragments, DNA fragments ranging from 300-400 bp were recovered by beads purification. Finally, the adapter-modified DNA fragments were enriched by 3-cycle-PCR. Final concentration of each library was measured by Qubit® 2.0 Florometer and Qubit DNA Broad range assay (Life Technologies, Paisley, UK). Average dsDNA library size was determined using the Agilent DNA 7500 kit on an Agilent 2100 Bioanalyzer. Libraries were normalized and pooled in 10 mM Tris-Cl, pH 8.0, plus 0.05% Tween 20 to the final concentration of 10 nM. Denaturated in 0.2N NaOH, 10 pM pool of 20 libraries in 600 µl ice-cold HT1 buffer was loaded onto the flow cell provided in the MiSeq Reagent kit v2 300 cycles and sequenced on a MiSeq (Illumina Inc., San Diego, CA, USA) platform with a paired-end protocol and read lengths of 151 nt.

[0246] The breseq pipeline (Deatherage and Barrick, 2014) version 0.23 with bowtie2 (Langmead and Salzberg) was used to map sequencing reads and identify sequence variants relative to the *E. coli* K12 MG1655 reference genome (NCBI accession number NC_000913.2). Gene deletions present in the strains were verified manually based on missing coverage regions in the genome. Common variants found in MG1655 stock cultures (Freddolino et al., 2012) were excluded from further analysis. All sequencing samples had an average mapped coverage of at least 25x. The experiment identifies mutations that cause increased tolerance towards high concentrations of serine as shown in Table S5.

Table S5: Mutations found in the strains evolved for increased serine tolerance. The reference number for the genome sequence is NC_000913.

Gene Name	Change	Annotation	Q1	Q3	ALE5-4	ALE5-8	ALE6-1	ALE6-2
thrA	A->G	Y356C	0	0	0	0	0	0

thrA	A->C	S357R	0	0	1	1	0	0
thrA	T->A	S359R	0	0	0	0	1	1
trxA/rho	IS1 -1 9 bp	Insertion ¹	0	0	0	0	0	0
rho	G->T	R87L	0	0	0	0	1	1
gcvA/ygdI	1 bp	Insertion ²	0	0	0	0	1	1
gcvA/ygdI	IS4 1 13 bp	Insertion ³	0	0	1	1	0	0
dapA/gcvR	1 bp	Insertion ⁴	0	0	1	0	1	1
lrp	A->G	D143G	0	0	0	0	0	0
trxB/lrp	C->T	Intergenic ⁹ (-271/-274)	0	0	0	0	1	1
frc	IS1 -1 9 bp	Insertion ⁵	0	0	0	0	0	0
eno	C->G	V164L	0	0	0	0	0	0
argP	C->A	Q132K	0	0	0	0	0	0
tufA	C->A	G19V	0	0	0	0	0	0
cycA	A->G	I220V	0	0	0	0	0	0
rhtA/ompX/ybiP/mntS	2854 bp	Deletion ⁶	0	1	0	0	0	0
rpe	A->G	I202T	0	0	0	0	1	1
ytfB/fkIB	T->C	Intergenic ¹⁰ (-154/-64)	0	0	0	0	1	1
yojL	G->C	D334H	0	0	0	0	1	0
aroP	IS5 1 4 bp	Insertion ⁷	0	0	1	1	0	0
hyaF	T->G	V120G	0	0	1	1	0	0
mdtJ/tqsA	IS1 -1 9 bp	Insertion ⁸	0	0	0	0	0	0
pykF	G->T	E250*	0	0	1	1	0	0
malT	C->T	Q420*	0	0	1	1	0	0
rpoB	C->T	P520L	0	0	1	1	0	0
fumB	T->G	T218P	0	0	0	0	0	0
gshA	G->A	A178V	0	0	0	1	0	0
lamB	C->T	Q112*	0	0	0	1	0	0
Gene Name	Change	Annotation	Q1	ALE8-3	ALE8-8	ALE9-3	ALE9-8	
thrA	A->G	Y356C	0	1	1	1	1	
thrA	A->C	S357R	0	0	0	0	0	
thrA	T->A	S359R	0	0	0	0	0	
trxA/rho	IS1 -1 9 bp	Insertion ¹	0	1	1	1	1	
rho	G->T	R87L	0	0	0	0	0	
gcvA/ygdI	1 bp	Insertion ²	0	0	0	0	0	
gcvA/ygdI	IS4 1 13 bp	Insertion ³	0	0	0	0	0	
dapA/gcvR	1 bp	Insertion ⁴	0	0	0	0	0	
lrp	A->G	D143G	0	1	1	1	1	
trxB/lrp	C->T	Intergenic ⁹ (-271/-274)	0	0	0	0	0	
frc	IS1 -1 9 bp	Insertion ⁵	0	1	1	1	1	
eno	C->G	V164L	0	1	1	1	1	

argP	C->A	Q132K	0	1	1	1	1	
tufA	C->A	G19V	0	1	1	1	1	
cycA	A->G	I220V	0	1	1	1	1	
rhtA/ompX/ybiP/mntS	2854 bp	Deletion ⁶	0	0	0	0	0	
rpe	A->G	I202T	0	0	0	0	0	
ytfB/fkIB	T->C	Intergenic ¹⁰ (-154/-64)	0	0	0	0	0	
yojI	G->C	D334H	0	0	0	0	0	
aroP	IS5 1 4 bp	Insertion ⁷	0	0	0	0	0	
hyaF	T->G	V120G	0	0	0	0	0	
mdtJ/tqsA	IS1 -1 9 bp	Insertion ⁸	0	0	0	0	1	
pykF	G->T	E250*	0	0	0	0	0	
malT	C->T	Q420*	0	0	0	0	0	
rpoB	C->T	P520L	0	0	0	0	0	
fumB	T->G	T218P	0	0	0	1	0	
gshA	G->A	A178V	0	0	0	0	0	
lamB	C->T	Q112*	0	0	0	0	0	

*: designates a stop codon

Insertion¹: Insertion of a 768 bp long insertion sequence element IS1 in the lagging strand at the location 3966174 which is an intergenic region between genes *trxA* and *rho*. Duplication of

the location 2942174, which is an intergenic region between genes *gcvA* and *ygdI*. Duplication of 9 bp upstream and downstream of insertion sequence is observed.

Insertion²: Insertion of 1bp at location 2942629, which is an intergenic region between genes *gcvA* and *ygdI*.

Insertion³: Insertion of 1342 bp long insertion sequence element IS4 at the location 2942878, which is an intergenic region between genes *gcvA* and *ygdI*. Duplication of around 13 bp upstream and downstream of insertion sequence is observed.

Insertion⁴: Insertion of 1bp at location 2599854, which is an intergenic region between genes *dapA* and *gcvR*.

Insertion⁵: Insertion of 768 bp long insertion sequence element IS1 in the lagging strand at location 2492323, which leads to a truncation of gene *frc*. Duplication of 9 bp upstream and downstream of insertion sequence is observed.

Deletion⁶ : Deletion of 2854 bp from location 850092, resulting in deletion of the first 5 bp of *rhtA*, complete deletion of genes *ompX* and *ybiP*, deletion of 239 bp of sRNA *rybA* and 77 bp deletion of *mntS* gene.

Insertion⁷: Insertion of 1195 bp long insertion sequence element IS5 at location 121518 which leads to deletion of the majority of *aroP*. Duplication of 4 bp upstream and downstream of insertion sequence is observed.

Insertion⁸: Insertion of 768 bp long insertion sequence element IS1 in the lagging strand at location 1673670, which is an intergenic region between genes *mdtJ* and *tqsA*. Duplication of 9 bp upstream and downstream of insertion sequence is observed.

Intergenic⁹: C->T mutation at location 923321 which is an intergenic region between *trxB* and *lrp*. The mutation is 271 bp upstream of *trxB* and 274 bp upstream of *lrp*.

Intergenic¹⁰: T->C mutation at the location 4428871 which is an intergenic region between *yftB* and *fkfB*. The mutation is 154 bp upstream of *yftB* and 64 bp upstream of *fkfB*.

Example 6 - Reverse engineering of ALE mutations by cam-sacB system and multiplex genome engineering

[0247] In order to identify mutations that cause increased tolerance towards serine, the mutations identified in Example 5 were introduced into the Q1(DE3) strain using two different methods as described below.

A. Introduction of *thrA* mutations

[0248] Mutations in *thrA* were introduced into the genome of the Q1(DE3) strain using a cam-sacB based selection system. Cam-sacB was inserted using pKD46 plasmid harboring *exo*, *beta* and *gamma* genes for recombination. Positive selection for cassette insertion was done by selecting clones for chloramphenicol resistance. The loss of cassette was selected by replica plating clones on LB-chloramphenicol and LB-sucrose plate containing 15 % sucrose (no NaCl). The Cam-sacB cassette was amplified using *thrA_camsacB_F* and *R* (Table S6) primers respectively. Apart from template and extension time, the reaction mixture and PCR program was the same as described in example 1. The extension time was 2 min and 30sec, while template was 1 µl of overnight culture of the *E. coli* culture containing cam-sacB cassette. Competent Q1(DE3) cells were then transformed with 200 ng of *thrA*-cam-sacB cassette, and were plated on LB-chloramphenicol-ampicillin plates after two hour and incubated overnight at 30°C. A single colony was picked and made electrocompetent after 1 h of induction (Example 1) and transformed with *thrA* alleles. After 2 hours of recovery, cells were plated on LB-sucrose plates and incubated at 37°C to cure pKD46 plasmid. 24 clones of

each experiment were replica plated on LB-chloramphenicol and LB plates. Clones that did not grow on chloramphenicol plates were checked for the loss of the cassette by colony PCR and were subsequently sequenced by Sanger sequencing.

[0249] All three strains containing *thrA* mutations (Y356C, S357R or S359R) were grown in M9 media supplemented with 2mM glycine, 2 g/L glucose and 6.25 g/L L-serine. In this experiment, the background was not subtracted from the measurements, resulting in apparent increase in the OD for the Q1 strain. However, under these conditions, the Q1 strain did not show significant growth. On the other hand, each of the *thrA* mutations resulted in a similar and very significant increase in tolerance towards L-serine (Figure7), allowing them to grow at a L-serine concentration of at least 6.25 g/L opposed to the parent Q1 strain. Since all *thrA* mutants had same growth profile, S357R mutant was chosen as the parent strain (referred to as strain Q1(DE3)-*thrA*S357R) for the multiplex genome engineering described below.

Table S6: Primers used for genome integration of *thrA* mutations in the genome.

Primer name	Sequence
thrA_gF	ATGCGAGTGTTGAAGTTCGGCG
thrA_gR	TCAGACTCCTAACTTCCATGAGAGGG
thrA_camsacB_F	ATGCGAGTGTTGAAGTTCGGCGGTACATCAGTGGCAAATGCAGAACGTTTAAAAT GAGACGTTGATCGGCACG
thrA_camsacB_R	TCAGACTCCTAACTTCCATGAGAGGGTACGTAGCAGATCAGCAAAGACACCAAAG GGAAAACGTCCATATGCAC

B. Multiplex genome engineering, screening and amplicon sequencing of selected ALE mutations

[0250] In order to identify mutations that cause increased tolerance towards serine, selected mutations identified in Example 5 were introduced into the Q1(DE3)-*thrA*S357R strain using multiplex genome engineering (Wang et al., 2011). The protocol applied for multiplex genome engineering was similar to the method published by Wang and Church, 2011. Strain Q1(DE3)-*thrA*S357R was transformed with plasmid pMA1 (containing only beta protein under control by the arabinose inducible promoter). Clones were plated on LB-ampicillin plates and incubated at 37°C. Colonies were cultured overnight in TY-amp-gly media at 37°C and re-inoculated in 25 ml fresh TY-gly media the following day. After reaching an O.D. of 0.4 at 37°C, arabinose was added to a final concentration of 0.2 %. Cells were re-incubated at 37°C and 250 rpm for additional 15 min and were then made electro-competent by washing twice with 10 % glycerol (ice cold). Final volume of electro competent cells was adjusted to 200 µl using 10 % glycerol. The primers targeting the loci (*lrp* D143G, *eno* V164L, *argP* Q132K, *cycA* 1220V, *pykF* E250*, *rpoB*520L, *gcvA**, *yojL* D334H, *rho* R87L) are given in Table S7. All primers were pooled and adjusted to a final concentration of 10 pmol/µl. To 50 µl of cells, 1 µl of this mix was added and transformed by electroporation. The transformed cells were directly added to 25 ml TY-gly media and were incubated at 37°C, 250 rpm for two hours to reach O.D. of 0.4, followed by arabinose induction and transformation as above for second round of multiplex engineering. The process was repeated six times. After the 6th round of multiplex genome engineering, the cells were incubated overnight. The resulting library of mutants was subsequently grown in M9 minimal media with and without serine. This way, mutations resulting in increased growth rates in minimal media or increased tolerance towards serine were enriched: A total of 1.5 µl of overnight culture was added in triplicates to wells in micro titer plates containing 150 µl triplicates of M9 media supplemented with 2 mM glycine and 2 g/L glucose (minimal media) or M9 media with 2 mM glycine, 2 g/L glucose and 25 g/L serine (minimal media with serine). Growth profiles were monitored at 37°C with continuous fast shaking in a microtiter plate reader for 40 hr. Optical density was measured at 630 nm every 5 min. Triplicates were pooled and used as template for amplicon generation. The PCR program and reaction composition was as described in Example1. Illumina protocol was followed for amplicon processing and next gen sequencing was as described in Example 5. Sequencing results were analyzed computationally, and the enrichment of the various mutations was calculated as the frequency of the mutations in either minimal media or minimal media with serine, divided by the frequency of the mutations in the unselected library (Figure 8). This experiment shows that the mutations in *lrp*, *rho*, *argP* and *eno*

results in increased tolerance towards serine.

Table S7: Primers used for Multiplex genome engineering

Primer name	Sequence
lrpD143G	GCTTGACTTCTTCCATAACAACGTAAGTGCGCGTCCCGTTAACCCCAGGCAGACGC AGCAGGGTTTC
enoV164L	CCAACCGGCTGAATCATGAATTCTTGA ATGTCGAGATTATTATCAGCGTGCTCACCACCGTTGATGATG
argPQ132K	CTCAACTTGCAGGTAGAAGATGAAACGAGGAAAGAGAGGCTCCGCCGCGGCGA AGTGGTCGGC
cycA1220V	CAGCTCAATCCCCACGAAAGCTAATACGGCAACTTGAAAACCGGCAAAGAAGCCA CTTAAACCTTTC
pykFE250***	GCATCATGGTTGCGCGTGGCGAC CTCGGCGTTTAGTGACCCTAAATCTTCGCCCAGAAGATGATGA
rpoBP520L	GATACGACGTTTGTGCGTAATTTCTGAGAGGAGATTATTTTGGTCCATAAACTGA GACAGCTG
gcvA***	CTCGTAAGGCATTTAGCGGTGGTA ATGGTTATC ATTAGGCTATTAACTTTGATGTTAAATG
yojLD334H	GGTGAGATTAATCGGACCAACGGAGAAGGCATTGTGTTGGTATGCAAACGTCAC GTTACGCAGCTCCAGC
rhoR87L	GAGATGGTATCACCAGTGCGGAGATTAAATCTTAGTATCTGAGAAGGGGAAACG TAGATGTCATCAG

Table S8: Primers used for amplicon sequencing

Primer name	Sequence
lrp_F	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGCGGTGATTTCGACTACCTGTTG
lrp_R	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGGCGCGTCTTAATAACCAGACGAT
enoV164_F	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGGCTGTACGAGCACATCGCTGAAC
enoV164_R	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGCCCATGCGGATGGCTTCTTTTAC
argP_F	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG GACAGTCTGGCGACGTGGTTGCT
argP_R	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGTATCGACAAGACAACCTCGGCAGCG
cycA_F	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGGGAAGCGTCATTCGCGCATTG
cycA_R	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGGTTAATCGCGCGTGGCAGTG
pyfK_F	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGGGCCTCAACAACCTTCGACGA
pyfK_R	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGGAATCCAGCATCTGGGTTCG
rpoB_F	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGCAACGCCAAGCCGATTTCCG
rpoB_R	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGGTCTCGAACTTCGAAGCCTGC
gcvA_F	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGGCTGGTAGAAGCTCAACGGAC
gcvA_R	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGTGCTGCGGCATCAAAAACCTCG

Primer name	Sequence
yojl_F	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGGCCTTTCAAAGCAGAGTTTCCGC
yojl_R	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGCTACCGTTGCCGCCAATCAG
rho_F	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGGCAGGATGGATTTGGTTTCCTCC
rho_R	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGGCAGCGCAAAATAGCGTTCACC

Example 7 - Serine production by tolerant and susceptible *E. coli* during fed batch fermentation

[0251] A new vector was constructed in order to upregulate expression of exporter along with serine pathway in the pACYC-Duet vector by following the same above strategy as mentioned in example 2. *serB* was cloned using NcoI and HindIII double digestion followed by ligation. The resulting vector (pACYC-Duet-*serB*) was then subjected to double digestion by NdeI and PaeI for cloning of *ydeD*-c-His. Both strains Q1 (DE3) and Q3 (DE3) were made electrocompetent by growing cells in 30 ml 2xYT media supplemented with 0.2 % glucose (37°C, 250 rpm) to mid log phase. Cells were harvested by centrifugation at 6500 rpm for 5 min at 4°C, and were washed twice by ice cold 10 % glycerol. Cells were finally resuspended in 500 µL of 10 % glycerol and 50 µL of cells were transformed with plasmids pCDF-Duet1-*serA*mut-*serC* and pACYC-*serB*-*ydeD*-c-His for serine production.

[0252] Serine production was demonstrated during fed batch fermentation in 1L fermenters (Sartorius, Gottingen, Germany). The media contained 2g/L MgSO4*7H2O, 2 g/L KH2PO4, 5 g/L (NH4)2SO4, 7.5 g/L Glucose, 2 g/L Yeast extract, 0.6 g/L glycine, 0.12 g/L threonine, 4X trace element (as mentioned in Example 2), 50 mg/L spectinomycin and 25 mg/L chloramphenicol. For the *E. coli* Q1(DE3) strain, the initial glucose concentration was 10 g/L in order to enhance the growth before induction. The feed generally contained 140 g/L glucose, 24 g/L Ammonium sulfate, 2g/L glycine, 0.12 g/L threonine, 2.5 g/L each of MgSO4*7H2O and KH2PO4, 1X trace elements 50 mg/L spectinomycin and 25 mg/L chloramphenicol, while the feed for the Q1(DE3) strain contained 120 g/L of glucose.

[0253] A log phase culture was used to inoculate 500 ml media by 1:50 inoculum ratio. The cultures were allowed to grow over night in the fermenter at 37°C, 1000 rpm baffle speed and 20 % air saturation. Feed at the rate of 8 g/h was started after glucose concentration was below 250 mg/L in the media. Production was induced at late log phase (O.D. 7.5 to 9.5) by the addition of 40 µM IPTG and feed rate was reduced to 6 g/h. Samples were taken at regular intervals and were subjected to HPLC and LCMS analysis as mentioned before.

[0254] The experiment demonstrates that serine can be produced at high titer and with a high yield in *E. coli* using fed batch fermentation (Figure 9). The titers were 6.8 g/L and 12.5 g/L for the Q1(DE3) and the Q3(DE3) strain, respectively. The fermentations resulted in a surprisingly high mass yield of 36.7 % from glucose in the Q3(DE3) strain when compared to 24.3 % in the Q1(DE3) strain (Figure 9B). This shows that a higher titer and yield can be achieved using the production strain that is tolerant towards serine.

Example 8 - Reverse engineered strains showing increased serine production

[0255] Strains reverse engineered in example 6 were checked for serine tolerance as mentioned in example 3. The best serine tolerant strain (F7) was checked for serine production and was also genome sequenced as described in example 5. Plasmids for overexpression of the serine production pathway were introduced as described in Example 7. Shake flask experiments were carried out as described in Example 2, with the only difference being that the concentration of glucose was 2.5 g/L. The O.D. measurements and sampling were done at regular intervals, and the samples were analyzed using methods described in Example 2. Surprisingly, said strain resulted in a significantly increased production and yield of serine from glucose as shown in Table S9. The genome sequence revealed a deletion of *zwf*, which encodes the first enzyme in the pentose phosphate pathway. Additionally the strain carried the thrAS357R and rhoR87L mutations. Furthermore the strain also had a truncation of the branched chain amino acid exporter brnQ (105 bp was deleted starting at location 419986. The 439 amino acid protein was truncated after 308

amino acids).

Table S9: Effect of Δzwf on production of serine

	Serine concentration in g/L (+/- standard diviation)	Yield from glucose (g serine/ g glucose)
ΔsdaA ΔsdaB ΔtdcG ΔglyA (Q1)	0.925 (+/- 0.048)	0.37
ΔsdaA ΔsdaB ΔtdcG ΔglyA Δzwf Δbrnq thrA S357R rho R87L	1.323 (+/- 0.06)	0.53

Example 9 - Production by ALE 8

[0256] To use pET vectors as expression system, a DE3 cassette containing T7 polymerase was integrated into the genome of the ALE 8-8 mutant (Example 5) using a Lambda DE3 lysogenization kit (Millipore, Damstadt Germany) resulting in the strain ALE 8-8(DE3). Subsequently, the ALE 8-8(DE3) was made electrocompetent by growing cells in 30 ml 2xYT media supplemented with 0.2 % glucose (37°C, 250 rpm) to mid log phase. Cells were harvested by centrifugation at 6500 rpm for 5 min at 4°C, and were washed twice by ice cold 10 % glycerol. Cells were finally resuspended in 500 μL of 10 % glycerol and 50 μL of cells were transformed with plasmids pCDF-Duet1-*serAmut-serC* and pACYC-*serB* for serine production.

[0257] Serine production was demonstrated during fed batch fermentation in 1L fermenters (Sartorius, Gottingen, Germany) as mentioned in example 7. The media contained 2g/L MGSO4*7H2O, 2 g/L KH2PO4, 5 g/L (NH4)2SO4, 10 g/L Glucose, 2 g/L Yeast extract, 0.6 g/L glycine, 4X trace elements (as mentioned in Example 2), 50 mg/L spectinomycin and 25 mg/L chloramphenicol. The 375 g of feed contained 400 g/L glucose, 24 g/L Ammonium sulfate, 2g/L glycine, 2.5 g/L of MGSO4*7H2O and 5 g/L of KH2PO4, 1X trace elements 50 mg/L spectinomycin and 25 mg/L chloramphenicol. A log phase culture was used to inoculate 500 ml media by 1:50 inoculum ratio. The cultures were allowed to grow overnight in the fermenter at 37°C, 1000 rpm baffle speed and 20 % air saturation. Production was induced at late log phase (O.D. 8.5 to 9.5) by the addition of 80 μM IPTG and feed at the rate of 8 g/h was started. Samples were taken at regular intervals and were subjected to HPLC and LCMS analysis as mentioned before.

[0258] The experiment shows that both a high cell density and a high serine titer (55.0 g/L) can be obtained in ALE 8-8 (DE3) strain (Figure 10A) with a mass yield from glucose of 36.0% (Figure 10B).

Example 10 - Over expression and site saturation mutagenesis of thrA at site Y356, S357 and S359

[0259] Using site saturation mutagenesis (SSM), the selected amino acid residue/s can be mutated to all other 20 amino acids. This makes it possible to investigate the effect of amino acid substitutions on the enzyme activity and in turn on phenotype. The *thrA* gene was amplified from MG-1655 wt strain using primers *thrA_NcoI_F* and *thrA_HindIII* (primer sequences in Table S10) and cloned in pACYC-Duet1 plasmid using NcoI and HindIII enzyme. The protocol of cloning was same as for cloning *serB* in example 2. The vector constructed was pACYC-*thrA*. This plasmid was used as a template for SSM. SSM for observed *thrA* mutations in ALE strains (Y356, S357 and S359) was performed using primers given in Table S10. The SSM protocol was similar to SDM protocol applied in Example 2 for mutagenesis of feedback insensitive *serA*. PCR product was DpnI digested and transformed in Q1 (DE3) strain, which carries the wild type *thrA* gene in its genome, and plated on LB-chloramphenicol plates supplemented with 0.1 % glucose and 4 mM glycine. Chloramphenicol was supplemented in all following media for plasmid stability.

[0260] Individual colonies were picked and inoculated into 96 well MTP plate containing 2x TY media supplemented with 0.1 % glucose and 2 mM Glycine. 90 clones (1 microtiter plate (MTP)) from each SSM library was picked (in total 3 plates). The Q1(DE3) strain harboring pACYC-Duet empty vector and pACYC-*thrAwt* was inoculated as a control strain in each plate. The plate was incubated at 37 °C and 300 rpm overnight. This pre-culture plate was used as inoculum from which 1 μL was added to a new 96 well MTP plate containing 100 μL of M9 media supplemented with 0.2% glucose, 2 mM glycine. The culture was incubated for 4 h after which 25 uL of 1 mM IPTG was added to induce the expression. The plate was subsequently incubated for 2 h and then M9 media containing 0.2 % glucose, 2mM

glycine and 12.5 g/L L-serine was added thus reaching final serine concentration of 6.25 g/L. The plate was then incubated with shaking in an MTP reader for growth analysis as described before. Selected clones showing tolerance from each plate were collected in 1 plate and the tolerance studies were repeated for these strains. The plasmids from the tolerant cloned were sequenced and the growth rates were estimated from the log phase of culture. This way, a number of amino acid substitutions that result in tolerance towards serine were identified (Figure 11). Figure 11 (A-C) shows the growth rate of mutant strains having different amino acid substitutions observed at positions 356, 357 and 359 of ThrA, respectively (denoted by the respective one-letter code). The growth rate of *E. coli* carrying the wild type *thrA* gene is denoted "wt".

[0261] The experiment demonstrates that amino acid substitutions at position 356, 357 and/or 359 in the native ThrA protein confer increased tolerance of modified strains towards L-serine. The data further demonstrates that overexpression of mutants of ThrA can increase the tolerance towards L-serine even in strains having a native *thrA* gene present in their genome.

Table S10: Primers used for cloning and site saturation mutagenesis (SSM) of *thrA*.

thrA_NcoI_F	GCGCCATGGGAGTGTTGAAGTTCGGCG
thrA_HindIII_R	GCCAAGCTTTCAGACTCCTAACTTCCATGAGAGGG
thrA_Y356NNK_F	CGCAGAAACTGATGCTMNNTTCGGAAGATGATTGCG
thrA_Y356NNK_R	CGCAGAAACTGATGCTMNNTTCGGAAGATGATTGCG
thrA_S357NNK_F	CAATCATCTTCCGAATACNNKATCAGTTTCTGCGTTCC
thrA_S357NNK_R	GGAACGCAGAAACTGATMNNGTATTTCGGAAGATGATTG
thrA_S359NNK_F	CCGAATACAGCATCNNKTTCTGCGTTCCAC
thrA_S359NNK_R	GTGGAACGCAGAAMNNGATGCTGTATTCCG

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SEQUENCE LISTING

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Glu	Ala	Asn	Ala	Lys	Ala	His	Arg	Gly	Val	Trp	Asn	Lys	Leu	Ala	Ala																																																
			130																135																140																												
Gly	Ser	Phe	Glu	Ala	Arg	Gly	Lys	Lys	Leu	Gly	Ile	Ile	Gly	Tyr	Gly																																																
			145																150																155																160												
His	Ile	Gly	Thr	Gln	Leu	Gly	Ile	Leu	Ala	Glu	Ser	Leu	Gly	Met	Tyr																																																
				165																170																175																											
Val	Tyr	Phe	Tyr	Asp	Ile	Glu	Asn	Lys	Leu	Pro	Leu	Gly	Asn	Ala	Thr																																																
			180																185																190																												
Gln	Val	Gln	His	Leu	Ser	Asp	Leu	Leu	Asn	Met	Ser	Asp	Val	Val	Ser																																																
			195																200																205																												
Leu	His	Val	Pro	Glu	Asn	Pro	Ser	Thr	Lys	Asn	Met	Met	Gly	Ala	Lys																																																
			210																215																220																												
Glu	Ile	Ser	Leu	Met	Lys	Pro	Gly	Ser	Leu	Leu	Ile	Asn	Ala	Ser	Arg																																																
			225																230																235																240												
Gly	Thr	Val	Val	Asp	Ile	Pro	Ala	Leu	Cys	Asp	Ala	Leu	Ala	Ser	Lys																																																
				245																250																255																											
His	Leu	Ala	Gly	Ala	Ala	Ile	Asp	Val	Phe	Pro	Thr	Glu	Pro	Ala	Thr																																																
			260																265																270																												
Asn	Ser	Asp	Pro	Phe	Thr	Ser	Pro	Leu	Cys	Glu	Phe	Asp	Asn	Val	Leu																																																
			275																280																285																												
Leu	Thr	Pro	His	Ile	Gly	Gly	Ser	Thr	Gln	Glu	Ala	Gln	Glu	Asn	Ile																																																
			290																295																300																												
Gly	Leu	Glu	Val	Ala	Gly	Lys	Leu	Ile	Lys	Tyr	Ser	Asp	Asn	Gly	Ser																																																
			305																310																315																320												
Thr	Leu	Ser	Ala	Val	Asn	Phe	Pro	Glu	Val	Ser	Leu	Pro	Leu	His	Gly																																																
				325																330																335																											
Gly	Arg	Arg	Leu	Met	His	Ile	His	Glu	Asn	Arg	Pro	Gly	Val	Leu	Thr																																																
			340																345																350																												
Ala	Leu	Asn	Lys	Ile	Phe	Ala	Glu	Gln	Gly	Val	Asn	Ile	Ala	Ala	Gln																																																
			355																360																365																												
Tyr	Leu	Gln	Thr	Ser	Ala	Gln	Met	Gly	Tyr	Val	Val	Ile	Asp	Ile	Glu																																																
			370																375																380																												
Ala	Asp	Glu	Asp	Val	Ala	Glu	Lys	Ala	Leu	Gln	Ala	Met	Lys	Ala	Ile																																																
			385																390																395																400												
Pro	Gly	Thr	Ile	Arg	Ala	Arg	Leu	Leu	Tyr																																																						
				405																410																																											

<210> 6

$\langle 211 \rangle$ 410

<212> PRT

<213> Artificial Sequence

<220>

<223> Mutant SerA

<400> 6

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Met Ala Lys Val Ser Leu Glu Lys Asp Lys Ile Lys Phe Leu Leu Val
1          5          10          15

Glu Gly Val His Gln Lys Ala Leu Glu Ser Leu Arg Ala Ala Gly Tyr
20          25          30

Thr Asn Ile Glu Phe His Lys Gly Ala Leu Asp Asp Glu Gln Leu Lys
35          40          45

Glu Ser Ile Arg Asp Ala His Phe Ile Gly Leu Arg Ser Arg Thr His
50          55          60

Leu Thr Glu Asp Val Ile Asn Ala Ala Glu Lys Leu Val Ala Ile Gly
65          70          75          80

Cys Phe Cys Ile Gly Thr Asn Gln Val Asp Leu Asp Ala Ala Ala Lys
85          90          95

Arg Gly Ile Pro Val Phe Asn Ala Pro Phe Ser Asn Thr Arg Ser Val
100         105         110

Ala Glu Leu Val Ile Gly Glu Leu Leu Leu Leu Leu Arg Gly Val Pro
115         120         125

Glu Ala Asn Ala Lys Ala His Arg Gly Val Trp Asn Lys Leu Ala Ala
130         135         140

Gly Ser Phe Glu Ala Arg Gly Lys Lys Leu Gly Ile Ile Gly Tyr Gly
145         150         155         160

His Ile Gly Thr Gln Leu Gly Ile Leu Ala Glu Ser Leu Gly Met Tyr
165         170         175

Val Tyr Phe Tyr Asp Ile Glu Asn Lys Leu Pro Leu Gly Asn Ala Thr
180         185         190

Gln Val Gln His Leu Ser Asp Leu Leu Asn Met Ser Asp Val Val Ser
195         200         205

Leu His Val Pro Glu Asn Pro Ser Thr Lys Asn Met Met Gly Ala Lys
210         215         220

Glu Ile Ser Leu Met Lys Pro Gly Ser Leu Leu Ile Asn Ala Ser Arg
225         230         235         240

Gly Thr Val Val Asp Ile Pro Ala Leu Cys Asp Ala Leu Ala Ser Lys
245         250         255

His Leu Ala Gly Ala Ala Ile Asp Val Phe Pro Thr Glu Pro Ala Thr
260         265         270

Asn Ser Asp Pro Phe Thr Ser Pro Leu Cys Glu Phe Asp Asn Val Leu
275         280         285

Leu Thr Pro His Ile Gly Gly Ser Thr Gln Glu Ala Gln Glu Asn Ile
290         295         300

Gly Leu Glu Val Ala Gly Lys Leu Ile Lys Tyr Ser Asp Asn Gly Ser
305         310         315         320

Thr Leu Ser Ala Val Asn Phe Pro Glu Val Ser Leu Pro Leu His Gly
325         330         335

Gly Arg Arg Leu Met His Ile Ala Glu Ala Arg Pro Gly Val Leu Thr
340         345         350

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Ala Leu Asn Lys Ile Phe Ala Glu Gln Gly Val Ala Ile Ala Ala Gln
 355 360 365

Tyr Leu Gln Thr Ser Ala Gln Met Gly Tyr Val Val Ile Asp Ile Glu
 370 375 380

Ala Asp Glu Asp Val Ala Glu Lys Ala Leu Gln Ala Met Lys Ala Ile
 385 390 395 400

Pro Gly Thr Ile Arg Ala Arg Leu Leu Tyr
 405 410

<210> 7

<211> 322

<212> PRT

<213> Escherichia coli

<400> 7

Met Pro Asn Ile Thr Trp Cys Asp Leu Pro Glu Asp Val Ser Leu Trp
 1 5 10 15

Pro Gly Leu Pro Leu Ser Leu Ser Gly Asp Glu Val Met Pro Leu Asp
 20 25 30

Tyr His Ala Gly Arg Ser Gly Trp Leu Leu Tyr Gly Arg Gly Leu Asp
 35 40 45

Lys Gln Arg Leu Thr Gln Tyr Gln Ser Lys Leu Gly Ala Ala Met Val
 50 55 60

Ile Val Ala Ala Trp Cys Val Glu Asp Tyr Gln Val Ile Arg Leu Ala
 65 70 75 80

Gly Ser Leu Thr Ala Arg Ala Thr Arg Leu Ala His Glu Ala Gln Leu
 85 90 95

Asp Val Ala Pro Leu Gly Lys Ile Pro His Leu Arg Thr Pro Gly Leu
 100 105 110

Leu Val Met Asp Met Asp Ser Thr Ala Ile Gln Ile Glu Cys Ile Asp
 115 120 125

Glu Ile Ala Lys Leu Ala Gly Thr Gly Glu Met Val Ala Glu Val Thr
 130 135 140

Glu Arg Ala Met Arg Gly Glu Leu Asp Phe Thr Ala Ser Leu Arg Ser
 145 150 155 160

Arg Val Ala Thr Leu Lys Gly Ala Asp Ala Asn Ile Leu Gln Gln Val
 165 170 175

Arg Glu Asn Leu Pro Leu Met Pro Gly Leu Thr Gln Leu Val Leu Lys
 180 185 190

Leu Glu Thr Leu Gly Trp Lys Val Ala Ile Ala Ser Gly Gly Phe Thr
 195 200 205

Phe Phe Ala Glu Tyr Leu Arg Asp Lys Leu Arg Leu Thr Ala Val Val
 210 215 220

Ala Asn Glu Leu Glu Ile Met Asp Gly Lys Phe Thr Gly Asn Val Ile
 225 230 235 240

Gly Asp Ile Val Asp Ala Gln Tyr Lys Ala Lys Thr Leu Thr Arg Leu
 245 250 255

Ala Gln Glu Tyr Glu Ile Pro Leu Ala Gln Thr Val Ala Ile Gly Asp
 260 265 270

Gly Ala Asn Asp Leu Pro Met Ile Lys Ala Ala Gly Leu Gly Ile Ala
275 280 285

Tyr His Ala Lys Pro Lys Val Asn Glu Lys Ala Glu Val Thr Ile Arg
290 295 300

His Ala Asp Leu Met Gly Val Phe Cys Ile Leu Ser Gly Ser Leu Asn
305 310 315 320

Gln Lys

<210> 8

<211> 362

<212> PRT

<213> Escherichia coli

<400> 8

Met Ala Gln Ile Phe Asn Phe Ser Ser Gly Pro Ala Met Leu Pro Ala
1 5 10 15

Glu Val Leu Lys Gln Ala Gln Gln Glu Leu Arg Asp Trp Asn Gly Leu
20 25 30

Gly Thr Ser Val Met Glu Val Ser His Arg Gly Lys Glu Phe Ile Gln
35 40 45

Val Ala Glu Glu Ala Glu Lys Asp Phe Arg Asp Leu Leu Asn Val Pro
50 55 60

Ser Asn Tyr Lys Val Leu Phe Cys His Gly Gly Gly Arg Gly Gln Phe
65 70 75 80

Ala Ala Val Pro Leu Asn Ile Leu Gly Asp Lys Thr Thr Ala Asp Tyr
85 90 95

Val Asp Ala Gly Tyr Trp Ala Ala Ser Ala Ile Lys Glu Ala Lys Lys
100 105 110

Tyr Cys Thr Pro Asn Val Phe Asp Ala Lys Val Thr Val Asp Gly Leu
115 120 125

Arg Ala Val Lys Pro Met Arg Glu Trp Gln Leu Ser Asp Asn Ala Ala
130 135 140

Tyr Met His Tyr Cys Pro Asn Glu Thr Ile Asp Gly Ile Ala Ile Asp
145 150 155 160

Glu Thr Pro Asp Phe Gly Ala Asp Val Val Val Ala Ala Asp Phe Ser
165 170 175

Ser Thr Ile Leu Ser Arg Pro Ile Asp Val Ser Arg Tyr Gly Val Ile
180 185 190

Tyr Ala Gly Ala Gln Lys Asn Ile Gly Pro Ala Gly Leu Thr Ile Val
195 200 205

Ile Val Arg Glu Asp Leu Leu Gly Lys Ala Asn Ile Ala Cys Pro Ser
210 215 220

Ile Leu Asp Tyr Ser Ile Leu Asn Asp Asn Gly Ser Met Phe Asn Thr
225 230 235 240

Pro Pro Thr Phe Ala Trp Tyr Leu Ser Gly Leu Val Phe Lys Trp Leu
245 250 255

Lys Ala Asn Gly Gly Val Ala Glu Met Asp Lys Ile Asn Gln Gln Lys
260 265 270

Ala Glu Leu Leu Tyr Gly Val Ile Asp Asn Ser Asp Phe Tyr Arg Asn

275 280 285
 Asp Val Ala Lys Ala Asn Arg Ser Arg Met Asn Val Pro Phe Gln Leu
 290 295 300
 Ala Asp Ser Ala Leu Asp Lys Leu Phe Leu Glu Glu Ser Phe Ala Ala
 305 310 315 320
 Gly Leu His Ala Leu Lys Gly His Arg Val Val Gly Gly Met Arg Ala
 325 330 335
 Ser Ile Tyr Asn Ala Met Pro Leu Glu Gly Val Lys Ala Leu Thr Asp
 340 345 350
 Phe Met Val Glu Phe Glu Arg Arg His Gly
 355 360
 <210> 9
 <211> 299
 <212> PRT
 <213> Escherichia coli
 <400> 9
 Met Ser Arg Lys Asp Gly Val Leu Ala Leu Leu Val Val Val Val Trp
 1 5 10 15
 Gly Leu Asn Phe Val Val Ile Lys Val Gly Leu His Asn Met Pro Pro
 20 25 30
 Leu Met Leu Ala Gly Leu Arg Phe Met Leu Val Ala Phe Pro Ala Ile
 35 40 45
 Phe Phe Val Ala Arg Pro Lys Val Pro Leu Asn Leu Leu Leu Gly Tyr
 50 55 60
 Gly Leu Thr Ile Ser Phe Ala Gln Phe Ala Phe Leu Phe Cys Ala Ile
 65 70 75 80
 Asn Phe Gly Met Pro Ala Gly Leu Ala Ser Leu Val Leu Gln Ala Gln
 85 90 95
 Ala Phe Phe Thr Ile Met Leu Gly Ala Phe Thr Phe Gly Glu Arg Leu
 100 105 110
 His Gly Lys Gln Leu Ala Gly Ile Ala Leu Ala Ile Phe Gly Val Leu
 115 120 125
 Val Leu Ile Glu Asp Ser Leu Asn Gly Gln His Val Ala Met Leu Gly
 130 135 140
 Phe Met Leu Thr Leu Ala Ala Ala Phe Ser Trp Ala Cys Gly Asn Ile
 145 150 155 160
 Phe Asn Lys Lys Ile Met Ser His Ser Thr Arg Pro Ala Val Met Ser
 165 170 175
 Leu Val Ile Trp Ser Ala Leu Ile Pro Ile Ile Pro Phe Phe Val Ala
 180 185 190
 Ser Leu Ile Leu Asp Gly Ser Ala Thr Met Ile His Ser Leu Val Thr
 195 200 205
 Ile Asp Met Thr Thr Ile Leu Ser Leu Met Tyr Leu Ala Phe Val Ala
 210 215 220
 Thr Ile Val Gly Tyr Gly Ile Trp Gly Thr Leu Leu Gly Arg Tyr Glu
 225 230 235 240
 Thr Trp Arg Val Ala Pro Leu Ser Leu Leu Val Pro Val Val Gly Leu
 245 250 255

Ala Ser Ala Ala Leu Leu Leu Asp Glu Arg Leu Thr Gly Leu Gln Phe
260 265 270

Leu Gly Ala Val Leu Ile Met Thr Gly Leu Tyr Ile Asn Val Phe Gly
275 280 285

Leu Arg Trp Arg Lys Ala Val Lys Val Gly Ser
290 295

<210> 10

<211> 305

<212> PRT

<213> Artificial Sequence

<220>

<223> YdeD containing His-tag at C-terminus

<400> 10

Met Ser Arg Lys Asp Gly Val Leu Ala Leu Leu Val Val Val Val Trp
1 5 10 15

Gly Leu Asn Phe Val Val Ile Lys Val Gly Leu His Asn Met Pro Pro
20 25 30

Leu Met Leu Ala Gly Leu Arg Phe Met Leu Val Ala Phe Pro Ala Ile
35 40 45

Phe Phe Val Ala Arg Pro Lys Val Pro Leu Asn Leu Leu Leu Gly Tyr
50 55 60

Gly Leu Thr Ile Ser Phe Ala Gln Phe Ala Phe Leu Phe Cys Ala Ile
65 70 75 80

Asn Phe Gly Met Pro Ala Gly Leu Ala Ser Leu Val Leu Gln Ala Gln
85 90 95

Ala Phe Phe Thr Ile Met Leu Gly Ala Phe Thr Phe Gly Glu Arg Leu
100 105 110

His Gly Lys Gln Leu Ala Gly Ile Ala Leu Ala Ile Phe Gly Val Leu
115 120 125

Val Leu Ile Glu Asp Ser Leu Asn Gly Gln His Val Ala Met Leu Gly
130 135 140

Phe Met Leu Thr Leu Ala Ala Ala Phe Ser Trp Ala Cys Gly Asn Ile
145 150 155 160

Phe Asn Lys Lys Ile Met Ser His Ser Thr Arg Pro Ala Val Met Ser
165 170 175

Leu Val Ile Trp Ser Ala Leu Ile Pro Ile Ile Pro Phe Phe Val Ala
180 185 190

Ser Leu Ile Leu Asp Gly Ser Ala Thr Met Ile His Ser Leu Val Thr
195 200 205

Ile Asp Met Thr Thr Ile Leu Ser Leu Met Tyr Leu Ala Phe Val Ala
210 215 220

Thr Ile Val Gly Tyr Gly Ile Trp Gly Thr Leu Leu Gly Arg Tyr Glu
225 230 235 240

Thr Trp Arg Val Ala Pro Leu Ser Leu Leu Val Pro Val Val Gly Leu
245 250 255

Ala Ser Ala Ala Leu Leu Leu Asp Glu Arg Leu Thr Gly Leu Gln Phe
260 265 270

Leu Gly Ala Val Leu Ile Met Thr Gly Leu Tyr Ile Asn Val Phe Gly
 275 280 285

Leu Arg Trp Arg Lys Ala Val Lys Val Gly Ser His His His His His
 290 295 300

His
 305

<210> 11
 <211> 820
 <212> PRT
 <213> Escherichia coli

<400> 11
 Met Arg Val Leu Lys Phe Gly Gly Thr Ser Val Ala Asn Ala Glu Arg
 1 5 10 15

Phe Leu Arg Val Ala Asp Ile Leu Glu Ser Asn Ala Arg Gln Gly Gln
 20 25 30

Val Ala Thr Val Leu Ser Ala Pro Ala Lys Ile Thr Asn His Leu Val
 35 40 45

Ala Met Ile Glu Lys Thr Ile Ser Gly Gln Asp Ala Leu Pro Asn Ile
 50 55 60

Ser Asp Ala Glu Arg Ile Phe Ala Glu Leu Leu Thr Gly Leu Ala Ala
 65 70 75 80

Ala Gln Pro Gly Phe Pro Leu Ala Gln Leu Lys Thr Phe Val Asp Gln
 85 90 95

Glu Phe Ala Gln Ile Lys His Val Leu His Gly Ile Ser Leu Leu Gly
 100 105 110

Gln Cys Pro Asp Ser Ile Asn Ala Ala Leu Ile Cys Arg Gly Glu Lys
 115 120 125

Met Ser Ile Ala Ile Met Ala Gly Val Leu Glu Ala Arg Gly His Asn
 130 135 140

Val Thr Val Ile Asp Pro Val Glu Lys Leu Leu Ala Val Gly His Tyr
 145 150 155 160

Leu Glu Ser Thr Val Asp Ile Ala Glu Ser Thr Arg Arg Ile Ala Ala
 165 170 175

Ser Arg Ile Pro Ala Asp His Met Val Leu Met Ala Gly Phe Thr Ala
 180 185 190

Gly Asn Glu Lys Gly Glu Leu Val Val Leu Gly Arg Asn Gly Ser Asp
 195 200 205

Tyr Ser Ala Ala Val Leu Ala Ala Cys Leu Arg Ala Asp Cys Cys Glu
 210 215 220

Ile Trp Thr Asp Val Asp Gly Val Tyr Thr Cys Asp Pro Arg Gln Val
 225 230 235 240

Pro Asp Ala Arg Leu Leu Lys Ser Met Ser Tyr Gln Glu Ala Met Glu
 245 250 255

Leu Ser Tyr Phe Gly Ala Lys Val Leu His Pro Arg Thr Ile Thr Pro
 260 265 270

Ile Ala Gln Phe Gln Ile Pro Cys Leu Ile Lys Asn Thr Gly Asn Pro
 275 280 285

Gln Ala Pro Gly Thr Leu Ile Gly Ala Ser Arg Asp Glu Asp Glu Leu
 290 295 300
 Pro Val Lys Gly Ile Ser Asn Leu Asn Asn Met Ala Met Phe Ser Val
 305 310 315 320
 Ser Gly Pro Gly Met Lys Gly Met Val Gly Met Ala Ala Arg Val Phe
 325 330 335
 Ala Ala Met Ser Arg Ala Arg Ile Ser Val Val Leu Ile Thr Gln Ser
 340 345 350
 Ser Ser Glu Tyr Ser Ile Ser Phe Cys Val Pro Gln Ser Asp Cys Val
 355 360 365
 Arg Ala Glu Arg Ala Met Gln Glu Glu Phe Tyr Leu Glu Leu Lys Glu
 370 375 380
 Gly Leu Leu Glu Pro Leu Ala Val Thr Glu Arg Leu Ala Ile Ile Ser
 385 390 395 400
 Val Val Gly Asp Gly Met Arg Thr Leu Arg Gly Ile Ser Ala Lys Phe
 405 410 415
 Phe Ala Ala Leu Ala Arg Ala Asn Ile Asn Ile Val Ala Ile Ala Gln
 420 425 430
 Gly Ser Ser Glu Arg Ser Ile Ser Val Val Val Asn Asn Asp Asp Ala
 435 440 445
 Thr Thr Gly Val Arg Val Thr His Gln Met Leu Phe Asn Thr Asp Gln
 450 455 460
 Val Ile Glu Val Phe Val Ile Gly Val Gly Gly Val Gly Gly Ala Leu
 465 470 475 480
 Leu Glu Gln Leu Lys Arg Gln Gln Ser Trp Leu Lys Asn Lys His Ile
 485 490 495
 Asp Leu Arg Val Cys Gly Val Ala Asn Ser Lys Ala Leu Leu Thr Asn
 500 505 510
 Val His Gly Leu Asn Leu Glu Asn Trp Gln Glu Glu Leu Ala Gln Ala
 515 520 525
 Lys Glu Pro Phe Asn Leu Gly Arg Leu Ile Arg Leu Val Lys Glu Tyr
 530 535 540
 His Leu Leu Asn Pro Val Ile Val Asp Cys Thr Ser Ser Gln Ala Val
 545 550 555 560
 Ala Asp Gln Tyr Ala Asp Phe Leu Arg Glu Gly Phe His Val Val Thr
 565 570 575
 Pro Asn Lys Lys Ala Asn Thr Ser Ser Met Asp Tyr Tyr His Gln Leu
 580 585 590
 Arg Tyr Ala Ala Glu Lys Ser Arg Arg Lys Phe Leu Tyr Asp Thr Asn
 595 600 605
 Val Gly Ala Gly Leu Pro Val Ile Glu Asn Leu Gln Asn Leu Leu Asn
 610 615 620
 Ala Gly Asp Glu Leu Met Lys Phe Ser Gly Ile Leu Ser Gly Ser Leu
 625 630 635 640
 Ser Tyr Ile Phe Gly Lys Leu Asp Glu Gly Met Ser Phe Ser Glu Ala
 645 650 655
 Thr Thr Leu Ala Arg Glu Met Gly Tyr Thr Glu Pro Asp Pro Arg Asp
 660 665 670

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Asp Leu Ser Gly Met Asp Val Ala Arg Lys Leu Leu Ile Leu Ala Arg
675                      680                      685

Glu Thr Gly Arg Glu Leu Glu Leu Ala Asp Ile Glu Ile Glu Pro Val
690                      695                      700

Leu Pro Ala Glu Phe Asn Ala Glu Gly Asp Val Ala Ala Phe Met Ala
705                      710                      715                      720

Asn Leu Ser Gln Leu Asp Asp Leu Phe Ala Ala Arg Val Ala Lys Ala
725                      730                      735

Arg Asp Glu Gly Lys Val Leu Arg Tyr Val Gly Asn Ile Asp Glu Asp
740                      745                      750

Gly Val Cys Arg Val Lys Ile Ala Glu Val Asp Gly Asn Asp Pro Leu
755                      760                      765

Phe Lys Val Lys Asn Gly Glu Asn Ala Leu Ala Phe Tyr Ser His Tyr
770                      775                      780

Tyr Gln Pro Leu Pro Leu Val Leu Arg Gly Tyr Gly Ala Gly Asn Asp
785                      790                      795                      800

Val Thr Ala Ala Gly Val Phe Ala Asp Leu Leu Arg Thr Leu Ser Trp
805                      810                      815

Lys Leu Gly Val
820

<210> 12
<211> 164
<212> PRT
<213> Escherichia coli

<400> 12
Met Val Asp Ser Lys Lys Arg Pro Gly Lys Asp Leu Asp Arg Ile Asp
1                      5                      10                      15

Arg Asn Ile Leu Asn Glu Leu Gln Lys Asp Gly Arg Ile Ser Asn Val
20                      25                      30

Glu Leu Ser Lys Arg Val Gly Leu Ser Pro Thr Pro Cys Leu Glu Arg
35                      40                      45

Val Arg Arg Leu Glu Arg Gln Gly Phe Ile Gln Gly Tyr Thr Ala Leu
50                      55                      60

Leu Asn Pro His Tyr Leu Asp Ala Ser Leu Leu Val Phe Val Glu Ile
65                      70                      75                      80

Thr Leu Asn Arg Gly Ala Pro Asp Val Phe Glu Gln Phe Asn Thr Ala
85                      90                      95

Val Gln Lys Leu Glu Glu Ile Gln Glu Cys His Leu Val Ser Gly Asp
100                     105                     110

Phe Asp Tyr Leu Leu Lys Thr Arg Val Pro Asp Met Ser Ala Tyr Arg
115                     120                     125

Lys Leu Leu Gly Glu Thr Leu Leu Arg Leu Pro Gly Val Asn Asp Thr
130                     135                     140

Arg Thr Tyr Val Val Met Glu Glu Val Lys Gln Ser Asn Arg Leu Val
145                     150                     155                     160

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Ile Lys Thr Arg

<210> 13

<211> 419

<212> PRT

<213> Escherichia coli

<400> 13

Met Asn Leu Thr Glu Leu Lys Asn Thr Pro Val Ser Glu Leu Ile Thr
1 5 10 15

Leu Gly Glu Asn Met Gly Leu Glu Asn Leu Ala Arg Met Arg Lys Gln
20 25 30

Asp Ile Ile Phe Ala Ile Leu Lys Gln His Ala Lys Ser Gly Glu Asp
35 40 45

Ile Phe Gly Asp Gly Val Leu Glu Ile Leu Gln Asp Gly Phe Gly Phe
50 55 60

Leu Arg Ser Ala Asp Ser Ser Tyr Leu Ala Gly Pro Asp Asp Ile Tyr
65 70 75 80

Val Ser Pro Ser Gln Ile Arg Arg Phe Asn Leu Arg Thr Gly Asp Thr
85 90 95

Ile Ser Gly Lys Ile Arg Pro Pro Lys Glu Gly Glu Arg Tyr Phe Ala
100 105 110

Leu Leu Lys Val Asn Glu Val Asn Phe Asp Lys Pro Glu Asn Ala Arg
115 120 125

Asn Lys Ile Leu Phe Glu Asn Leu Thr Pro Leu His Ala Asn Ser Arg
130 135 140

Leu Arg Met Glu Arg Gly Asn Gly Ser Thr Glu Asp Leu Thr Ala Arg
145 150 155 160

Val Leu Asp Leu Ala Ser Pro Ile Gly Arg Gly Gln Arg Gly Leu Ile
165 170 175

Val Ala Pro Pro Lys Ala Gly Lys Thr Met Leu Leu Gln Asn Ile Ala
180 185 190

Gln Ser Ile Ala Tyr Asn His Pro Asp Cys Val Leu Met Val Leu Leu

195 200 205

Ile Asp Glu Arg Pro Glu Glu Val Thr Glu Met Gln Arg Leu Val Lys
210 215 220

Gly Glu Val Val Ala Ser Thr Phe Asp Glu Pro Ala Ser Arg His Val
225 230 235 240

Gln Val Ala Glu Met Val Ile Glu Lys Ala Lys Arg Leu Val Glu His
245 250 255

Lys Lys Asp Val Ile Ile Leu Leu Asp Ser Ile Thr Arg Leu Ala Arg
260 265 270

Ala Tyr Asn Thr Val Val Pro Ala Ser Gly Lys Val Leu Thr Gly Gly
275 280 285

Val Asp Ala Asn Ala Leu His Arg Pro Lys Arg Phe Phe Gly Ala Ala
290 295 300

Arg Asn Val Glu Glu Gly Gly Ser Leu Thr Ile Ile Ala Thr Ala Leu
305 310 315 320 325

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305              310              315              320
Ile Asp Thr Gly Ser Lys Met Asp Glu Val Ile Tyr Glu Glu Phe Lys
          325          330          335

Gly Thr Gly Asn Met Glu Leu His Leu Ser Arg Lys Ile Ala Glu Lys
          340          345          350

Arg Val Phe Pro Ala Ile Asp Tyr Asn Arg Ser Gly Thr Arg Lys Glu
          355          360          365

Glu Leu Leu Thr Thr Gln Glu Glu Leu Gln Lys Met Trp Ile Leu Arg
          370          375          380

Lys Ile Ile His Pro Met Gly Glu Ile Asp Ala Met Glu Phe Leu Ile
385          390          395          400

Asn Lys Leu Ala Met Thr Lys Thr Asn Asp Asp Phe Phe Glu Met Met
          405          410          415

Lys Arg Ser

<210> 14
<211> 432
<212> PRT
<213> Escherichia coli

<400> 14
Met Ser Lys Ile Val Lys Ile Ile Gly Arg Glu Ile Ile Asp Ser Arg
1          5          10          15

Gly Asn Pro Thr Val Glu Ala Glu Val His Leu Glu Gly Gly Phe Val
          20          25          30

Gly Met Ala Ala Ala Pro Ser Gly Ala Ser Thr Gly Ser Arg Glu Ala
          35          40          45

Leu Glu Leu Arg Asp Gly Asp Lys Ser Arg Phe Leu Gly Lys Gly Val
          50          55          60

Thr Lys Ala Val Ala Ala Val Asn Gly Pro Ile Ala Gln Ala Leu Ile
65          70          75          80

Gly Lys Asp Ala Lys Asp Gln Ala Gly Ile Asp Lys Ile Met Ile Asp
          85          90          95

Leu Asp Gly Thr Glu Asn Lys Ser Lys Phe Gly Ala Asn Ala Ile Leu
          100          105          110

Ala Val Ser Leu Ala Asn Ala Lys Ala Ala Ala Ala Lys Gly Met
          115          120          125

Pro Leu Tyr Glu His Ile Ala Glu Leu Asn Gly Thr Pro Gly Lys Tyr
          130          135          140

Ser Met Pro Val Pro Met Met Asn Ile Ile Asn Gly Gly Glu His Ala
145          150          155          160

Asp Asn Asn Val Asp Ile Gln Glu Phe Met Ile Gln Pro Val Gly Ala
          165          170          175

Lys Thr Val Lys Glu Ala Ile Arg Met Gly Ser Glu Val Phe His His
          180          185          190

Leu Ala Lys Val Leu Lys Ala Lys Gly Met Asn Thr Ala Val Gly Asp
          195          200          205

Glu Gly Gly Tyr Ala Pro Asn Leu Gly Ser Asn Ala Glu Ala Leu Ala
          210          215          220

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210      215      220
Val Ile Ala Glu Ala Val Lys Ala Ala Gly Tyr Glu Leu Gly Lys Asp
225      230      235      240

Ile Thr Leu Ala Met Asp Cys Ala Ala Ser Glu Phe Tyr Lys Asp Gly
245      250      255

Lys Tyr Val Leu Ala Gly Glu Gly Asn Lys Ala Phe Thr Ser Glu Glu
260      265      270

Phe Thr His Phe Leu Glu Glu Leu Thr Lys Gln Tyr Pro Ile Val Ser
275      280      285

Ile Glu Asp Gly Leu Asp Glu Ser Asp Trp Asp Gly Phe Ala Tyr Gln
290      295      300

Thr Lys Val Leu Gly Asp Lys Ile Gln Leu Val Gly Asp Asp Leu Phe
305      310      315      320

Val Thr Asn Thr Lys Ile Leu Lys Glu Gly Ile Glu Lys Gly Ile Ala
325      330      335

Asn Ser Ile Leu Ile Lys Phe Asn Gln Ile Gly Ser Leu Thr Glu Thr
340      345      350

Leu Ala Ala Ile Lys Met Ala Lys Asp Ala Gly Tyr Thr Ala Val Ile
355      360      365

Ser His Arg Ser Gly Glu Thr Glu Asp Ala Thr Ile Ala Asp Leu Ala
370      375      380

Val Gly Thr Ala Ala Gly Gln Ile Lys Thr Gly Ser Met Ser Arg Ser
385      390      395      400

Asp Arg Val Ala Lys Tyr Asn Gln Leu Ile Arg Ile Glu Glu Ala Leu
405      410      415

Gly Glu Lys Ala Pro Tyr Asn Gly Arg Lys Glu Ile Lys Gly Gln Ala
420      425      430

<210> 15
<211> 297
<212> PRT
<213> Escherichia coli

<400> 15
Met Lys Arg Pro Asp Tyr Arg Thr Leu Gln Ala Leu Asp Ala Val Ile
1      5      10      15

Arg Glu Arg Gly Phe Glu Arg Ala Ala Gln Lys Leu Cys Ile Thr Gln
20      25      30

Ser Ala Val Ser Gln Arg Ile Lys Gln Leu Glu Asn Met Phe Gly Gln
35      40      45

Pro Leu Leu Val Arg Thr Val Pro Pro Arg Pro Thr Glu Gln Gly Gln
50      55      60

Lys Leu Leu Ala Leu Leu Arg Gln Val Glu Leu Leu Glu Glu Glu Trp
65      70      75      80

Leu Gly Asp Glu Gln Thr Gly Ser Thr Pro Leu Leu Leu Ser Leu Ala
85      90      95

Val Asn Ala Asp Ser Leu Ala Thr Trp Leu Leu Pro Ala Leu Ala Pro
100     105     110

Val Leu Ala Asp Ser Pro Ile Arg Leu Asn Leu Gln Val Glu Asp Glu
115     120     125

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Thr Arg Thr Gln Glu Arg Leu Arg Arg Gly Glu Val Val Gly Ala Val
130 135 140

Ser Ile Gln His Gln Ala Leu Pro Ser Cys Leu Val Asp Lys Leu Gly
145 150 155 160

Ala Leu Asp Tyr Leu Phe Val Ser Ser Lys Pro Phe Ala Glu Lys Tyr
165 170 175

Phe Pro Asn Gly Val Thr Arg Ser Ala Leu Leu Lys Ala Pro Val Val
180 185 190

Ala Phe Asp His Leu Asp Asp Met His Gln Ala Phe Leu Gln Gln Asn
195 200 205

Phe Asp Leu Pro Pro Gly Ser Val Pro Cys His Ile Val Asn Ser Ser
210 215 220

Glu Ala Phe Val Gln Leu Ala Arg Gln Gly Thr Thr Cys Cys Met Ile
225 230 235 240

Pro His Leu Gln Ile Glu Lys Glu Leu Ala Ser Gly Glu Leu Ile Asp
245 250 255

Leu Thr Pro Gly Leu Phe Gln Arg Arg Met Leu Tyr Trp His Arg Phe
260 265 270

Ala Pro Glu Ser Arg Met Met Arg Lys Val Thr Asp Ala Leu Leu Asp
275 280 285

Tyr Gly His Lys Val Leu Arg Gln Asp
290 295

<210> 16

<211> 394

<212> PRT

<213> Escherichia coli

<400> 16

Met Ser Lys Glu Lys Phe Glu Arg Thr Lys Pro His Val Asn Val Gly
1 5 10 15

Thr Ile Gly His Val Asp His Gly Lys Thr Thr Leu Thr Ala Ala Ile
20 25 30

Thr Thr Val Leu Ala Lys Thr Tyr Gly Gly Ala Ala Arg Ala Phe Asp
35 40 45

Gln Ile Asp Asn Ala Pro Glu Glu Lys Ala Arg Gly Ile Thr Ile Asn
50 55 60

Thr Ser His Val Glu Tyr Asp Thr Pro Thr Arg His Tyr Ala His Val
65 70 75 80

Asp Cys Pro Gly His Ala Asp Tyr Val Lys Asn Met Ile Thr Gly Ala
85 90 95

Ala Gln Met Asp Gly Ala Ile Leu Val Val Ala Ala Thr Asp Gly Pro
100 105 110

Met Pro Gln Thr Arg Glu His Ile Leu Leu Gly Arg Gln Val Gly Val
115 120 125

Pro Tyr Ile Ile Val Phe Leu Asn Lys Cys Asp Met Val Asp Asp Glu
130 135 140

Glu Leu Leu Glu Leu Val Glu Met Glu Val Arg Glu Leu Leu Ser Gln
145 150 155 160

Tyr Asp Phe Pro Gly Asp Asp Thr Pro Ile Val Arg Gly Ser Ala Leu
165 170 175

Lys Ala Leu Glu Gly Asp Ala Glu Trp Glu Ala Lys Ile Leu Glu Leu
180 185 190

Ala Gly Phe Leu Asp Ser Tyr Ile Pro Glu Pro Glu Arg Ala Ile Asp
195 200 205

Lys Pro Phe Leu Leu Pro Ile Glu Asp Val Phe Ser Ile Ser Gly Arg
210 215 220

Gly Thr Val Val Thr Gly Arg Val Glu Arg Gly Ile Ile Lys Val Gly
225 230 235 240

Glu Glu Val Glu Ile Val Gly Ile Lys Glu Thr Gln Lys Ser Thr Cys
245 250 255

Thr Gly Val Glu Met Phe Arg Lys Leu Leu Asp Glu Gly Arg Ala Gly
260 265 270

Glu Asn Val Gly Val Leu Leu Arg Gly Ile Lys Arg Glu Glu Ile Glu
275 280 285

Arg Gly Gln Val Leu Ala Lys Pro Gly Thr Ile Lys Pro His Thr Lys
290 295 300

Phe Glu Ser Glu Val Tyr Ile Leu Ser Lys Asp Glu Gly Gly Arg His
305 310 315 320

Thr Pro Phe Phe Lys Gly Tyr Arg Pro Gln Phe Tyr Phe Arg Thr Thr
325 330 335

Asp Val Thr Gly Thr Ile Glu Leu Pro Glu Gly Val Glu Met Val Met
340 345 350

Pro Gly Asp Asn Ile Lys Met Val Val Thr Leu Ile His Pro Ile Ala
355 360 365

Met Asp Asp Gly Leu Arg Phe Ala Ile Arg Glu Gly Gly Arg Thr Val
370 375 380

Gly Ala Gly Val Val Ala Lys Val Leu Gly
385 390

<210> 17

<211> 470

<212> PRT

<213> Escherichia coli

<400> 17

Met Val Asp Gln Val Lys Val Val Ala Asp Asp Gln Ala Pro Ala Glu
1 5 10 15

Gln Ser Leu Arg Arg Asn Leu Thr Asn Arg His Ile Gln Leu Ile Ala
20 25 30

Ile Gly Gly Ala Ile Gly Thr Gly Leu Phe Met Gly Ser Gly Lys Thr
35 40 45

Ile Ser Leu Ala Gly Pro Ser Ile Ile Phe Val Tyr Met Ile Ile Gly
50 55 60

Phe Met Leu Phe Phe Val Met Arg Ala Met Gly Glu Leu Leu Leu Ser
65 70 75 80

Asn Leu Glu Tyr Lys Ser Phe Ser Asp Phe Ala Ser Asp Leu Leu Gly
85 90 95

Pro Trp Ala Gly Tyr Phe Thr Gly Trp Thr Tyr Trp Phe Cys Trp Val

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100 105 110
Val Thr Gly Met Ala Asp Val Val Ala Ile Thr Ala Tyr Ala Gln Phe
115 120 125
Trp Phe Pro Asp Leu Ser Asp Trp Val Ala Ser Leu Ala Val Ile Val
130 135 140
Leu Leu Leu Thr Leu Asn Leu Ala Thr Val Lys Met Phe Gly Glu Met
145 150 155 160
Glu Phe Trp Phe Ala Met Ile Lys Ile Val Ala Ile Val Ser Leu Ile
165 170 175
Val Val Gly Leu Val Met Val Ala Met His Phe Gln Ser Pro Thr Gly
180 185 190
Val Glu Ala Ser Phe Ala His Leu Trp Asn Asp Gly Gly Trp Phe Pro
195 200 205
Lys Gly Leu Ser Gly Phe Phe Ala Gly Phe Gln Ile Ala Val Phe Ala
210 215 220
Phe Val Gly Ile Glu Leu Val Gly Thr Thr Ala Ala Glu Thr Lys Asp
225 230 235 240
Pro Glu Lys Ser Leu Pro Arg Ala Ile Asn Ser Ile Pro Ile Arg Ile
245 250 255
Ile Met Phe Tyr Val Phe Ala Leu Ile Val Ile Met Ser Val Thr Pro
260 265 270
Trp Ser Ser Val Val Pro Glu Lys Ser Pro Phe Val Glu Leu Phe Val
275 280 285
Leu Val Gly Leu Pro Ala Ala Ala Ser Val Ile Asn Phe Val Val Leu
290 295 300
Thr Ser Ala Ala Ser Ser Ala Asn Ser Gly Val Phe Ser Thr Ser Arg
305 310 315 320
Met Leu Phe Gly Leu Ala Gln Glu Gly Val Ala Pro Lys Ala Phe Ala
325 330 335
Lys Leu Ser Lys Arg Ala Val Pro Ala Lys Gly Leu Thr Phe Ser Cys
340 345 350
Ile Cys Leu Leu Gly Gly Val Val Met Leu Tyr Val Asn Pro Ser Val
355 360 365
Ile Gly Ala Phe Thr Met Ile Thr Thr Val Ser Ala Ile Leu Phe Met
370 375 380
Phe Val Trp Thr Ile Ile Leu Cys Ser Tyr Leu Val Tyr Arg Lys Gln
385 390 395 400
Arg Pro His Leu His Glu Lys Ser Ile Tyr Lys Met Pro Leu Gly Lys
405 410 415
Leu Met Cys Trp Val Cys Met Ala Phe Phe Val Phe Val Val Val Leu
420 425 430
Leu Thr Leu Glu Asp Asp Thr Arg Gln Ala Leu Leu Val Thr Pro Leu
435 440 445
Trp Phe Ile Ala Leu Gly Leu Gly Trp Leu Phe Ile Gly Lys Lys Arg
450 455 460
Ala Ala Glu Leu Arg Lys
465 470

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<210> 18

<211> 225

<212> PRT

<213> Escherichia coli

<400> 18

Met Lys Gln Tyr Leu Ile Ala Pro Ser Ile Leu Ser Ala Asp Phe Ala
1 5 10 15

Arg Leu Gly Glu Asp Thr Ala Lys Ala Leu Ala Ala Gly Ala Asp Val
20 25 30

Val His Phe Asp Val Met Asp Asn His Tyr Val Pro Asn Leu Thr Ile
35 40 45

Gly Pro Met Val Leu Lys Ser Leu Arg Asn Tyr Gly Ile Thr Ala Pro
50 55 60

Ile Asp Val His Leu Met Val Lys Pro Val Asp Arg Ile Val Pro Asp
65 70 75 80

Phe Ala Ala Ala Gly Ala Ser Ile Ile Thr Phe His Pro Glu Ala Ser
85 90 95

Glu His Val Asp Arg Thr Leu Gln Leu Ile Lys Glu Asn Gly Cys Lys
100 105 110

Ala Gly Leu Val Phe Asn Pro Ala Thr Pro Leu Ser Tyr Leu Asp Tyr
115 120 125

Val Met Asp Lys Leu Asp Val Ile Leu Leu Met Ser Val Asn Pro Gly
130 135 140

Phe Gly Gly Gln Ser Phe Ile Pro Gln Thr Leu Asp Lys Leu Arg Glu
145 150 155 160

Val Arg Arg Arg Ile Asp Glu Ser Gly Phe Asp Ile Arg Leu Glu Val
165 170 175

Asp Gly Gly Val Lys Val Asn Asn Ile Gly Glu Ile Ala Ala Ala Gly
180 185 190

Ala Asp Met Phe Val Ala Gly Ser Ala Ile Phe Asp Gln Pro Asp Tyr
195 200 205

Lys Lys Val Ile Asp Glu Met Arg Ser Glu Leu Ala Lys Val Ser His
210 215 220

Glu
225

<210> 19

<211> 547

<212> PRT

<213> Escherichia coli

<400> 19

Met Glu Leu Leu Val Leu Val Trp Arg Gln Tyr Arg Trp Pro Phe Ile
1 5 10 15

Ser Val Met Ala Leu Ser Leu Ala Ser Ala Ala Leu Gly Ile Gly Leu
20 25 30

Ile Ala Phe Ile Asn Gln Arg Leu Ile Glu Thr Ala Asp Thr Ser Leu
35 40 45

Leu Val Leu Pro Glu Phe Leu Gly Leu Leu Leu Leu Leu Met Ala Val
50 55 60

Thr Leu Gly Ser Gln Leu Ala Leu Thr Thr Leu Gly His His Phe Val
 65 70 75 80
 Tyr Arg Leu Arg Ser Glu Phe Ile Lys Arg Ile Leu Asp Thr His Val
 85 90 95
 Glu Arg Ile Glu Gln Leu Gly Ser Ala Ser Leu Leu Ala Gly Leu Thr
 100 105 110
 Ser Asp Val Arg Asn Ile Thr Ile Ala Phe Val Arg Leu Pro Glu Leu
 115 120 125
 Val Gln Gly Ile Ile Leu Thr Ile Gly Ser Ala Ala Tyr Leu Trp Met
 130 135 140
 Leu Ser Gly Lys Met Leu Leu Val Thr Ala Ile Trp Met Ala Ile Thr
 145 150 155 160
 Ile Trp Gly Gly Phe Val Leu Val Ala Arg Val Tyr Lys His Met Ala
 165 170 175
 Thr Leu Arg Glu Thr Glu Asp Lys Leu Tyr Thr Asp Phe Gln Thr Val
 180 185 190
 Leu Glu Gly Arg Lys Glu Leu Thr Leu Asn Arg Glu Arg Ala Glu Tyr
 195 200 205
 Val Phe Asn Asn Leu Tyr Ile Pro Asp Ala Gln Glu Tyr Arg His His
 210 215 220
 Ile Ile Arg Ala Asp Thr Phe His Leu Ser Ala Val Asn Trp Ser Asn
 225 230 235 240
 Ile Met Met Leu Gly Ala Ile Gly Leu Val Phe Trp Met Ala Asn Ser
 245 250 255
 Leu Gly Trp Ala Asp Thr Asn Val Ala Ala Thr Tyr Ser Leu Thr Leu
 260 265 270
 Leu Phe Leu Arg Thr Pro Leu Leu Ser Ala Val Gly Ala Leu Pro Thr
 275 280 285
 Leu Leu Thr Ala Gln Val Ala Phe Asn Lys Leu Asn Lys Phe Ala Leu
 290 295 300
 Ala Pro Phe Lys Ala Glu Phe Pro Arg Pro Gln Ala Phe Pro Asn Trp
 305 310 315 320
 Gln Thr Leu Glu Leu Arg Asn Val Thr Phe Ala Tyr Gln Asp Asn Ala
 325 330 335
 Phe Ser Val Gly Pro Ile Asn Leu Thr Ile Lys Arg Gly Glu Leu Leu
 340 345 350
 Phe Leu Ile Gly Gly Asn Gly Ser Gly Lys Ser Thr Leu Ala Met Leu
 355 360 365
 Leu Thr Gly Leu Tyr Gln Pro Gln Ser Gly Glu Ile Leu Leu Asp Gly
 370 375 380
 Lys Pro Val Ser Gly Glu Gln Pro Glu Asp Tyr Arg Lys Leu Phe Ser
 385 390 395 400
 Ala Val Phe Thr Asp Val Trp Leu Phe Asp Gln Leu Leu Gly Pro Glu
 405 410 415
 Gly Lys Pro Ala Asn Pro Gln Leu Val Glu Lys Trp Leu Ala Gln Leu
 420 425 430

Lys Met Ala His Lys Leu Glu Leu Ser Asn Gly Arg Ile Val Asn Leu
435 440 445

Lys Leu Ser Lys Gly Gln Lys Lys Arg Val Ala Leu Leu Leu Ala Leu
450 455 460

Ala Glu Glu Arg Asp Ile Ile Leu Leu Asp Glu Trp Ala Ala Asp Gln
465 470 475 480

Asp Pro His Phe Arg Arg Glu Phe Tyr Gln Val Leu Leu Pro Leu Met
485 490 495

Gln Glu Met Gly Lys Thr Ile Phe Ala Ile Ser His Asp Asp His Tyr
500 505 510

Phe Ile His Ala Asp Arg Leu Leu Glu Met Arg Asn Gly Gln Leu Ser
515 520 525

Glu Leu Thr Gly Glu Glu Arg Asp Ala Ala Ser Arg Asp Ala Val Ala
530 535 540

Arg Thr Ala
545

<210> 20

<211> 285

<212> PRT

<213> Escherichia coli

<400> 20

Met Ser Glu Thr Phe Phe His Leu Leu Gly Pro Gly Thr Gln Pro Asn
1 5 10 15

Asp Asp Ser Phe Ser Met Asn Pro Leu Pro Ile Thr Cys Gln Val Asn
20 25 30

Asp Glu Pro Ser Met Ala Ala Leu Glu Gln Cys Ala His Ser Pro Gln
35 40 45

Val Ile Ala Leu Leu Asn Glu Leu Gln His Gln Leu Ser Glu Arg Gln
50 55 60

Pro Pro Leu Gly Glu Val Leu Ala Val Asp Leu Leu Asn Leu Asn Ala
65 70 75 80

Asp Asp Arg His Phe Ile Asn Thr Leu Leu Gly Glu Gly Glu Val Ser
85 90 95

Val Arg Ile Gln Gln Ala Asp Asp Ser Glu Ser Glu Ile Gln Glu Ala
100 105 110

Ile Phe Cys Gly Leu Trp Arg Val Arg Arg Arg Gly Glu Lys Leu
115 120 125

Leu Glu Asp Lys Leu Glu Ala Gly Cys Ala Pro Leu Ala Leu Trp Gln
130 135 140

Ala Ala Thr Gln Asn Leu Leu Pro Thr Asp Ser Leu Leu Pro Pro Pro
145 150 155 160

Ile Asp Gly Leu Met Asn Gly Leu Pro Leu Ala His Glu Leu Leu Ala
165 170 175

His Val Arg Asn Pro Asp Ala Gln Pro His Ser Ile Asn Leu Thr Gln
180 185 190

Leu Pro Ile Ser Glu Ala Asp Arg Leu Phe Leu Ser Arg Leu Cys Gly
195 200 205

Pro Gly Asn Ile Gln Ile Arg Thr Ile Gly Tyr Gly Glu Ser Tyr Ile
210 215 220

Asn Ala Thr Gly Leu Arg His Val Trp His Leu Arg Cys Thr Asp Thr
225 230 235 240

Leu Lys Gly Pro Leu Leu Glu Ser Tyr Glu Ile Cys Pro Ile Pro Glu
245 250 255

Val Val Leu Ala Ala Pro Glu Asp Leu Val Asp Ser Ala Gln Arg Leu
260 265 270

Ser Glu Val Cys Gln Trp Leu Ala Glu Ala Ala Pro Thr
275 280 285

<210> 21

<211> 470

<212> PRT

<213> Escherichia coli

<400> 21

Met Lys Lys Thr Lys Ile Val Cys Thr Ile Gly Pro Lys Thr Glu Ser
1 5 10 15

Glu Glu Met Leu Ala Lys Met Leu Asp Ala Gly Met Asn Val Met Arg
20 25 30

Leu Asn Phe Ser His Gly Asp Tyr Ala Glu His Gly Gln Arg Ile Gln
35 40 45

Asn Leu Arg Asn Val Met Ser Lys Thr Gly Lys Thr Ala Ala Ile Leu
50 55 60

Leu Asp Thr Lys Gly Pro Glu Ile Arg Thr Met Lys Leu Glu Gly Gly
65 70 75 80

Asn Asp Val Ser Leu Lys Ala Gly Gln Thr Phe Thr Phe Thr Thr Asp
85 90 95

Lys Ser Val Ile Gly Asn Ser Glu Met Val Ala Val Thr Tyr Glu Gly
100 105 110

Phe Thr Thr Asp Leu Ser Val Gly Asn Thr Val Leu Val Asp Asp Gly
115 120 125

Leu Ile Gly Met Glu Val Thr Ala Ile Glu Gly Asn Lys Val Ile Cys
130 135 140

Lys Val Leu Asn Asn Gly Asp Leu Gly Glu Asn Lys Gly Val Asn Leu
145 150 155 160

Pro Gly Val Ser Ile Ala Leu Pro Ala Leu Ala Glu Lys Asp Lys Gln
165 170 175

Asp Leu Ile Phe Gly Cys Glu Gln Gly Val Asp Phe Val Ala Ala Ser
180 185 190

Phe Ile Arg Lys Arg Ser Asp Val Ile Glu Ile Arg Glu His Leu Lys
195 200 205

Ala His Gly Gly Glu Asn Ile His Ile Ile Ser Lys Ile Glu Asn Gln
210 215 220

Glu Gly Leu Asn Asn Phe Asp Glu Ile Leu Glu Ala Ser Asp Gly Ile
225 230 235 240

Met Val Ala Arg Gly Asp Leu Gly Val Glu Ile Pro Val Glu Glu Val
245 250 255

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Ile Phe Ala Gln Lys Met Met Ile Glu Lys Cys Ile Arg Ala Arg Lys
    260                265                270

Val Val Ile Thr Ala Thr Gln Met Leu Asp Ser Met Ile Lys Asn Pro
    275                280                285

Arg Pro Thr Arg Ala Glu Ala Gly Asp Val Ala Asn Ala Ile Leu Asp
    290                295                300

Gly Thr Asp Ala Val Met Leu Ser Gly Glu Ser Ala Lys Gly Lys Tyr
    305                310                315                320

Pro Leu Glu Ala Val Ser Ile Met Ala Thr Ile Cys Glu Arg Thr Asp
    325                330                335

Arg Val Met Asn Ser Arg Leu Glu Phe Asn Asn Asp Asn Arg Lys Leu
    340                345                350

Arg Ile Thr Glu Ala Val Cys Arg Gly Ala Val Glu Thr Ala Glu Lys
    355                360                365

Leu Asp Ala Pro Leu Ile Val Val Ala Thr Gln Gly Gly Lys Ser Ala
    370                375                380

Arg Ala Val Arg Lys Tyr Phe Pro Asp Ala Thr Ile Leu Ala Leu Thr
    385                390                395                400

Thr Asn Glu Lys Thr Ala His Gln Leu Val Leu Ser Lys Gly Val Val
    405                410                415

Pro Gln Leu Val Lys Glu Ile Thr Ser Thr Asp Asp Phe Tyr Arg Leu
    420                425                430

Gly Lys Glu Leu Ala Leu Gln Ser Gly Leu Ala His Lys Gly Asp Val
    435                440                445

Val Val Met Val Ser Gly Ala Leu Val Pro Ser Gly Thr Thr Asn Thr
    450                455                460

Ala Ser Val His Val Leu
    465                470

<210> 22
<211> 249
<212> PRT
<213> Escherichia coli

<400> 22
Met Lys Lys Thr Lys Ile Val Cys Thr Ile Gly Pro Lys Thr Glu Ser
1      5      10      15

Glu Glu Met Leu Ala Lys Met Leu Asp Ala Gly Met Asn Val Met Arg
20     25     30

Leu Asn Phe Ser His Gly Asp Tyr Ala Glu His Gly Gln Arg Ile Gln
35     40     45

Asn Leu Arg Asn Val Met Ser Lys Thr Gly Lys Thr Ala Ala Ile Leu
50     55     60

Leu Asp Thr Lys Gly Pro Glu Ile Arg Thr Met Lys Leu Glu Gly Gly
65     70     75     80

Asn Asp Val Ser Leu Lys Ala Gly Gln Thr Phe Thr Phe Thr Thr Asp
85     90     95

Lys Ser Val Ile Gly Asn Ser Glu Met Val Ala Val Thr Tyr Glu Gly
100    105    110

Phe Thr Thr Asn Leu Ser Val Gly Asn Thr Val Leu Val Asn Asn Gly

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115      120      125
Leu Ile Gly Met Glu Val Thr Ala Ile Glu Gly Asn Lys Val Ile Cys
130      135      140
Lys Val Leu Asn Asn Gly Asp Leu Gly Glu Asn Lys Gly Val Asn Leu
145      150      155      160
Pro Gly Val Ser Ile Ala Leu Pro Ala Leu Ala Glu Lys Asp Lys Gln
165      170      175
Asp Leu Ile Phe Gly Cys Glu Gln Gly Val Asp Phe Val Ala Ala Ser
180      185      190
Phe Ile Arg Lys Arg Ser Asp Val Ile Glu Ile Arg Glu His Leu Lys
195      200      205
Ala His Gly Gly Glu Asn Ile His Ile Ile Ser Lys Ile Glu Asn Gln
210      215      220
Glu Gly Leu Asn Asn Phe Asp Glu Ile Leu Glu Ala Ser Asp Gly Ile
225      230      235      240
Met Val Ala Arg Gly Asp Leu Gly Val
245
<210> 23
<211> 901
<212> PRT
<213> Escherichia coli

<400> 23
Met Leu Ile Pro Ser Lys Leu Ser Arg Pro Val Arg Leu Asp His Thr
1      5      10      15
Val Val Arg Glu Arg Leu Leu Ala Lys Leu Ser Gly Ala Asn Asn Phe
20      25      30
Arg Leu Ala Leu Ile Thr Ser Pro Ala Gly Tyr Gly Lys Thr Thr Leu
35      40      45
Ile Ser Gln Trp Ala Ala Gly Lys Asn Asp Ile Gly Trp Tyr Ser Leu
50      55      60
Asp Glu Gly Asp Asn Gln Gln Glu Arg Phe Ala Ser Tyr Leu Ile Ala
65      70      75      80
Ala Val Gln Gln Ala Thr Asn Gly His Cys Ala Ile Cys Glu Thr Met
85      90      95
Ala Gln Lys Arg Gln Tyr Ala Ser Leu Thr Ser Leu Phe Ala Gln Leu
100     105     110
Phe Ile Glu Leu Ala Glu Trp His Ser Pro Leu Tyr Leu Val Ile Asp
115     120     125
Asp Tyr His Leu Ile Thr Asn Pro Val Ile His Glu Ser Met Arg Phe
130     135     140
Phe Ile Arg His Gln Pro Glu Asn Leu Thr Leu Val Val Leu Ser Arg
145     150     155     160
Asn Leu Pro Gln Leu Gly Ile Ala Asn Leu Arg Val Arg Asp Gln Leu
165     170     175
Leu Glu Ile Gly Ser Gln Gln Leu Ala Phe Thr His Gln Glu Ala Lys
180     185     190
Gln Phe Phe Asp Cys Arg Leu Ser Ser Pro Ile Glu Ala Ala Glu Ser

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195					200					205					
Ser	Arg	Ile	Cys	Asp	Asp	Val	Ser	Gly	Trp	Ala	Thr	Ala	Leu	Gln	Leu
210						215					220				
Ile	Ala	Leu	Ser	Ala	Arg	Gln	Asn	Thr	His	Ser	Ala	His	Lys	Ser	Ala
225					230					235					240
Arg	Arg	Leu	Ala	Gly	Ile	Asn	Ala	Ser	His	Leu	Ser	Asp	Tyr	Leu	Val
				245					250					255	
Asp	Glu	Val	Leu	Asp	Asn	Val	Asp	Leu	Ala	Thr	Arg	His	Phe	Leu	Leu
			260					265					270		
Lys	Ser	Ala	Ile	Leu	Arg	Ser	Met	Asn	Asp	Ala	Leu	Ile	Thr	Arg	Val
	275						280					285			
Thr	Gly	Glu	Glu	Asn	Gly	Gln	Met	Arg	Leu	Glu	Glu	Ile	Glu	Arg	Gln
	290					295					300				
Gly	Leu	Phe	Leu	Gln	Arg	Met	Asp	Asp	Thr	Gly	Glu	Trp	Phe	Cys	Tyr
305					310					315					320
His	Pro	Leu	Phe	Gly	Asn	Phe	Leu	Arg	Gln	Arg	Cys	Gln	Trp	Glu	Leu
				325					330					335	
Ala	Ala	Glu	Leu	Pro	Glu	Ile	His	Arg	Ala	Ala	Ala	Glu	Ser	Trp	Met
			340					345					350		
Ala	Gln	Gly	Phe	Pro	Ser	Glu	Ala	Ile	His	His	Ala	Leu	Ala	Ala	Gly
		355					360					365			
Asp	Ala	Leu	Met	Leu	Arg	Asp	Ile	Leu	Leu	Asn	His	Ala	Trp	Ser	Leu
	370					375					380				
Phe	Asn	His	Ser	Glu	Leu	Ser	Leu	Leu	Glu	Glu	Ser	Leu	Lys	Ala	Leu
385					390					395					400
Pro	Trp	Asp	Ser	Leu	Leu	Glu	Asn	Pro	Gln	Leu	Val	Leu	Leu	Gln	Ala
				405					410					415	
Trp	Leu	Met	Gln	Ser	Gln	His	Arg	Tyr	Gly	Glu	Val	Asn	Thr	Leu	Leu
		420						425					430		
Ala	Arg	Ala	Glu	His	Glu	Ile	Lys	Asp	Ile	Arg	Glu	Asp	Thr	Met	His
	435						440					445			
Ala	Glu	Phe	Asn	Ala	Leu	Arg	Ala	Gln	Val	Ala	Ile	Asn	Asp	Gly	Asn
	450					455					460				
Pro	Asp	Glu	Ala	Glu	Arg	Leu	Ala	Lys	Leu	Ala	Leu	Glu	Glu	Leu	Pro
465					470					475					480
Pro	Gly	Trp	Phe	Tyr	Ser	Arg	Ile	Val	Ala	Thr	Ser	Val	Leu	Gly	Glu
			485						490					495	
Val	Leu	His	Cys	Lys	Gly	Glu	Leu	Thr	Arg	Ser	Leu	Ala	Leu	Met	Gln
			500					505					510		
Gln	Thr	Glu	Gln	Met	Ala	Arg	Gln	His	Asp	Val	Trp	His	Tyr	Ala	Leu
	515						520					525			
Trp	Ser	Leu	Ile	Gln	Gln	Ser	Glu	Ile	Leu	Phe	Ala	Gln	Gly	Phe	Leu
	530					535					540				
Gln	Thr	Ala	Trp	Glu	Thr	Gln	Glu	Lys	Ala	Phe	Gln	Leu	Ile	Asn	Glu
545					550					555					560
Gln	His	Leu	Glu	Gln	Leu	Pro	Met	His	Glu	Phe	Leu	Val	Arg	Ile	Arg
			565						570					575	

Ala Gln Leu Leu Trp Ala Trp Ala Arg Leu Asp Glu Ala Glu Ala Ser

580 585 590

Ala Arg Ser Gly Ile Glu Val Leu Ser Ser Tyr Gln Pro Gln Gln Gln
595 600 605

Leu Gln Cys Leu Ala Met Leu Ile Gln Cys Ser Leu Ala Arg Gly Asp
610 615 620

Leu Asp Asn Ala Arg Ser Gln Leu Asn Arg Leu Glu Asn Leu Leu Gly
625 630 635 640

Asn Gly Lys Tyr His Ser Asp Trp Ile Ser Asn Ala Asn Lys Val Arg
645 650 655

Val Ile Tyr Trp Gln Met Thr Gly Asp Lys Ala Ala Ala Ala Asn Trp
660 665 670

Leu Arg His Thr Ala Lys Pro Glu Phe Ala Asn Asn His Phe Leu Gln
675 680 685

Gly Gln Trp Arg Asn Ile Ala Arg Ala Gln Ile Leu Leu Gly Glu Phe
690 695 700

Glu Pro Ala Glu Ile Val Leu Glu Glu Leu Asn Glu Asn Ala Arg Ser
705 710 715 720

Leu Arg Leu Met Ser Asp Leu Asn Arg Asn Leu Leu Leu Leu Asn Gln
725 730 735

Leu Tyr Trp Gln Ala Gly Arg Lys Ser Asp Ala Gln Arg Val Leu Leu
740 745 750

Asp Ala Leu Lys Leu Ala Asn Arg Thr Gly Phe Ile Ser His Phe Val
755 760 765

Ile Glu Gly Glu Ala Met Ala Gln Gln Leu Arg Gln Leu Ile Gln Leu
770 775 780

Asn Thr Leu Pro Glu Leu Glu Gln His Arg Ala Gln Arg Ile Leu Arg
785 790 795 800

Glu Ile Asn Gln His His Arg His Lys Phe Ala His Phe Asp Glu Asn
805 810 815

Phe Val Glu Arg Leu Leu Asn His Pro Glu Val Pro Glu Leu Ile Arg
820 825 830

Thr Ser Pro Leu Thr Gln Arg Glu Trp Gln Val Leu Gly Leu Ile Tyr
835 840 845

Ser Gly Tyr Ser Asn Glu Gln Ile Ala Gly Glu Leu Glu Val Ala Ala
850 855 860

Thr Thr Ile Lys Thr His Ile Arg Asn Leu Tyr Gln Lys Leu Gly Val
865 870 875 880

Ala His Arg Gln Asp Ala Val Gln His Ala Gln Gln Leu Leu Lys Met
885 890 895

Met Gly Tyr Gly Val
900

<210> 24

<211> 779

<212> PRT

<213> Escherichia coli

<400> 24

Met Leu Ile Pro Ser Lys Leu Ser Arg Pro Val Arg Leu Asp His Thr
 1 5 10 15

Val Val Arg Glu Arg Leu Leu Ala Lys Leu Ser Gly Ala Asn Asn Phe
 20 25 30

Arg Leu Ala Leu Ile Thr Ser Pro Ala Gly Tyr Gly Lys Thr Thr Leu
 35 40 45

Ile Ser Gln Trp Ala Ala Gly Lys Asn Asp Ile Gly Trp Tyr Ser Leu
 50 55 60

Asp Glu Gly Asp Asn Gln Gln Glu Arg Phe Ala Ser Tyr Leu Ile Ala
 65 70 75 80

Ala Val Gln Gln Ala Thr Asn Gly His Cys Ala Ile Cys Glu Thr Met
 85 90 95

Ala Gln Lys Arg Gln Tyr Ala Ser Leu Thr Ser Leu Phe Ala Gln Leu
 100 105 110

Phe Ile Glu Leu Ala Glu Trp His Ser Pro Leu Tyr Leu Val Ile Asp
 115 120 125

Asp Tyr His Leu Ile Thr Asn Pro Val Ile His Glu Ser Met Arg Phe
 130 135 140

Phe Ile Arg His Gln Pro Glu Asn Leu Thr Leu Val Val Leu Ser Arg
 145 150 155 160

Asn Leu Pro Gln Leu Gly Ile Ala Asn Leu Arg Val Arg Asp Gln Leu
 165 170 175

Leu Glu Ile Gly Ser Gln Gln Leu Ala Phe Thr His Gln Glu Ala Lys
 180 185 190

Gln Phe Phe Asp Cys Arg Leu Ser Ser Pro Ile Glu Ala Ala Glu Ser
 195 200 205

Ser Arg Ile Cys Asp Asp Val Ser Gly Trp Ala Thr Ala Leu Gln Leu
 210 215 220

Ile Ala Leu Ser Ala Arg Gln Asn Thr His Ser Ala His Lys Ser Ala
 225 230 235 240

Arg Arg Leu Ala Gly Ile Asn Ala Ser His Leu Ser Asp Tyr Leu Val
 245 250 255

Asp Glu Val Leu Asp Asn Val Asp Leu Ala Thr Arg His Phe Leu Leu
 260 265 270

Lys Ser Ala Ile Leu Arg Ser Met Asn Asp Ala Leu Ile Thr Arg Val
 275 280 285

Thr Gly Glu Glu Asn Gly Gln Met Arg Leu Glu Glu Ile Glu Arg Gln
 290 295 300

Gly Leu Phe Leu Gln Arg Met Asp Asp Thr Gly Glu Trp Phe Cys Tyr
 305 310 315 320

His Pro Leu Phe Gly Asn Phe Leu Arg Gln Arg Cys Gln Trp Glu Leu
 325 330 335

Ala Ala Glu Leu Pro Glu Ile His Arg Ala Ala Ala Glu Ser Trp Met
 340 345 350

Ala Gln Gly Phe Pro Ser Glu Ala Ile His His Ala Leu Ala Ala Gly
 355 360 365

Asn Ala Leu Met Leu Arg Asn Ile Leu Leu Asn His Ala Trp Ser Leu

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Asp Ala Leu Met Leu Arg Asp His Leu Leu Asn His Ala Trp Ser Leu
370                               375                               380

Phe Asn His Ser Glu Leu Ser Leu Leu Glu Glu Ser Leu Lys Ala Leu
385                               390                               395                               400

Pro Trp Asp Ser Leu Leu Glu Asn Pro Gln Leu Val Leu Leu Gln Ala
405                               410                               415

Trp Leu Met Gln Ser Gln His Arg Tyr Gly Glu Val Asn Thr Leu Leu
420                               425                               430

Ala Arg Ala Glu His Glu Ile Lys Asp Ile Arg Glu Asp Thr Met His
435                               440                               445

Ala Glu Phe Asn Ala Leu Arg Ala Gln Val Ala Ile Asn Asp Gly Asn
450                               455                               460

Pro Asp Glu Ala Glu Arg Leu Ala Lys Leu Ala Leu Glu Glu Leu Pro
465                               470                               475                               480

Pro Gly Trp Phe Tyr Ser Arg Ile Val Ala Thr Ser Val Leu Gly Glu
485                               490                               495

Val Leu His Cys Lys Gly Glu Leu Thr Arg Ser Leu Ala Leu Met Gln
500                               505                               510

Gln Thr Glu Gln Met Ala Arg Gln His Asp Val Trp His Tyr Ala Leu
515                               520                               525

Trp Ser Leu Ile Gln Gln Ser Glu Ile Leu Phe Ala Gln Gly Phe Leu
530                               535                               540

Gln Thr Ala Trp Glu Thr Gln Glu Lys Ala Phe Gln Leu Ile Asn Glu
545                               550                               555                               560

Gln His Leu Glu Gln Leu Pro Met His Glu Phe Leu Val Arg Ile Arg
565                               570                               575

Ala Gln Leu Leu Trp Ala Trp Ala Arg Leu Asp Glu Ala Glu Ala Ser
580                               585                               590

Ala Arg Ser Gly Ile Glu Val Leu Ser Ser Tyr Gln Pro Gln Gln Gln
595                               600                               605

Leu Gln Cys Leu Ala Met Leu Ile Gln Cys Ser Leu Ala Arg Gly Asp
610                               615                               620

Leu Asp Asn Ala Arg Ser Gln Leu Asn Arg Leu Glu Asn Leu Leu Gly
625                               630                               635                               640

Asn Gly Lys Tyr His Ser Asp Trp Ile Ser Asn Ala Asn Lys Val Arg
645                               650                               655

Val Ile Tyr Trp Gln Met Thr Gly Asp Lys Ala Ala Ala Ala Asn Trp
660                               665                               670

Leu Arg His Thr Ala Lys Pro Glu Phe Ala Asn Asn His Phe Leu Gln
675                               680                               685

Gly Gln Trp Arg Asn Ile Ala Arg Ala Gln Ile Leu Leu Gly Glu Phe
690                               695                               700

Glu Pro Ala Glu Ile Val Leu Glu Glu Leu Asn Glu Asn Ala Arg Ser
705                               710                               715                               720

Leu Arg Leu Met Ser Asp Leu Asn Arg Asn Leu Leu Leu Leu Asn Gln
725                               730                               735

Leu Tyr Trp Gln Ala Gly Arg Lys Ser Asp Ala Gln Arg Val Leu Leu
740                               745                               750

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Asp Ala Leu Lys Leu Ala Asn Arg Thr Gly Phe Ile Ser His Phe Val
755 760 765

Ile Glu Gly Glu Ala Met Ala Gln Gln Leu Arg
770 775

<210> 25

<211> 1342

<212> PRT

<213> Escherichia coli

<400> 25

Met Val Tyr Ser Tyr Thr Glu Lys Lys Arg Ile Arg Lys Asp Phe Gly
1 5 10 15

Lys Arg Pro Gln Val Leu Asp Val Pro Tyr Leu Leu Ser Ile Gln Leu
20 25 30

Asp Ser Phe Gln Lys Phe Ile Glu Gln Asp Pro Glu Gly Gln Tyr Gly
35 40 45

Leu Glu Ala Ala Phe Arg Ser Val Phe Pro Ile Gln Ser Tyr Ser Gly
50 55 60

Asn Ser Glu Leu Gln Tyr Val Ser Tyr Arg Leu Gly Glu Pro Val Phe
65 70 75 80

Asp Val Gln Glu Cys Gln Ile Arg Gly Val Thr Tyr Ser Ala Pro Leu
85 90 95

Arg Val Lys Leu Arg Leu Val Ile Tyr Glu Arg Glu Ala Pro Glu Gly
100 105 110

Thr Val Lys Asp Ile Lys Glu Gln Glu Val Tyr Met Gly Glu Ile Pro
115 120 125

Leu Met Thr Asp Asn Gly Thr Phe Val Ile Asn Gly Thr Glu Arg Val
130 135 140

Ile Val Ser Gln Leu His Arg Ser Pro Gly Val Phe Phe Asp Ser Asp
145 150 155 160

Lys Gly Lys Thr His Ser Ser Gly Lys Val Leu Tyr Asn Ala Arg Ile
165 170 175

Ile Pro Tyr Arg Gly Ser Trp Leu Asp Phe Glu Phe Asp Pro Lys Asp
180 185 190

Asn Leu Phe Val Arg Ile Asp Arg Arg Arg Lys Leu Pro Ala Thr Ile
195 200 205

Ile Leu Arg Ala Leu Asn Tyr Thr Thr Glu Gln Ile Leu Asp Leu Phe
210 215 220

Phe Glu Lys Val Ile Phe Glu Ile Arg Asp Asn Lys Leu Gln Met Glu
225 230 235 240

Leu Val Pro Glu Arg Leu Arg Gly Glu Thr Ala Ser Phe Asp Ile Glu
245 250 255

Ala Asn Gly Lys Val Tyr Val Glu Lys Gly Arg Arg Ile Thr Ala Arg
260 265 270

His Ile Arg Gln Leu Glu Lys Asp Asp Val Lys Leu Ile Glu Val Pro
275 280 285

Val Glu Tyr Ile Ala Gly Lys Val Val Ala Lys Asp Tyr Ile Asp Glu
290 295 300

Ser Thr Gly Gly Leu Ile Glu Ala Ala Asp Met Gly Leu Ser Leu Asp

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Ser  Thr  Gly  Glu  Leu  Ile  Cys  Ala  Ala  Asn  Met  Glu  Leu  Ser  Leu  Asp
305              310              315              320

Leu  Leu  Ala  Lys  Leu  Ser  Gln  Ser  Gly  His  Lys  Arg  Ile  Glu  Thr  Leu
325              330              335

Phe  Thr  Asn  Asp  Leu  Asp  His  Gly  Pro  Tyr  Ile  Ser  Glu  Thr  Leu  Arg
340              345              350

Val  Asp  Pro  Thr  Asn  Asp  Arg  Leu  Ser  Ala  Leu  Val  Glu  Ile  Tyr  Arg
355              360              365

Met  Met  Arg  Pro  Gly  Glu  Pro  Pro  Thr  Arg  Glu  Ala  Ala  Glu  Ser  Leu
370              375              380

Phe  Glu  Asn  Leu  Phe  Phe  Ser  Glu  Asp  Arg  Tyr  Asp  Leu  Ser  Ala  Val
385              390              395              400

Gly  Arg  Met  Lys  Phe  Asn  Arg  Ser  Leu  Leu  Arg  Glu  Glu  Ile  Glu  Gly
405              410              415

Ser  Gly  Ile  Leu  Ser  Lys  Asp  Asp  Ile  Ile  Asp  Val  Met  Lys  Lys  Leu
420              425              430

Ile  Asp  Ile  Arg  Asn  Gly  Lys  Gly  Glu  Val  Asp  Asp  Ile  Asp  His  Leu
435              440              445

Gly  Asn  Arg  Arg  Ile  Arg  Ser  Val  Gly  Glu  Met  Ala  Glu  Asn  Gln  Phe
450              455              460

Arg  Val  Gly  Leu  Val  Arg  Val  Glu  Arg  Ala  Val  Lys  Glu  Arg  Leu  Ser
465              470              475              480

Leu  Gly  Asp  Leu  Asp  Thr  Leu  Met  Pro  Gln  Asp  Met  Ile  Asn  Ala  Lys
485              490              495

Pro  Ile  Ser  Ala  Ala  Val  Lys  Glu  Phe  Phe  Gly  Ser  Ser  Gln  Leu  Ser
500              505              510

Gln  Phe  Met  Asp  Gln  Asn  Asn  Pro  Leu  Ser  Glu  Ile  Thr  His  Lys  Arg
515              520              525

Arg  Ile  Ser  Ala  Leu  Gly  Pro  Gly  Gly  Leu  Thr  Arg  Glu  Arg  Ala  Gly
530              535              540

Phe  Glu  Val  Arg  Asp  Val  His  Pro  Thr  His  Tyr  Gly  Arg  Val  Cys  Pro
545              550              555              560

Ile  Glu  Thr  Pro  Glu  Gly  Pro  Asn  Ile  Gly  Leu  Ile  Asn  Ser  Leu  Ser
565              570              575

Val  Tyr  Ala  Gln  Thr  Asn  Glu  Tyr  Gly  Phe  Leu  Glu  Thr  Pro  Tyr  Arg
580              585              590

Lys  Val  Thr  Asp  Gly  Val  Val  Thr  Asp  Glu  Ile  His  Tyr  Leu  Ser  Ala
595              600              605

Ile  Glu  Glu  Gly  Asn  Tyr  Val  Ile  Ala  Gln  Ala  Asn  Ser  Asn  Leu  Asp
610              615              620

Glu  Glu  Gly  His  Phe  Val  Glu  Asp  Leu  Val  Thr  Cys  Arg  Ser  Lys  Gly
625              630              635              640

Glu  Ser  Ser  Leu  Phe  Ser  Arg  Asp  Gln  Val  Asp  Tyr  Met  Asp  Val  Ser
645              650              655

Thr  Gln  Gln  Val  Val  Ser  Val  Gly  Ala  Ser  Leu  Ile  Pro  Phe  Leu  Glu
660              665              670

His  Asp  Asp  Ala  Asn  Arg  Ala  Leu  Met  Gly  Ala  Asn  Met  Gln  Arg  Gln

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675	680	685
Ala Val Pro Thr Leu Arg	Ala Asp Lys Pro Leu Val Gly Thr Gly Met	
690	695	700
Glu Arg Ala Val Ala Val Asp Ser Gly Val Thr Ala Val Ala Lys Arg		
705	710	715 720
Gly Gly Val Val Gln Tyr Val Asp Ala Ser Arg Ile Val Ile Lys Val		
	725	730 735
Asn Glu Asp Glu Met Tyr Pro Gly Glu Ala Gly Ile Asp Ile Tyr Asn		
	740	745 750
Leu Thr Lys Tyr Thr Arg Ser Asn Gln Asn Thr Cys Ile Asn Gln Met		
	755	760 765
Pro Cys Val Ser Leu Gly Glu Pro Val Glu Arg Gly Asp Val Leu Ala		
	770	775 780
Asp Gly Pro Ser Thr Asp Leu Gly Glu Leu Ala Leu Gly Gln Asn Met		
	785	790 795 800
Arg Val Ala Phe Met Pro Trp Asn Gly Tyr Asn Phe Glu Asp Ser Ile		
	805	810 815
Leu Val Ser Glu Arg Val Val Gln Glu Asp Arg Phe Thr Thr Ile His		
	820	825 830
Ile Gln Glu Leu Ala Cys Val Ser Arg Asp Thr Lys Leu Gly Pro Glu		
	835	840 845
Glu Ile Thr Ala Asp Ile Pro Asn Val Gly Glu Ala Ala Leu Ser Lys		
	850	855 860
Leu Asp Glu Ser Gly Ile Val Tyr Ile Gly Ala Glu Val Thr Gly Gly		
	865	870 875 880
Asp Ile Leu Val Gly Lys Val Thr Pro Lys Gly Glu Thr Gln Leu Thr		
	885	890 895
Pro Glu Glu Lys Leu Leu Arg Ala Ile Phe Gly Glu Lys Ala Ser Asp		
	900	905 910
Val Lys Asp Ser Ser Leu Arg Val Pro Asn Gly Val Ser Gly Thr Val		
	915	920 925
Ile Asp Val Gln Val Phe Thr Arg Asp Gly Val Glu Lys Asp Lys Arg		
	930	935 940
Ala Leu Glu Ile Glu Glu Met Gln Leu Lys Gln Ala Lys Lys Asp Leu		
	945	950 955 960
Ser Glu Glu Leu Gln Ile Leu Glu Ala Gly Leu Phe Ser Arg Ile Arg		
	965	970 975
Ala Val Leu Val Ala Gly Gly Val Glu Ala Glu Lys Leu Asp Lys Leu		
	980	985 990
Pro Arg Asp Arg Trp Leu Glu Leu Gly Leu Thr Asp Glu Glu Lys Gln		
	995	1000 1005
Asn Gln Leu Glu Gln Leu Ala Glu Gln Tyr Asp Glu Leu Lys His		
	1010	1015 1020
Glu Phe Glu Lys Lys Leu Glu Ala Lys Arg Arg Lys Ile Thr Gln		
	1025	1030 1035
Gly Asp Asp Leu Ala Pro Gly Val Leu Lys Ile Val Lys Val Tyr		
	1040	1045 1050

Leu Ala Val Lys Arg Arg Ile Gln Pro Gly Asp Lys Met Ala Gly
 1055 1060 1065
 Arg His Gly Asn Lys Gly Val Ile Ser Lys Ile Asn Pro Ile Glu
 1070 1075 1080
 Asp Met Pro Tyr Asp Glu Asn Gly Thr Pro Val Asp Ile Val Leu
 1085 1090 1095
 Asn Pro Leu Gly Val Pro Ser Arg Met Asn Ile Gly Gln Ile Leu
 1100 1105 1110
 Glu Thr His Leu Gly Met Ala Ala Lys Gly Ile Gly Asp Lys Ile
 1115 1120 1125
 Asn Ala Met Leu Lys Gln Gln Gln Glu Val Ala Lys Leu Arg Glu
 1130 1135 1140
 Phe Ile Gln Arg Ala Tyr Asp Leu Gly Ala Asp Val Arg Gln Lys
 1145 1150 1155
 Val Asp Leu Ser Thr Phe Ser Asp Glu Glu Val Met Arg Leu Ala
 1160 1165 1170
 Glu Asn Leu Arg Lys Gly Met Pro Ile Ala Thr Pro Val Phe Asp
 1175 1180 1185
 Gly Ala Lys Glu Ala Glu Ile Lys Glu Leu Leu Lys Leu Gly Asp
 1190 1195 1200
 Leu Pro Thr Ser Gly Gln Ile Arg Leu Tyr Asp Gly Arg Thr Gly
 1205 1210 1215
 Glu Gln Phe Glu Arg Pro Val Thr Val Gly Tyr Met Tyr Met Leu
 1220 1225 1230
 Lys Leu Asn His Leu Val Asp Asp Lys Met His Ala Arg Ser Thr
 1235 1240 1245
 Gly Ser Tyr Ser Leu Val Thr Gln Gln Pro Leu Gly Gly Lys Ala
 1250 1255 1260
 Gln Phe Gly Gly Gln Arg Phe Gly Glu Met Glu Val Trp Ala Leu
 1265 1270 1275
 Glu Ala Tyr Gly Ala Ala Tyr Thr Leu Gln Glu Met Leu Thr Val
 1280 1285 1290
 Lys Ser Asp Asp Val Asn Gly Arg Thr Lys Met Tyr Lys Asn Ile
 1295 1300 1305
 Val Asp Gly Asn His Gln Met Glu Pro Gly Met Pro Glu Ser Phe
 1310 1315 1320
 Asn Val Leu Leu Lys Glu Ile Arg Ser Leu Gly Ile Asn Ile Glu
 1325 1330 1335
 Leu Glu Asp Glu
 1340
 <210> 26
 <211> 548
 <212> PRT
 <213> Escherichia coli
 <400> 26
 Met Ser Asn Lys Pro Phe Ile Tyr Gln Ala Pro Phe Pro Met Gly Lys
 1 5 10 15

Asp Asn Thr Glu Tyr Tyr Leu Leu Thr Ser Asp Tyr Val Ser Val Ala
 20 25 30

Asp Phe Asp Gly Glu Thr Ile Leu Lys Val Glu Pro Glu Ala Leu Thr
 35 40 45

Leu Leu Ala Gln Gln Ala Phe His Asp Ala Ser Phe Met Leu Arg Pro
 50 55 60

Ala His Gln Lys Gln Val Ala Ala Ile Leu His Asp Pro Glu Ala Ser
 65 70 75 80

Glu Asn Asp Lys Tyr Val Ala Leu Gln Phe Leu Arg Asn Ser Glu Ile
 85 90 95

Ala Ala Lys Gly Val Leu Pro Thr Cys Gln Asp Thr Gly Thr Ala Ile
 100 105 110

Ile Val Gly Lys Lys Gly Gln Arg Val Trp Thr Gly Gly Gly Asp Glu
 115 120 125

Glu Thr Leu Ser Lys Gly Val Tyr Asn Thr Tyr Ile Glu Asp Asn Leu
 130 135 140

Arg Tyr Ser Gln Asn Ala Ala Leu Asp Met Tyr Lys Glu Val Asn Thr
 145 150 155 160

Gly Thr Asn Leu Pro Ala Gln Ile Asp Leu Tyr Ala Val Asp Gly Asp
 165 170 175

Glu Tyr Lys Phe Leu Cys Val Ala Lys Gly Gly Gly Ser Ala Asn Lys
 180 185 190

Thr Tyr Leu Tyr Gln Glu Thr Lys Ala Leu Leu Thr Pro Gly Lys Leu
 195 200 205

Lys Asn Phe Leu Val Glu Lys Met Arg Thr Leu Gly Thr Ala Ala Cys
 210 215 220

Pro Pro Tyr His Ile Ala Phe Val Ile Gly Gly Thr Ser Ala Glu Thr
 225 230 235 240

Asn Leu Lys Thr Val Lys Leu Ala Ser Ala His Tyr Tyr Asp Glu Leu
 245 250 255

Pro Thr Glu Gly Asn Glu His Gly Gln Ala Phe Arg Asp Val Gln Leu
 260 265 270

Glu Gln Glu Leu Leu Glu Glu Ala Gln Lys Leu Gly Leu Gly Ala Gln
 275 280 285

Phe Gly Gly Lys Tyr Phe Ala His Asp Ile Arg Val Ile Arg Leu Pro
 290 295 300

Arg His Gly Ala Ser Cys Pro Val Gly Met Gly Val Ser Cys Ser Ala
 305 310 315 320

Asp Arg Asn Ile Lys Ala Lys Ile Asn Arg Glu Gly Ile Trp Ile Glu
 325 330 335

Lys Leu Glu His Asn Pro Gly Gln Tyr Ile Pro Gln Glu Leu Arg Gln
 340 345 350

Ala Gly Glu Gly Glu Ala Val Lys Val Asp Leu Asn Arg Pro Met Lys
 355 360 365

Glu Ile Leu Ala Gln Leu Ser Gln Tyr Pro Val Ser Thr Arg Leu Ser
 370 375 380

Leu Thr Gly Thr Ile Ile Val Gly Arg Asp Ile Ala His Ala Lys Leu
385 390 395 400

Lys Glu Leu Ile Asp Ala Gly Lys Glu Leu Pro Gln Tyr Ile Lys Asp
405 410 415

His Pro Ile Tyr Tyr Ala Gly Pro Ala Lys Thr Pro Ala Gly Tyr Pro
420 425 430

Ser Gly Ser Leu Gly Pro Thr Thr Ala Gly Arg Met Asp Ser Tyr Val
435 440 445

Asp Leu Leu Gln Ser His Gly Gly Ser Met Ile Met Leu Ala Lys Gly
450 455 460

Asn Arg Ser Gln Gln Val Thr Asp Ala Cys His Lys His Gly Gly Phe
465 470 475 480

Tyr Leu Gly Ser Ile Gly Gly Pro Ala Ala Val Leu Ala Gln Gln Ser
485 490 495

Ile Lys His Leu Glu Cys Val Ala Tyr Pro Glu Leu Gly Met Glu Ala
500 505 510

Ile Trp Lys Ile Glu Val Glu Asp Phe Pro Ala Phe Ile Leu Val Asp
515 520 525

Asp Lys Gly Asn Asp Phe Phe Gln Gln Ile Val Asn Lys Gln Cys Ala
530 535 540

Asn Cys Thr Lys
545

<210> 27

<211> 518

<212> PRT

<213> Escherichia coli

<400> 27

Met Ile Pro Asp Val Ser Gln Ala Leu Ala Trp Leu Glu Lys His Pro
1 5 10 15

Gln Ala Leu Lys Gly Ile Gln Arg Gly Leu Glu Arg Glu Thr Leu Arg
20 25 30

Val Asn Ala Asp Gly Thr Leu Ala Thr Thr Gly His Pro Glu Ala Leu
35 40 45

Gly Ser Ala Leu Thr His Lys Trp Ile Thr Thr Asp Phe Ala Glu Ala
50 55 60

Leu Leu Glu Phe Ile Thr Pro Val Asp Gly Asp Ile Glu His Met Leu
65 70 75 80

Thr Phe Met Arg Asp Leu His Arg Tyr Thr Ala Arg Asn Met Gly Asp
85 90 95

Glu Arg Met Trp Pro Leu Ser Met Pro Cys Tyr Ile Ala Glu Gly Gln
100 105 110

Asp Ile Glu Leu Ala Gln Tyr Gly Thr Ser Asn Thr Gly Arg Phe Lys
115 120 125

Thr Leu Tyr Arg Glu Gly Leu Lys Asn Arg Tyr Gly Ala Leu Met Gln
130 135 140

Thr Ile Ser Gly Val His Tyr Asn Phe Ser Leu Pro Met Ala Phe Trp
145 150 155 160

Gln Ala Lys Cys Gly Asp Ile Ser Gly Ala Asp Ala Lys Glu Lys Ile
 165 170 175
 Ser Ala Gly Tyr Phe Arg Val Ile Arg Asn Tyr Tyr Arg Phe Gly Trp
 180 185 190
 Val Ile Pro Tyr Leu Phe Gly Ala Ser Pro Ala Ile Cys Ser Ser Phe
 195 200 205
 Leu Gln Gly Lys Pro Thr Ser Leu Pro Phe Glu Lys Thr Glu Cys Gly
 210 215 220
 Met Tyr Tyr Leu Pro Tyr Ala Thr Ser Leu Arg Leu Ser Asp Leu Gly
 225 230 235 240
 Tyr Thr Asn Lys Ser Gln Ser Asn Leu Gly Ile Thr Phe Asn Asp Leu
 245 250 255
 Tyr Glu Tyr Val Ala Gly Leu Lys Gln Ala Ile Lys Thr Pro Ser Glu
 260 265 270
 Glu Tyr Ala Lys Ile Gly Ile Glu Lys Asp Gly Lys Arg Leu Gln Ile
 275 280 285
 Asn Ser Asn Val Leu Gln Ile Glu Asn Glu Leu Tyr Ala Pro Ile Arg
 290 295 300
 Pro Lys Arg Val Thr Arg Ser Gly Glu Ser Pro Ser Asp Ala Leu Leu
 305 310 315 320
 Arg Gly Gly Ile Glu Tyr Ile Glu Val Arg Ser Leu Asp Ile Asn Pro
 325 330 335
 Phe Ser Pro Ile Gly Val Asp Glu Gln Gln Val Arg Phe Leu Asp Leu
 340 345 350
 Phe Met Val Trp Cys Ala Leu Ala Asp Ala Pro Glu Met Ser Ser Ser
 355 360 365
 Glu Leu Ala Cys Thr Arg Val Asn Trp Asn Arg Val Ile Leu Glu Gly
 370 375 380
 Arg Lys Pro Gly Leu Thr Leu Gly Ile Gly Cys Glu Thr Ala Gln Phe
 385 390 395 400
 Pro Leu Pro Gln Val Gly Lys Asp Leu Phe Arg Asp Leu Lys Arg Val
 405 410 415
 Ala Gln Thr Leu Asp Ser Ile Asn Gly Gly Glu Ala Tyr Gln Lys Val
 420 425 430
 Cys Asp Glu Leu Val Ala Cys Phe Asp Asn Pro Asp Leu Thr Phe Ser
 435 440 445
 Ala Arg Ile Leu Arg Ser Met Ile Asp Thr Gly Ile Gly Gly Thr Gly
 450 455 460
 Lys Ala Phe Ala Glu Ala Tyr Arg Asn Leu Leu Arg Glu Glu Pro Leu
 465 470 475 480
 Glu Ile Leu Arg Glu Glu Asp Phe Val Ala Glu Arg Glu Ala Ser Glu
 485 490 495
 Arg Arg Gln Gln Glu Met Glu Ala Ala Asp Thr Glu Pro Phe Ala Val
 500 505 510
 Trp Leu Glu Lys His Ala
 515

<210> 28

<211> 446

<212> PRT

<213> Escherichia coli

<400> 28

Met Met Ile Thr Leu Arg Lys Leu Pro Leu Ala Val Ala Val Ala Ala
 1 5 10 15

Gly Val Met Ser Ala Gln Ala Met Ala Val Asp Phe His Gly Tyr Ala
 20 25 30

Arg Ser Gly Ile Gly Trp Thr Gly Ser Gly Gly Glu Gln Gln Cys Phe
 35 40 45

Gln Thr Thr Gly Ala Gln Ser Lys Tyr Arg Leu Gly Asn Glu Cys Glu
 50 55 60

Thr Tyr Ala Glu Leu Lys Leu Gly Gln Glu Val Trp Lys Glu Gly Asp
 65 70 75 80

Lys Ser Phe Tyr Phe Asp Thr Asn Val Ala Tyr Ser Val Ala Gln Gln
 85 90 95

Asn Asp Trp Glu Ala Thr Asp Pro Ala Phe Arg Glu Ala Asn Val Gln
 100 105 110

Gly Lys Asn Leu Ile Glu Trp Leu Pro Gly Ser Thr Ile Trp Ala Gly
 115 120 125

Lys Arg Phe Tyr Gln Arg His Asp Val His Met Ile Asp Phe Tyr Tyr
 130 135 140

Trp Asp Ile Ser Gly Pro Gly Ala Gly Leu Glu Asn Ile Asp Val Gly
 145 150 155 160

Phe Gly Lys Leu Ser Leu Ala Ala Thr Arg Ser Ser Glu Ala Gly Gly
 165 170 175

Ser Ser Ser Phe Ala Ser Asn Asn Ile Tyr Asp Tyr Thr Asn Glu Thr
 180 185 190

Ala Asn Asp Val Phe Asp Val Arg Leu Ala Gln Met Glu Ile Asn Pro
 195 200 205

Gly Gly Thr Leu Glu Leu Gly Val Asp Tyr Gly Arg Ala Asn Leu Arg
 210 215 220

Asp Asn Tyr Arg Leu Val Asp Gly Ala Ser Lys Asp Gly Trp Leu Phe
 225 230 235 240

Thr Ala Glu His Thr Gln Ser Val Leu Lys Gly Phe Asn Lys Phe Val
 245 250 255

Val Gln Tyr Ala Thr Asp Ser Met Thr Ser Gln Gly Lys Gly Leu Ser
 260 265 270

Gln Gly Ser Gly Val Ala Phe Asp Asn Glu Lys Phe Ala Tyr Asn Ile
 275 280 285

Asn Asn Asn Gly His Met Leu Arg Ile Leu Asp His Gly Ala Ile Ser
 290 295 300

Met Gly Asp Asn Trp Asp Met Met Tyr Val Gly Met Tyr Gln Asp Ile
 305 310 315 320

Asn Trp Asp Asn Asp Asn Gly Thr Lys Trp Trp Thr Val Gly Ile Arg
 325 330 335

Pro Met Tyr Lys Trp Thr Pro Ile Met Ser Thr Val Met Glu Ile Gly
 340 345 350

Tyr Asp Asn Val Glu Ser Gln Arg Thr Gly Asp Lys Asn Asn Gln Tyr
355 360 365

Lys Ile Thr Leu Ala Gln Gln Trp Gln Ala Gly Asp Ser Ile Trp Ser
370 375 380

Arg Pro Ala Ile Arg Val Phe Ala Thr Tyr Ala Lys Trp Asp Glu Lys
385 390 395 400

Trp Gly Tyr Asp Tyr Thr Gly Asn Ala Asp Asn Asn Ala Asn Phe Gly
405 410 415

Lys Ala Val Pro Ala Asp Phe Asn Gly Gly Ser Phe Gly Arg Gly Asp
420 425 430

Ser Asp Glu Trp Thr Phe Gly Ala Gln Met Glu Ile Trp Trp
435 440 445

<210> 29

<211> 111

<212> PRT

<213> Escherichia coli

<400> 29

Met Met Ile Thr Leu Arg Lys Leu Pro Leu Ala Val Ala Val Ala Ala
1 5 10 15

Gly Val Met Ser Ala Gln Ala Met Ala Val Asp Phe His Gly Tyr Ala
20 25 30

Arg Ser Gly Ile Gly Trp Thr Gly Ser Gly Gly Glu Gln Gln Cys Phe
35 40 45

Gln Thr Thr Gly Ala Gln Ser Lys Tyr Arg Leu Gly Asn Glu Cys Glu
50 55 60

Thr Tyr Ala Glu Leu Lys Leu Gly Gln Glu Val Trp Lys Glu Gly Asp
65 70 75 80

Lys Ser Phe Tyr Phe Asp Thr Asn Val Ala Tyr Ser Val Ala Gln Gln
85 90 95

Asn Asp Trp Glu Ala Thr Asp Pro Ala Phe Arg Glu Ala Asn Val
100 105 110

<210> 30

<211> 1476

<212> DNA

<213> Escherichia coli

<400> 30

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ccggacaccc ggattatcgg cgtagggcgt gctgactggg ataaagcggc atataccaaa	180
gttggtccgcg aggcgctcga aactttcatg aaagaaacca ttgatgaagg tttatgggac	240
accctgagtg cacgtctgga tttttgtaat ctcgatgtca atgacactgc tgcattcagc	300
cgtctcggcg cgatgctgga tcaaaaaaat cgtatcacca ttaactactt tgccatgccg	360
cccagcactt ttggcgcaat ttgcaaaggg cttggcgagg caaaactgaa tgctaaaccg	420
gcacgcgtag tcatggagaa accgctgggg acgtcgctgg cgacctcgca ggaaatcaat	480
gatcagggttg gcgaataactt cgaggagtgc caggtttaacc gtatcgacca ctatcttggt	540
aaagaaacgg tgctgaacct gttggcgctg cgttttgcta actccctggt tgtgaataac	600
tgggacaatc gcaccattga tcatgttgag attaccgtag cagaagaagt ggggatcgaa	660
ccccgctggc gctatcttgc taaagggcgt gacatcgagg aactgctgca gaaagcagtc	720

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gggagctggg gctatttga caagccggc cagatggcg acatgatca gaaccactg      720
ctgcaaattc tttgcatgat tgcgatgtct ccgccgtctg acctgagcgc agacagcatc      780
cgcgatgaaa aagtgaagt actgaagtct ctgcgcgcga tcgaccgctc caacgtacgc      840
gaaaaaaccg tacgcgggca atatactgcg ggcttcgccc agggcaaaaa agtgccggga      900
tatctggaag aagagggcgc gaacaagagc agcaatacag aaactttcgt ggcgatccgc      960
gtcgacattg ataactggcg ctgggccggg gtgccattct acctgcgtac tggtaaactg     1020
ctgccgacca aatgttctga agtcgtggtc tatttcaaaa cacctgaact gaatctgttt     1080
aaagaatcgt ggcaggatct gccgcagaat aaactgacta tccgtctgca acctgatgaa     1140
ggcgtggata tccagggtact gaataaagtt cctggccttg accacaaaca taacctgcaa     1200
atcaccaagc tggatctgag ctattcagaa acctttaatc agacgcatct ggcggtatgcc     1260
tatgaacgtt tgctgctgga aaccatgcgt ggtattcagg cactgtttgt acgtcgcgac     1320
gaagtggaag aagcctggaa atgggtagac tccattactg aggcgtgggc gatggacaat     1380
gatgcgccga aaccgtatca ggccggaacc tggggaccog ttgcctcggg ggcgatgatt     1440
acctgtgatg gtcgttcctg gaatgagttt gagtaa                               1476

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<210> 31

<211> 439

<212> PRT

<213> Escherichia coli

<400> 31

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Met Thr His Gln Leu Arg Ser Arg Asp Ile Ile Ala Leu Gly Phe Met
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Thr Phe Ala Leu Phe Val Gly Ala Gly Asn Ile Ile Phe Pro Pro Met
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Val Gly Leu Gln Ala Gly Glu His Val Trp Thr Ala Ala Phe Gly Phe
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Leu Ile Thr Ala Val Gly Leu Pro Val Leu Thr Val Val Ala Leu Ala
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Lys Val Gly Gly Gly Val Asp Ser Leu Ser Thr Pro Ile Gly Lys Val
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Ala Gly Val Leu Leu Ala Thr Val Cys Tyr Leu Ala Val Gly Pro Leu
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Phe Ala Thr Pro Arg Thr Ala Thr Val Ser Phe Glu Val Gly Ile Ala
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Pro Leu Thr Gly Asp Ser Ala Leu Pro Leu Phe Ile Tyr Ser Leu Val
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Val Asp Gln Ser Ala Asn Gly Ala Ala Ile Leu His Ala Tyr Val Gln
260 265 270

His Thr Phe Gly Gly Gly Gly Ser Phe Leu Leu Ala Ala Leu Ile Phe
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Ile Ala Cys Leu Val Thr Ala Val Gly Leu Thr Cys Ala Cys Ala Glu
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325 330 335

Ile Gln Ile Ser Val Pro Val Leu Thr Ala Ile Tyr Pro Pro Cys Ile
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Arg Val Ile Ala Pro Pro Met Phe Ile Ser Leu Leu Phe Gly Ile Leu
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Asp Gly Ile Lys Ala Ser Ala Phe Ser Asp Ile Leu Pro Ser Trp Ala
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Gln Arg Leu Pro Leu Ala Glu Gln Gly Leu Ala Trp Leu Met Pro Thr
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REFERENCES CITED IN THE DESCRIPTION

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P a t e n t k r a v

1. Bakterie tilhørende *Enterobacteriaceae*-familien, som er modificeret til at inaktivere generne *sdaA*, *sdaB*, *tdcG* og *glyA*.

5

2. Bakterie ifølge krav 1, hvor bakterieen er blevet yderligere modificeret til at overeksprimere en 3-phosphoglycerat-dehydrogenase, en phosphoserin-phosphatase og en phosphoserin-aminotransferase.

10

3. Bakterie ifølge krav 1 eller 2, hvor bakterien er i stand til at vokse i et minimalt dyrkningsmedium omfattende L-serin i en koncentration på mindst ca. 6,25 g/l.

15

4. Bakterie ifølge et hvilket som helst af kravene 1 til 3, hvor bakterien er i stand til at vokse i et minimalt dyrkningsmedium omfattende L-serin i en koncentration på mindst 6,25 g/l med en vækstrate på mindst 0,1 hr⁻¹ under eksponentiel vækst.

20

5. Bakterie ifølge et hvilket som helst af kravene 1 til 4, hvor bakterieen i *thrA*-genet omfatter en eller flere nukleotidsubstitutioner, som resulterer i en eller flere aminosyresubstitutioner i en position udvalgt fra gruppen bestående af Y356, S357 og S359.

25

6. Bakterie ifølge et hvilket som helst af kravene 1 til 5, hvor bakterien i *thrA*-genet omfatter en eller flere nukleotidsubstitutioner, som resulterer i en aminosyresubstitution i det kodede polypeptid i position Y356, en eller flere nukleotidsubstitutioner, som resulterer i en aminosyresubstitution i det kodede polypeptid i position S357, og/eller en eller flere nukleotidsubstitutioner, som resulterer i en aminosyresubstitution i det kodede polypeptid i position S359; hvor substitutionen i position Y356 er udvalgt fra gruppen bestående af Y356C, Y356T, Y356V, Y356S, Y356W, Y356Q, Y356G, Y356N, Y356D, Y356E, Y356F, Y356A, Y356I, Y356P, Y356H, Y356R, Y356L; substitutionen i position S357 er udvalgt fra gruppen bestående af S357R, S357V, S357P, S357G, S357L, S357Y, S357A, S357N, S357F, S357H, S357K, S357I og S357M; og

30

substitutionen i position S359 er udvalgt fra gruppen bestående af S359R, S359G, S359M, S359F, S359T, S359P, S359V, S359Q, S359A, S359C, S359K, S359E og S359L.

- 5 **7.** Bakterie ifølge et hvilket som helst af kravene 1 til 6, hvor bakterien eksprimerer et polypeptid, der kodes af *thrA*-genet, hvor polypeptidet omfatter en eller flere aminosyresubstitutioner udvalgt fra gruppen bestående af Y356C, S357R og S359R.
- 10 **8.** Bakterie ifølge et hvilket som helst af kravene 1 til 7, hvor bakterien eksprimerer et polypeptid med en aminosyresekvens, som har mindst ca. 90 % sekvensidentitet med aminosyresekvensen anført i SEQ ID NO: 11, som omfatter en aminosyresubstitution ved position Y356, S357 og/eller S359, hvor substitutionen i position Y356 er udvalgt fra gruppen bestående af Y356C, Y356T, 15 Y356V, Y356S, Y356W, Y356Q, Y356G, Y356N, Y356D, Y356E, Y356F, Y356A, Y356I, Y356P, Y356H, Y356R og Y356L; substitutionen i position S357 er udvalgt fra gruppen bestående af S357R, S357V, S357P, S357G, S357L, S357Y, S357A, S357N, S357F og S357H; og substitutionen i position S359 er udvalgt fra gruppen bestående af S359R, S359G, S359M, S359F, 20 S359T, S359P, S359V, S359Q, S359A, S359C, S359K, S359E og S359L.
- 9.** Bakterie ifølge et hvilket som helst af kravene 1 til 8, hvor bakterien er blevet yderligere modificeret til at overeksprimere genet *ydeD*.
- 25 **10.** Bakterie ifølge et hvilket som helst af kravene 1 til 9, hvor bakterien omfatter en eller flere genmutationer udvalgt fra gruppen bestående af: en eller flere nukleotidsubstitutioner i *lrp*-genet, som resulterer i aminosyresubstitutionen D143G i det kodede polypeptid, en eller flere nukleotidsubstitutioner i *rho*-genet, som resulterer i aminosyresubstitutionen R87L i det kodede polypeptid, 30 en eller flere nukleotidsubstitutioner i *eno*-genet, som resulterer i aminosyresubstitutionen V164L i det kodede polypeptid, en eller flere nukleotidsubstitutioner i *argP*-genet, som resulterer i aminosyresubstitutionen Q132K i det kodede polypeptid, en eller flere nukleotidsubstitutioner i *tufA*-genet, som resul-

terer i amisubstitutionen G19V i det kodede polypeptid, en eller flere nukleotid-substitutioner i *cycA*-genet, som resulterer i aminosyresubstitutionen I220V i det kodede polypeptid, en eller flere nukleotidsubstitutioner i *rpe*-genet, som resulterer i aminosyresubstitutionen I202T i det kodede polypeptid, en eller
 5 flere nukleotidsubstitutioner i *yojl*-genet, som resulterer i aminosyresubstituti-
 onen D334H i det kodede polypeptid, en eller flere nukleotidsubstitutioner i
hyaF-genet, som resulterer i aminosyresubstitutionen V120G i det kodede po-
 lypeptid, en eller flere nukleotidsubstitutioner i *pykF*-genet, som resulterer i
 aminosyresubstitutionen E250* i det kodede polypeptid, hvor * betegner et
 10 stopkodon, en eller flere nukleotidsubstitutioner i *malT*-genet, som resulterer i
 aminosyresubstitutionen Q420* i det kodede polypeptid, hvor * betegner et
 stopkodon, en eller flere nukleotidsubstitutioner i *rpoB*-genet, som resulterer i
 aminosyresubstitutionen P520L i det kodede polypeptid, en eller flere nu-
 kleotidsubstitutioner i *fumB*-genet, som resulterer i aminosyresubstitutionen
 15 T218P i det kodede polypeptid, en eller flere nukleotidsubstitutioner i *gshA*-
 genet, som resulterer i aminosyresubstitutionen A178V i det kodede polypep-
 tid, og en eller flere nukleotidsubstitutioner i *lamB*-genet, som resulterer i ami-
 nosyresubstitutionen Q112* i det kodede polypeptid, hvor * betegner et stop-
 kodon.

20

11. Bakterie ifølge et hvilket som helst af kravene 1 til 10, hvor bakterien tilhø-
 rer slægten *Escherichia*.

25

12. Bakterie ifølge et hvilket som helst af kravene 1 til 11, hvor bakterien er
Escherichia coli.

30

13. Fremgangsmåde til fremstilling af L-serin eller et L-serin-derivat, hvilken
 fremgangsmåde omfatter at dyrke en bakterie ifølge et hvilket som helst af
 kravene 1 til 12 i et dyrkningsmedium.

14. Fremgangsmåde ifølge krav 13, hvor L-serin-derivatet er udvalgt fra grup-
 pen bestående af L-cystein, L-methionin, L-glycin, O-acetylserin, L-tryptophan,
 thiamin, ethanolamin og ethylenglycol.

DRAWINGS

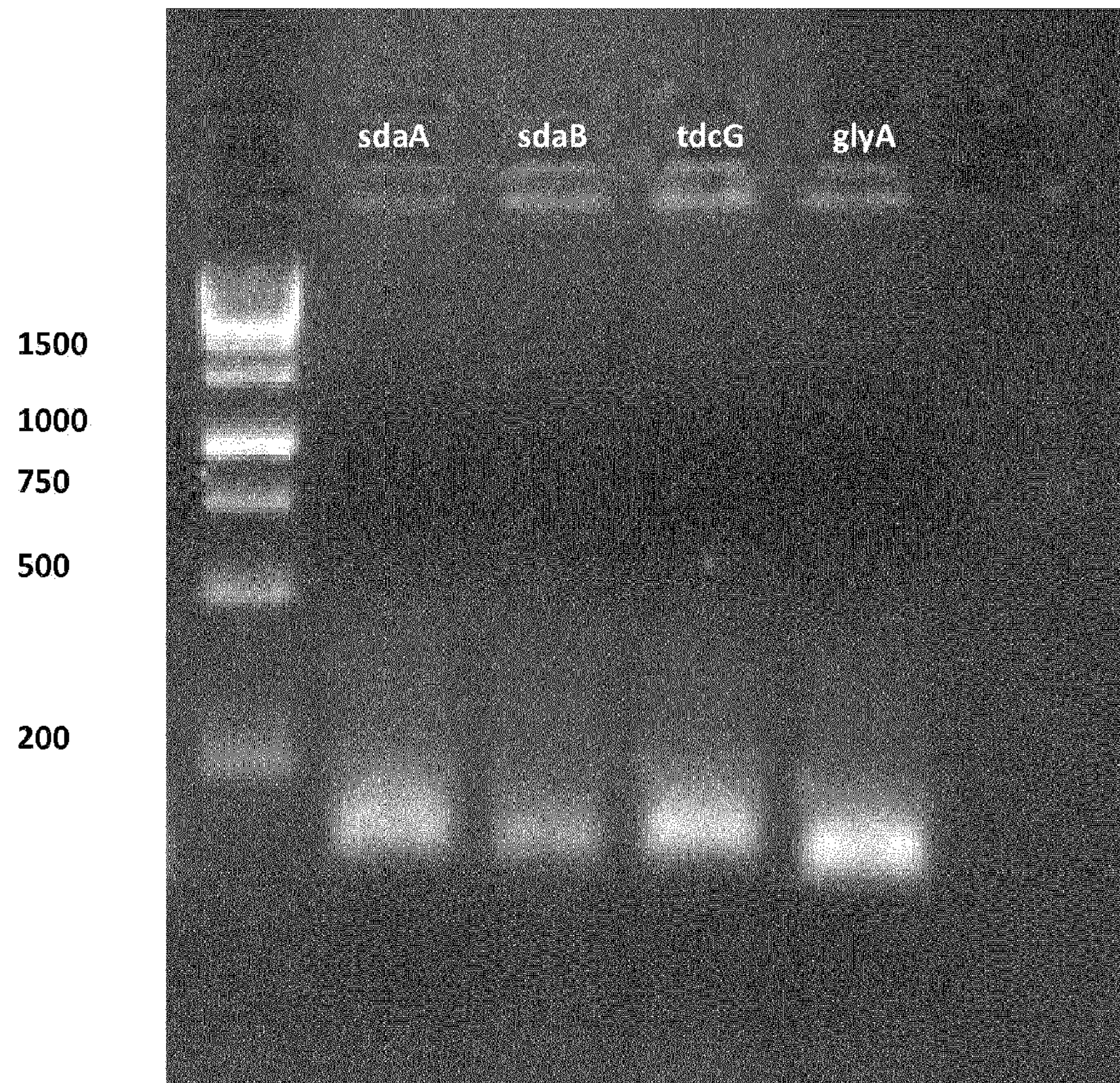


Figure 1

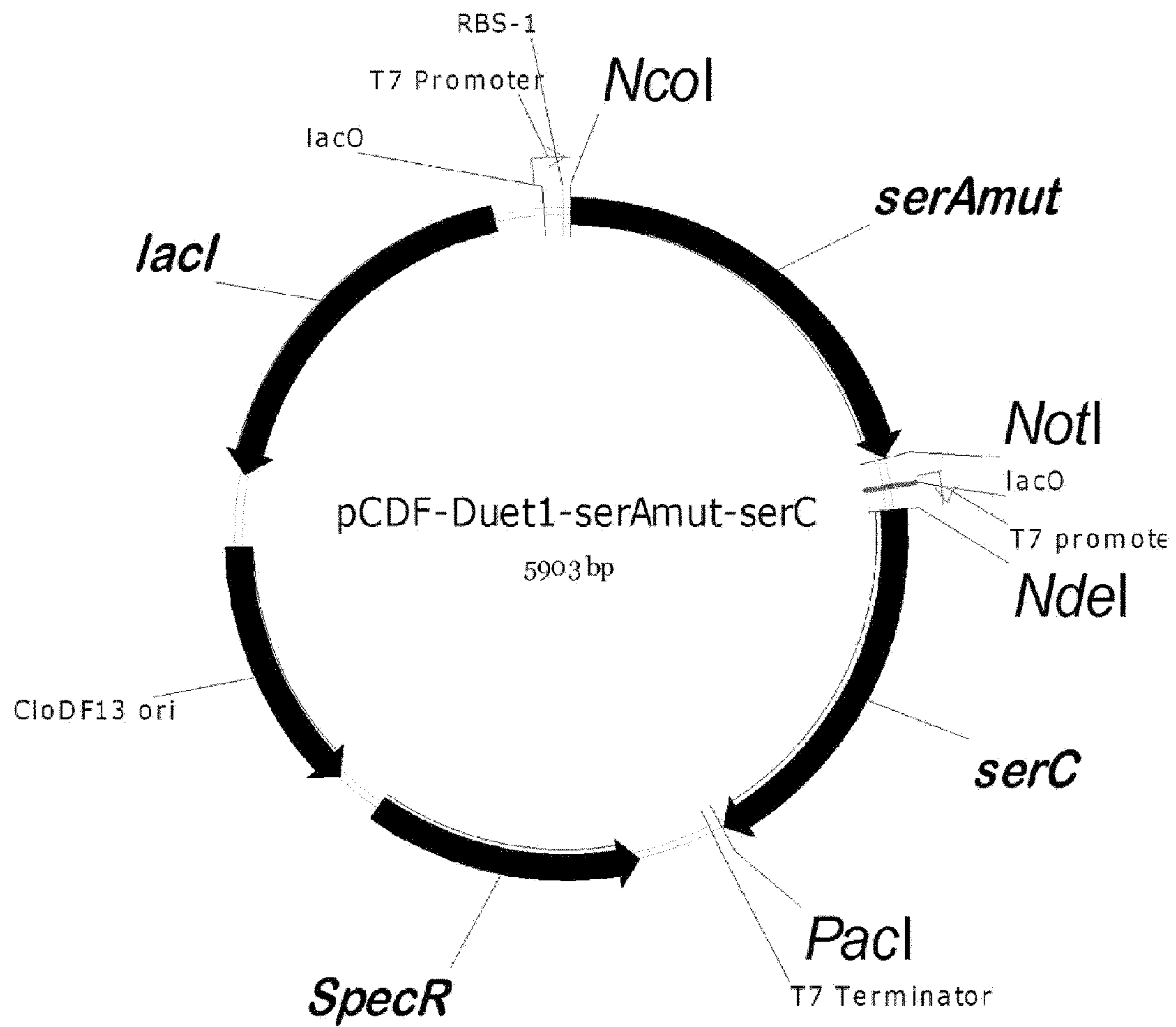


Figure 2

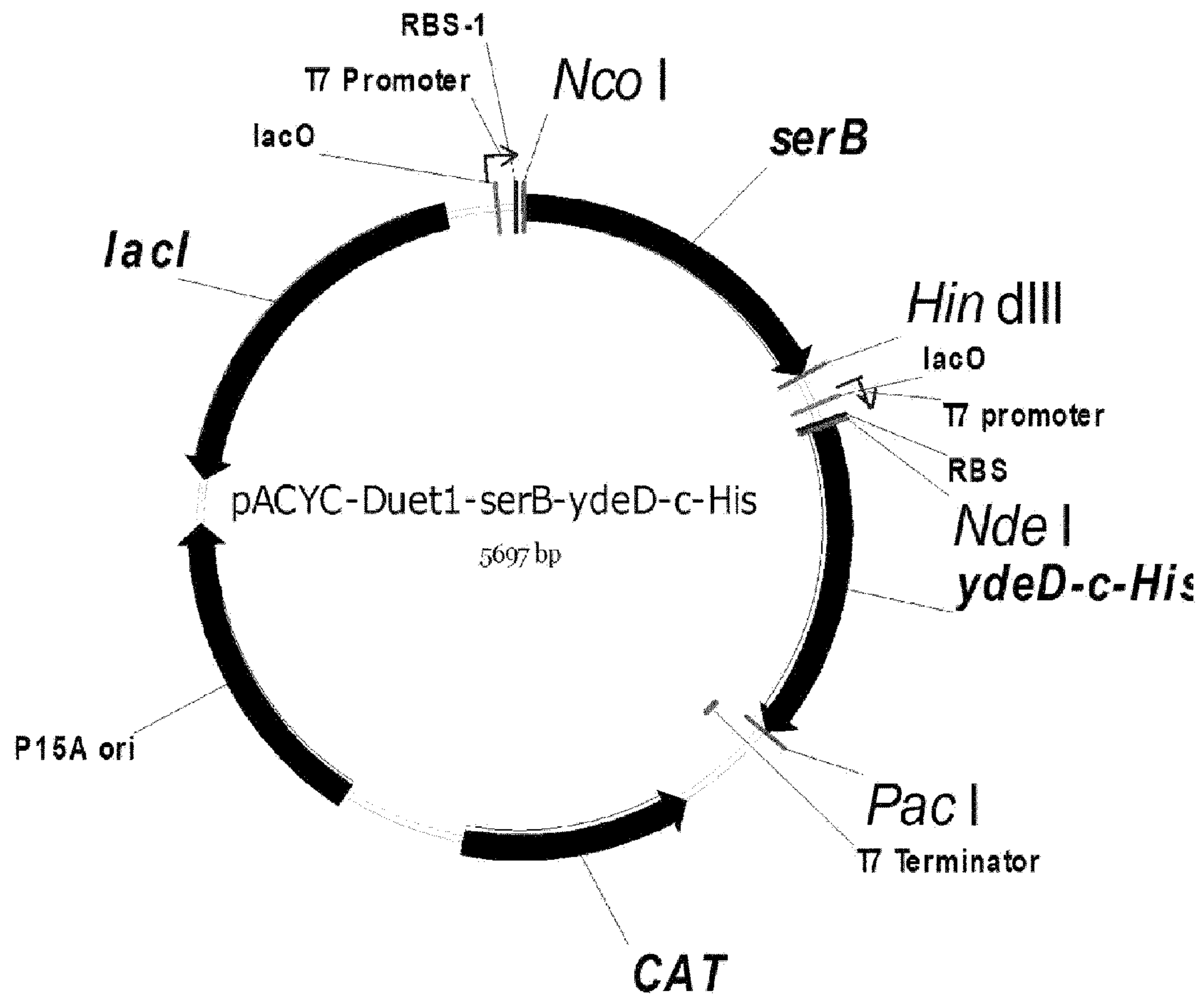


Figure 2 (cont.)

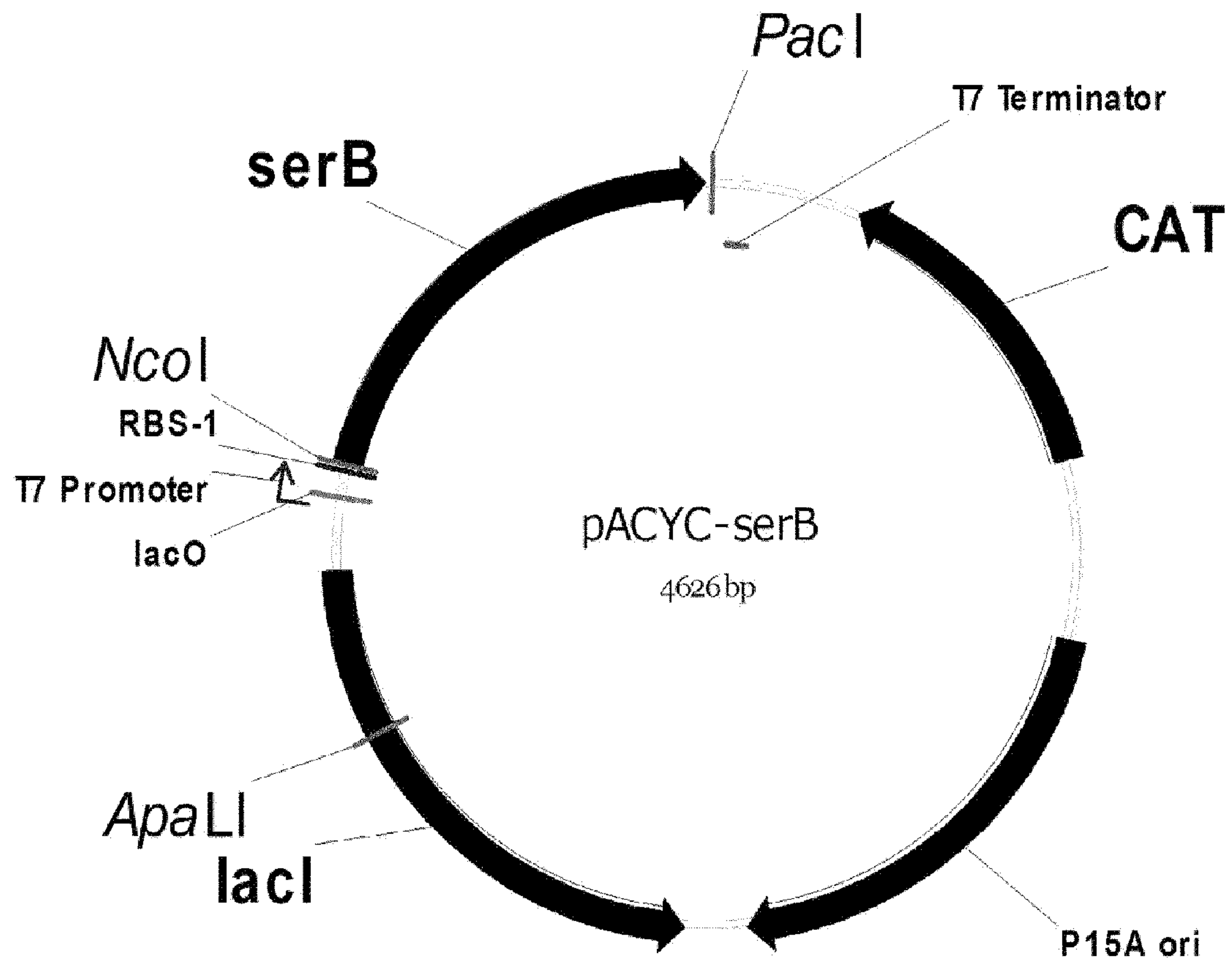


Figure 2 (cont.)

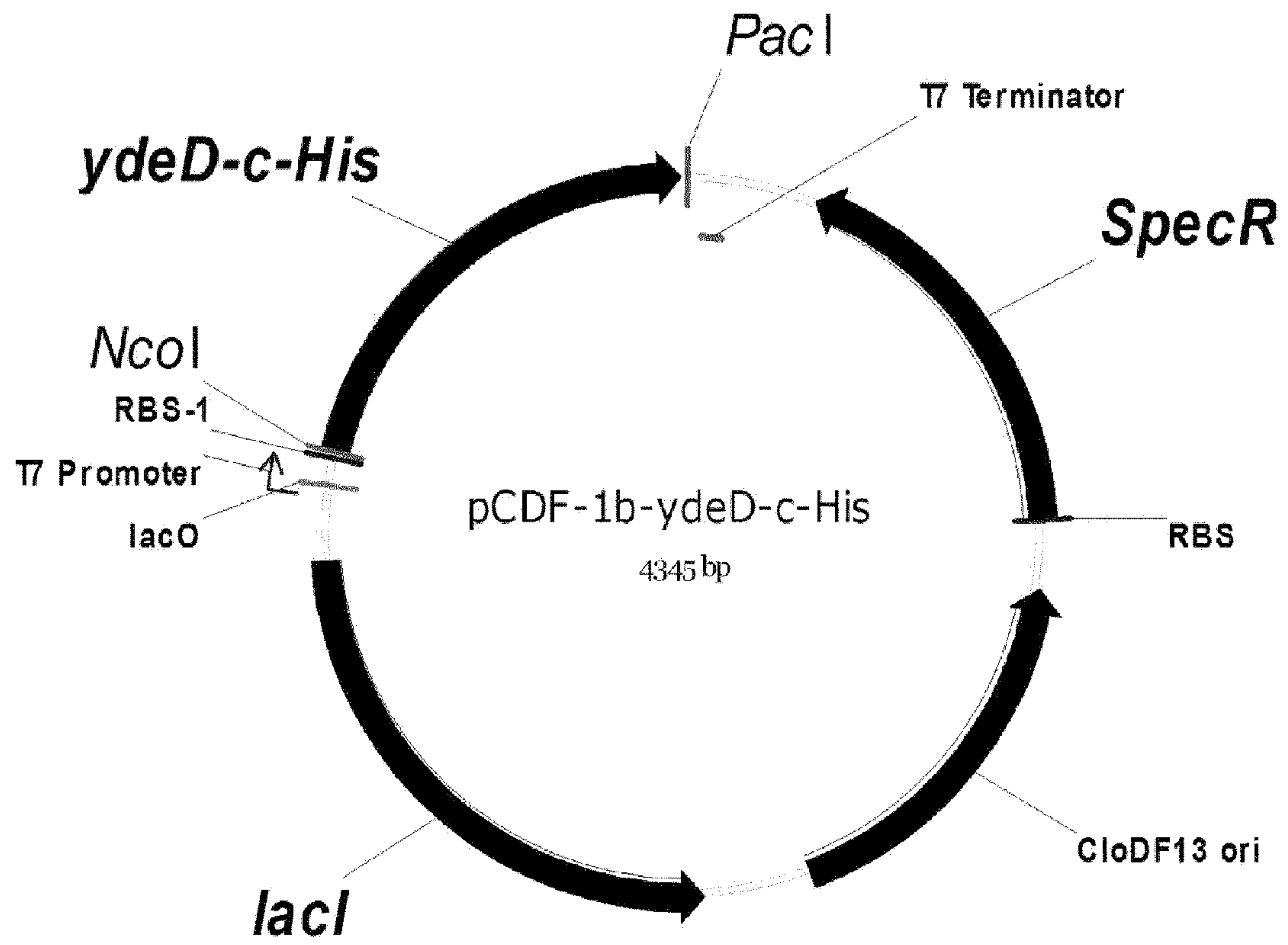


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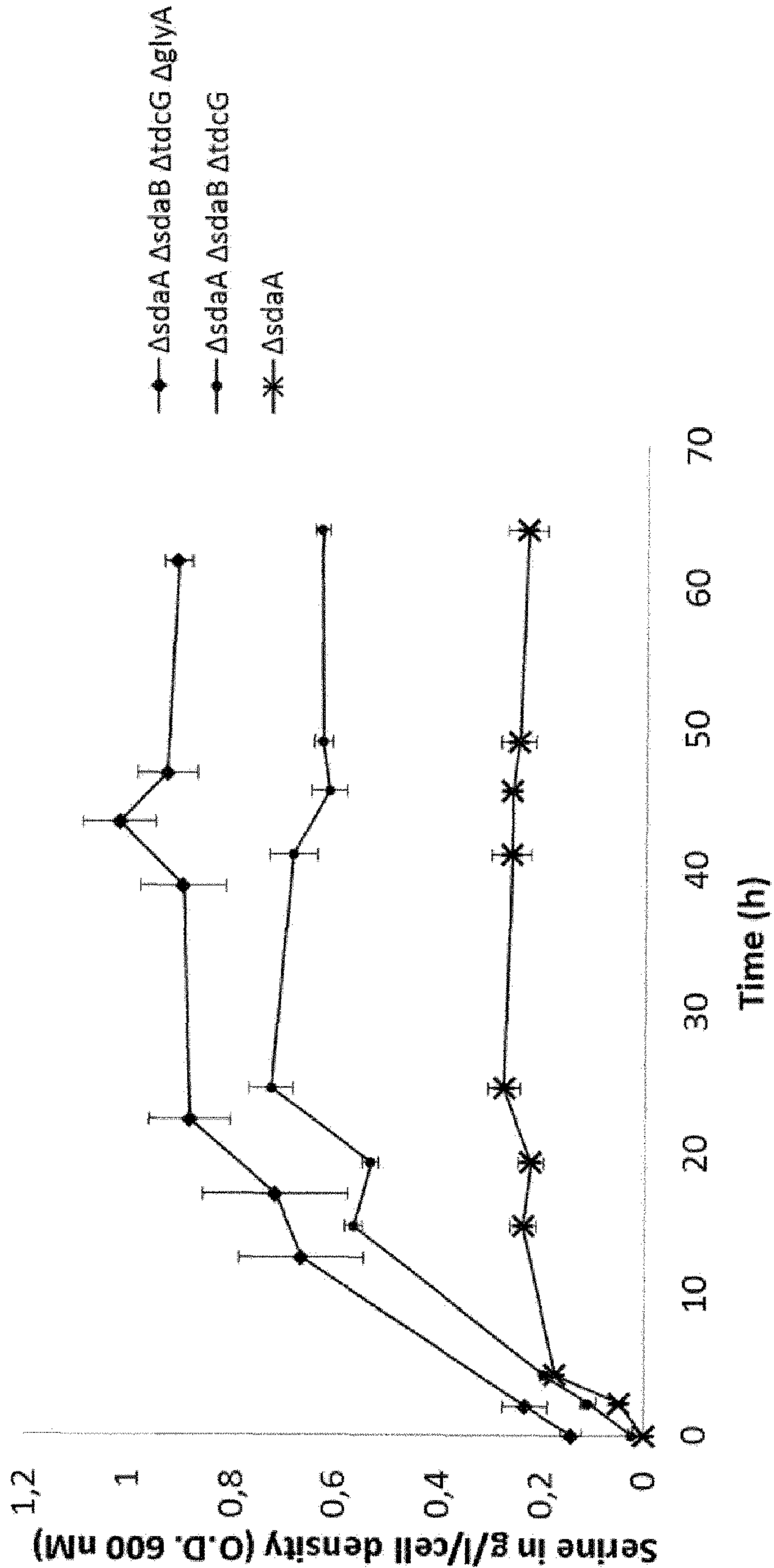


Figure 3

	Serine concentration in g/L (+/- standard deviation)	Yield from glucose (g serine / g glucose)
$\Delta sdaA \Delta sdaB \Delta tdcG \Delta glyA$ (Q1(DE3))	0.889 (+/- 0.057)	0.445
$sdaA \Delta sdaB \Delta tdcG ::(DE3)$	0.536 (+/- 0.019)	0.268
$\Delta sdaA ::(DE3)$	0.493 (+/- 0.033)	0.247

Growth profile with overexpression of exporter ydeD in Q1(DE3)

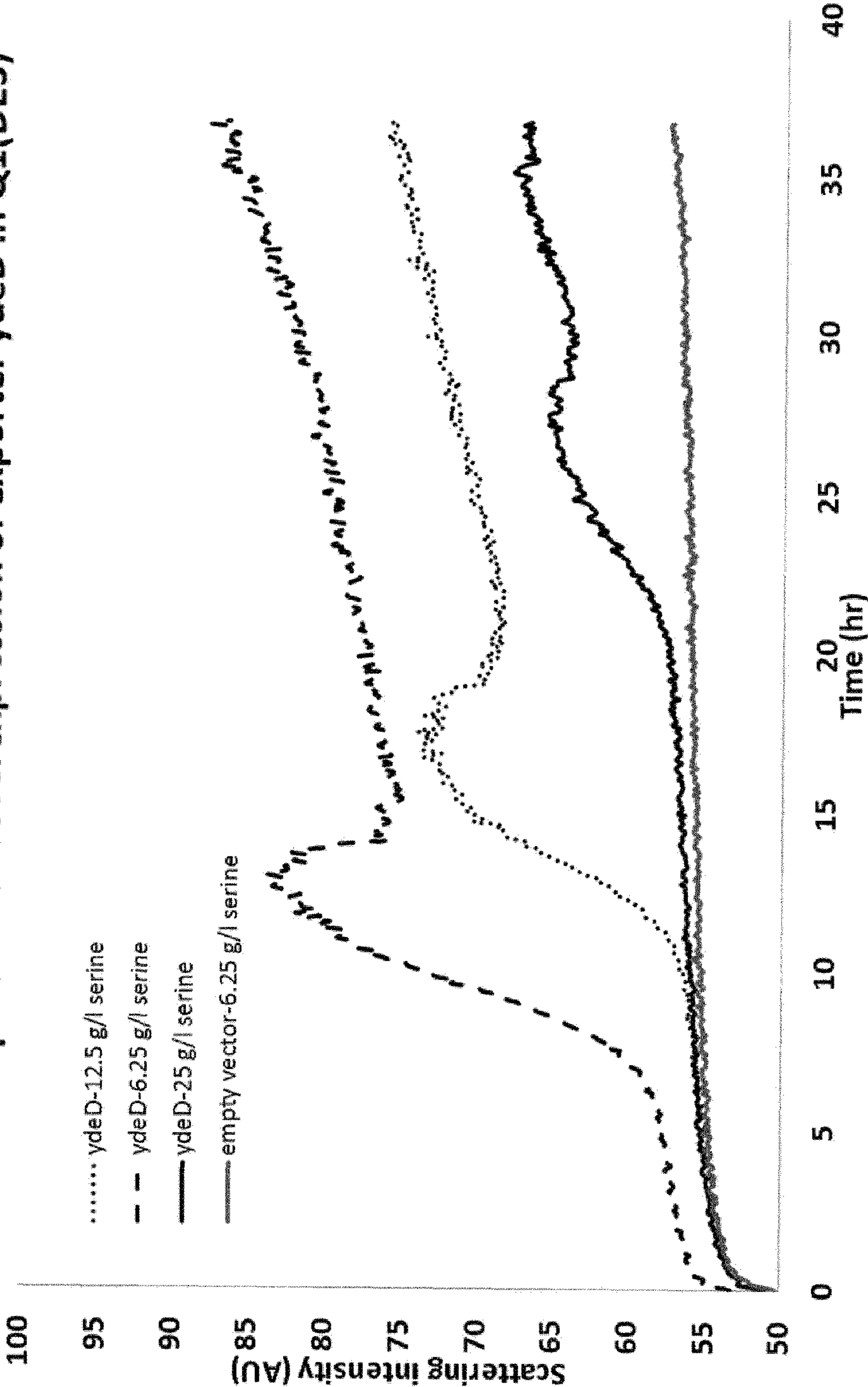


Figure 4

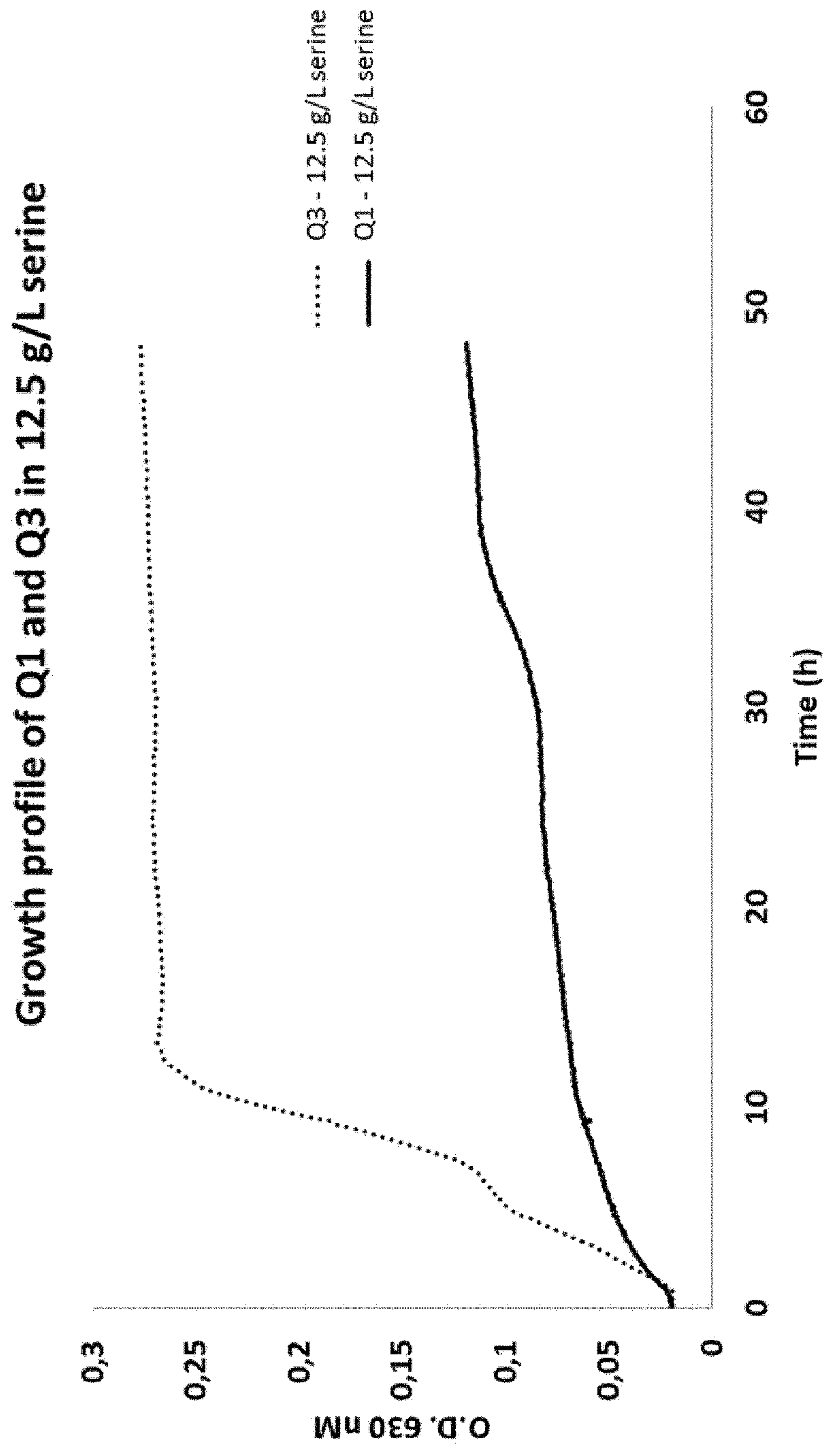


Figure 5

6A.

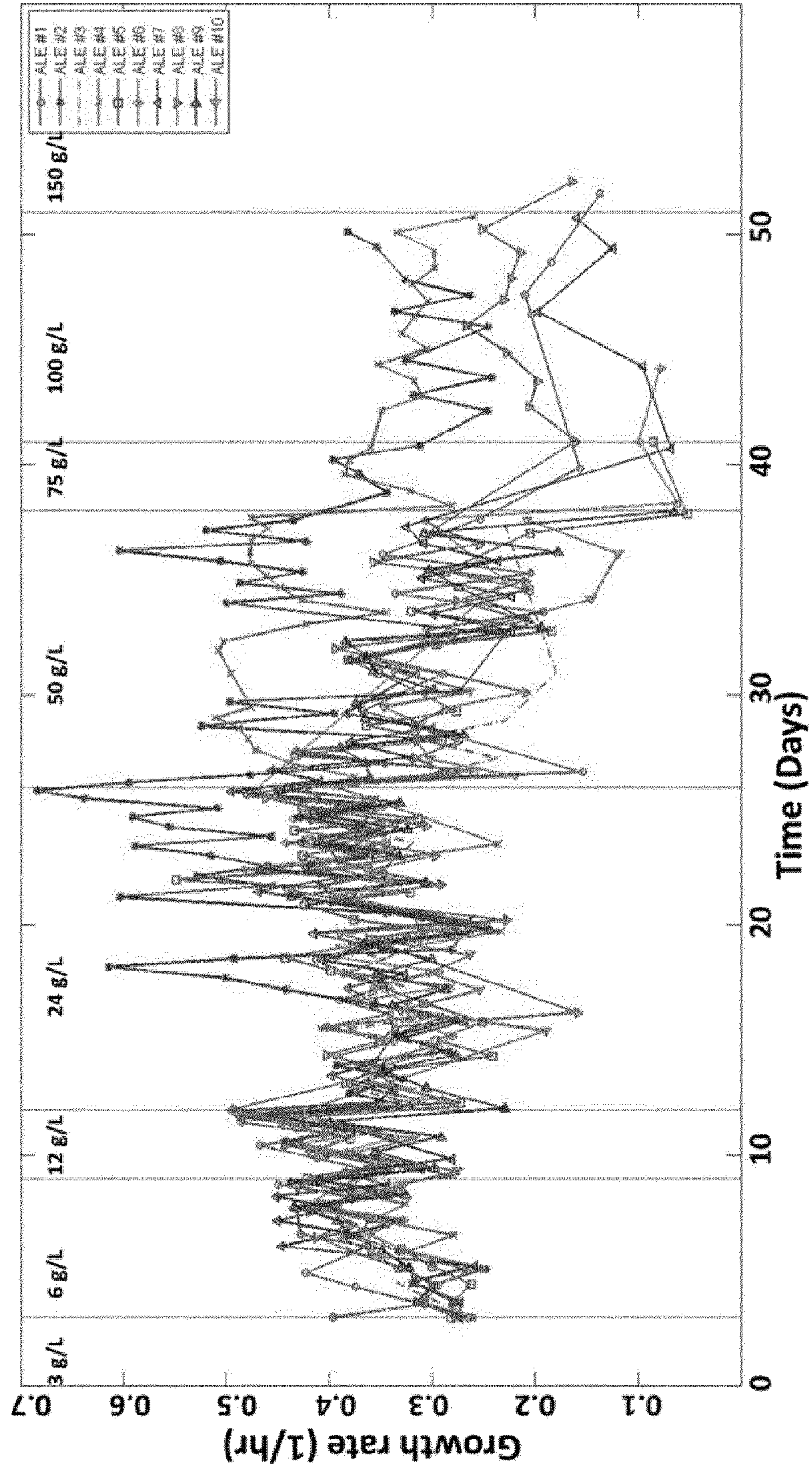


Figure 6

6B.

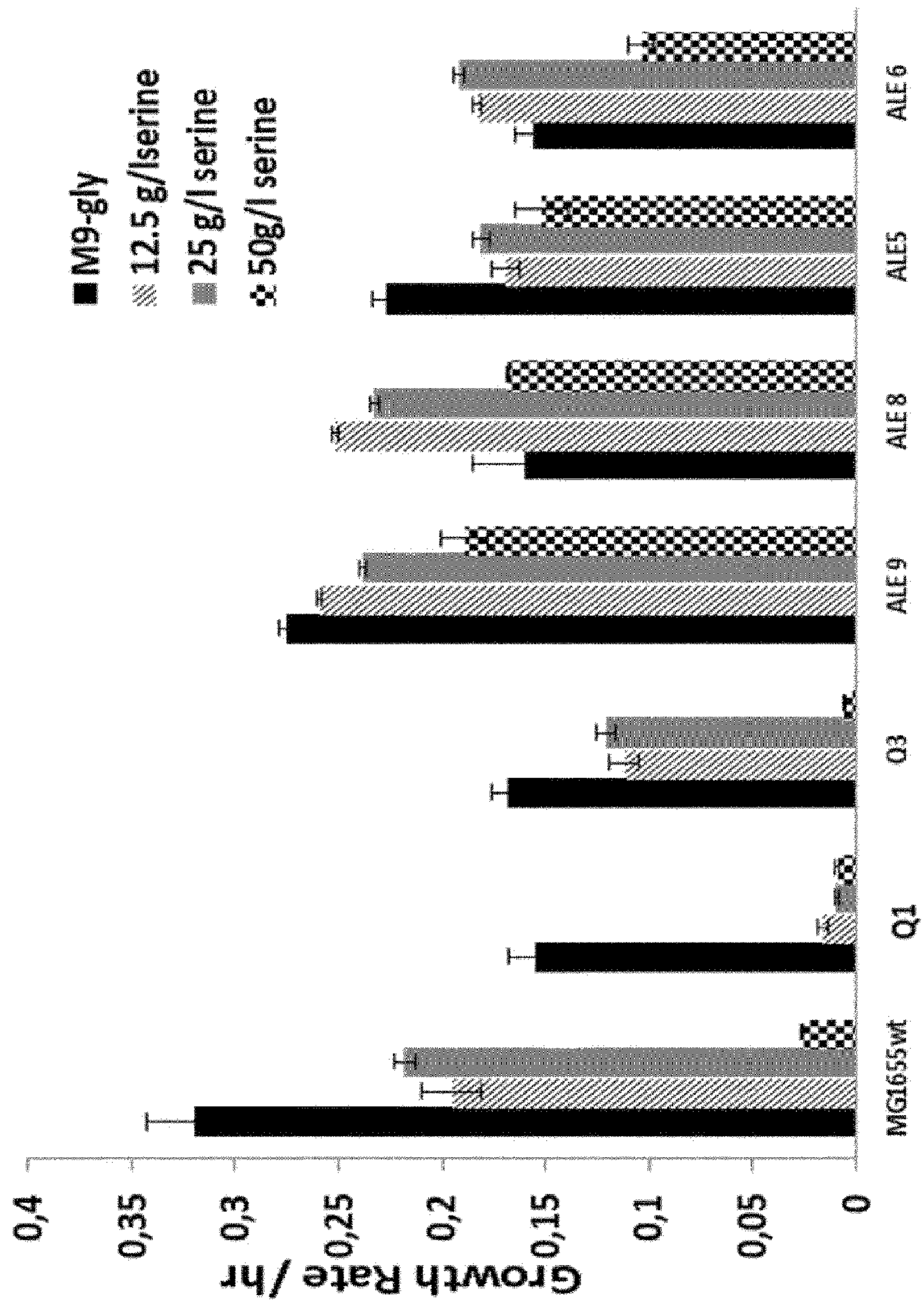


Figure 6

6C.

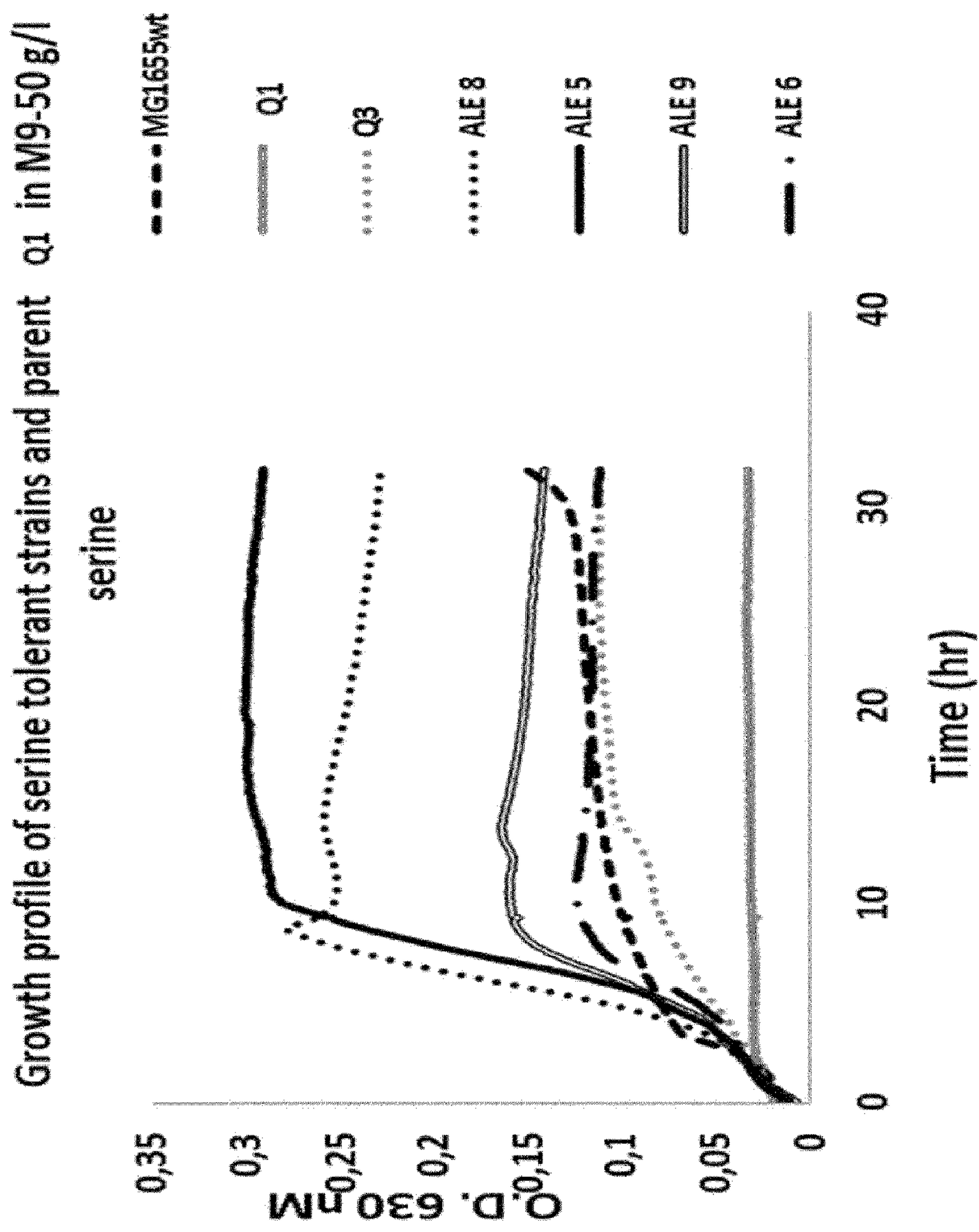


Figure 6

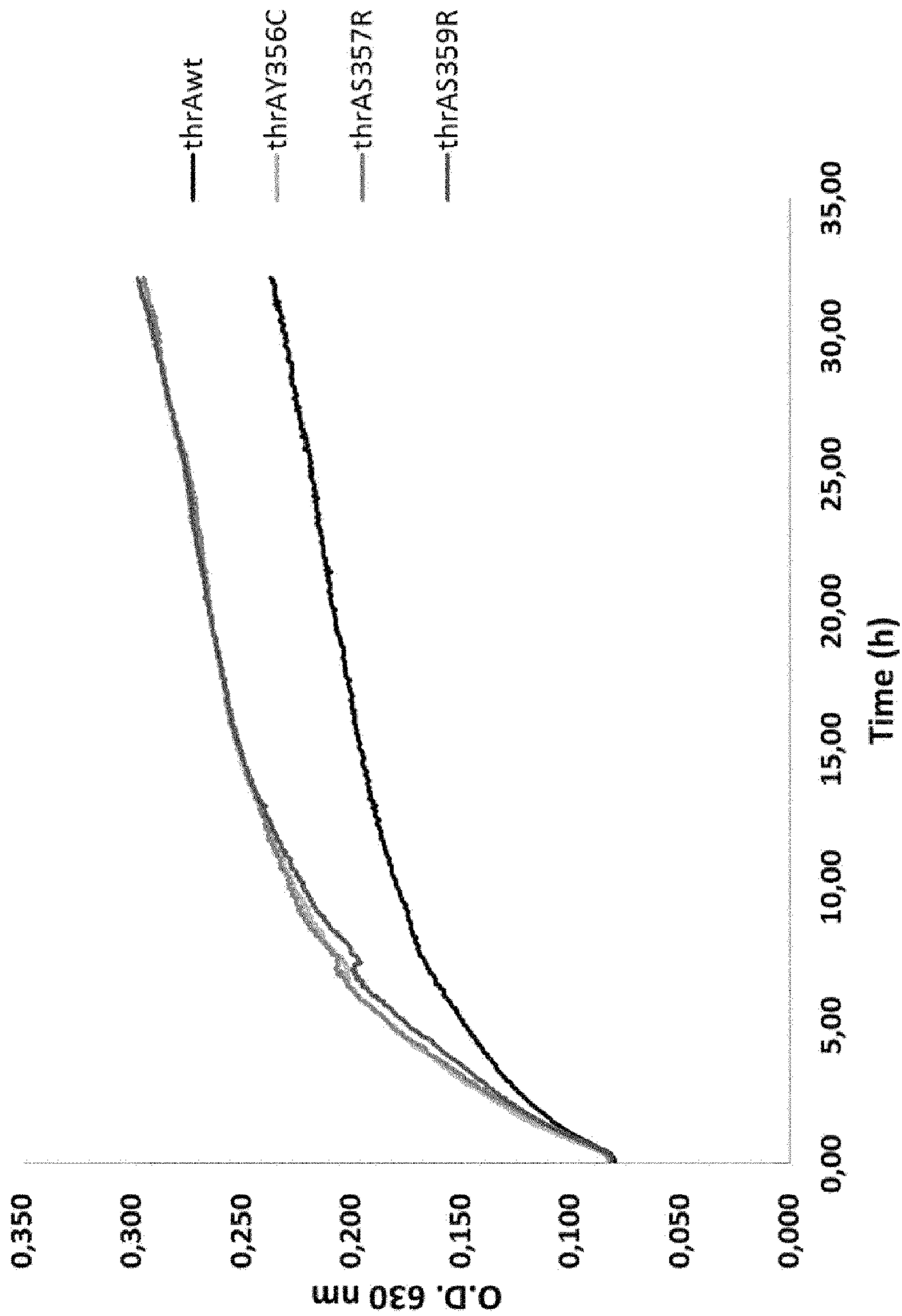


Figure 7

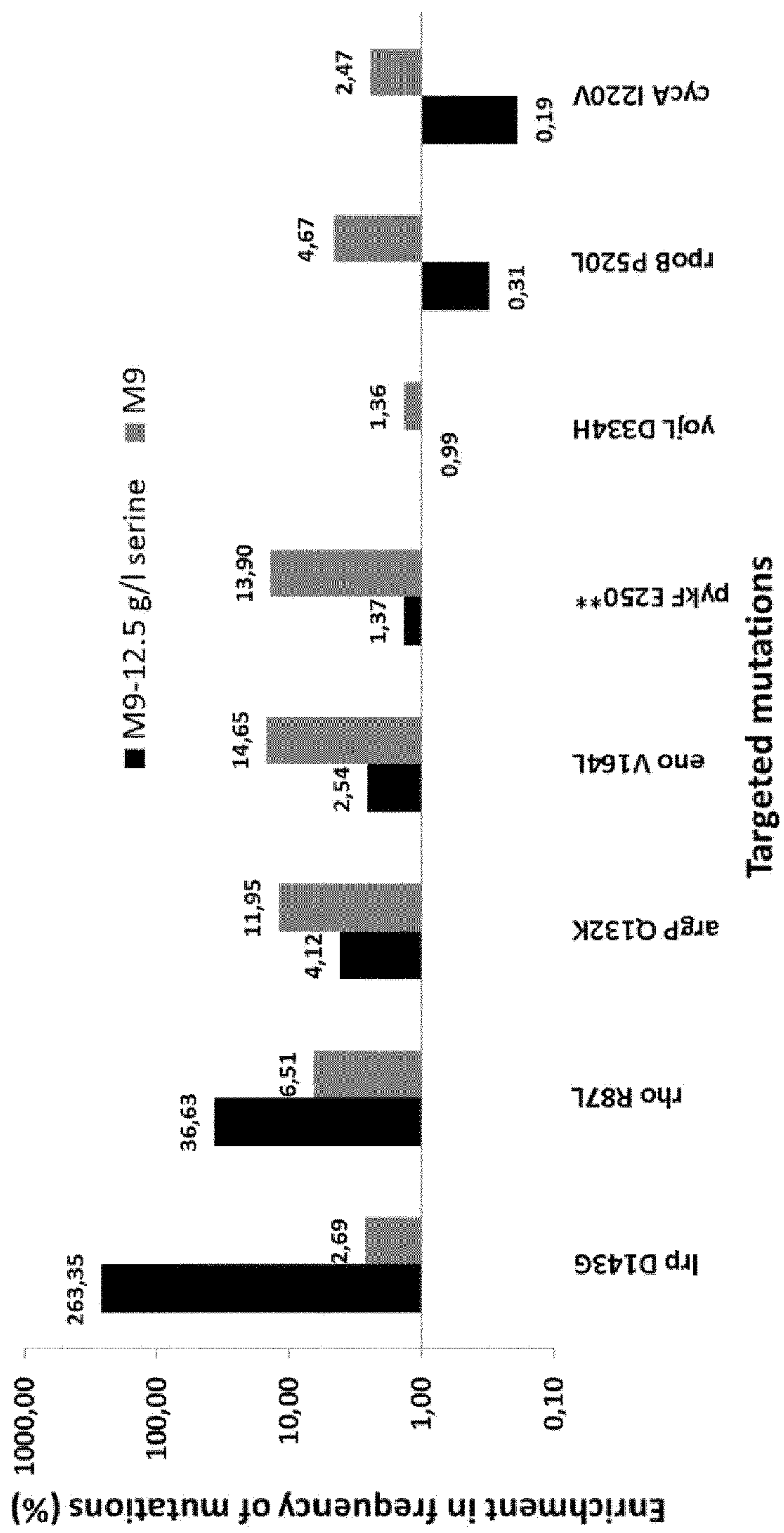


Figure 8

9A.

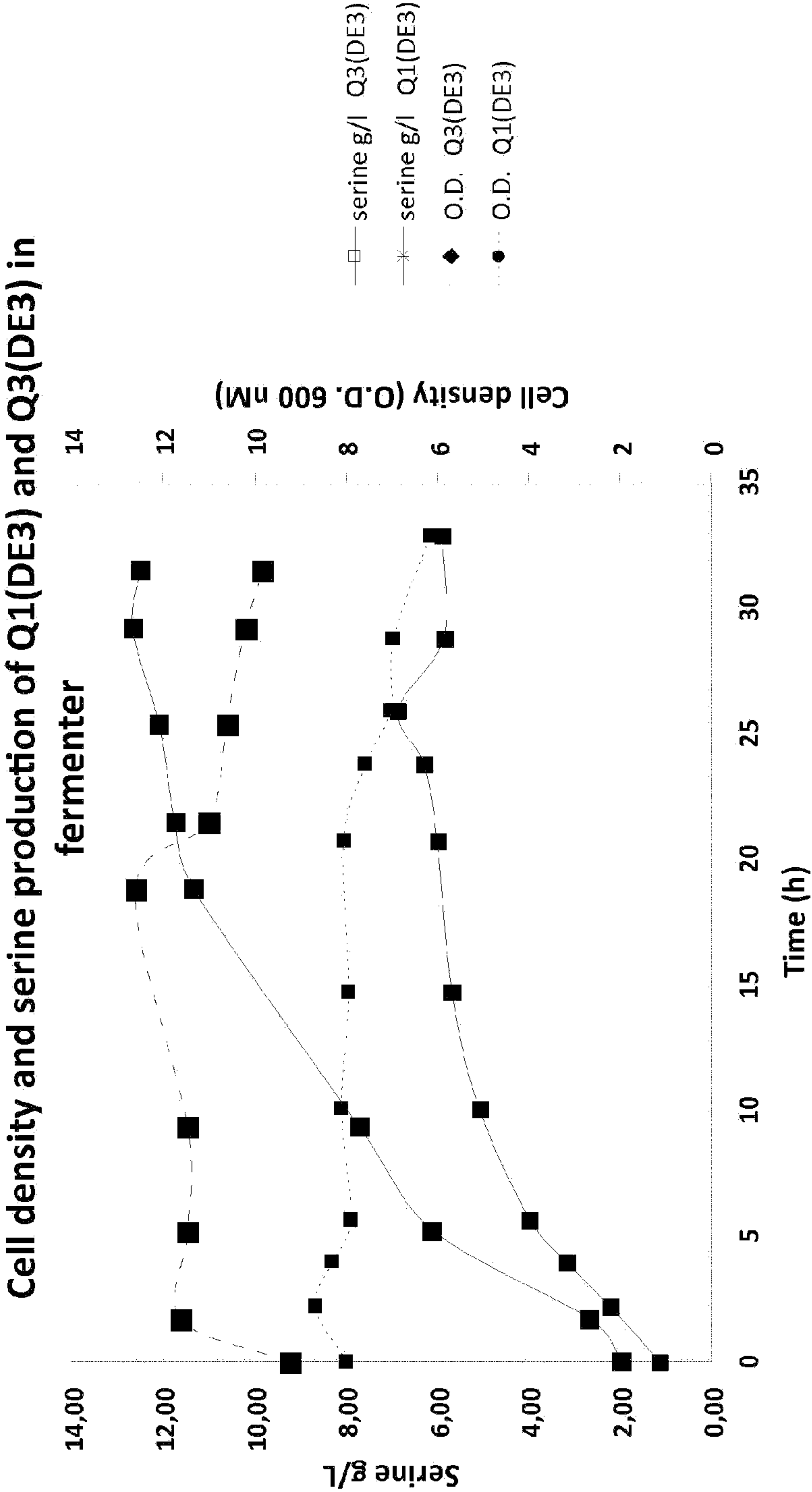
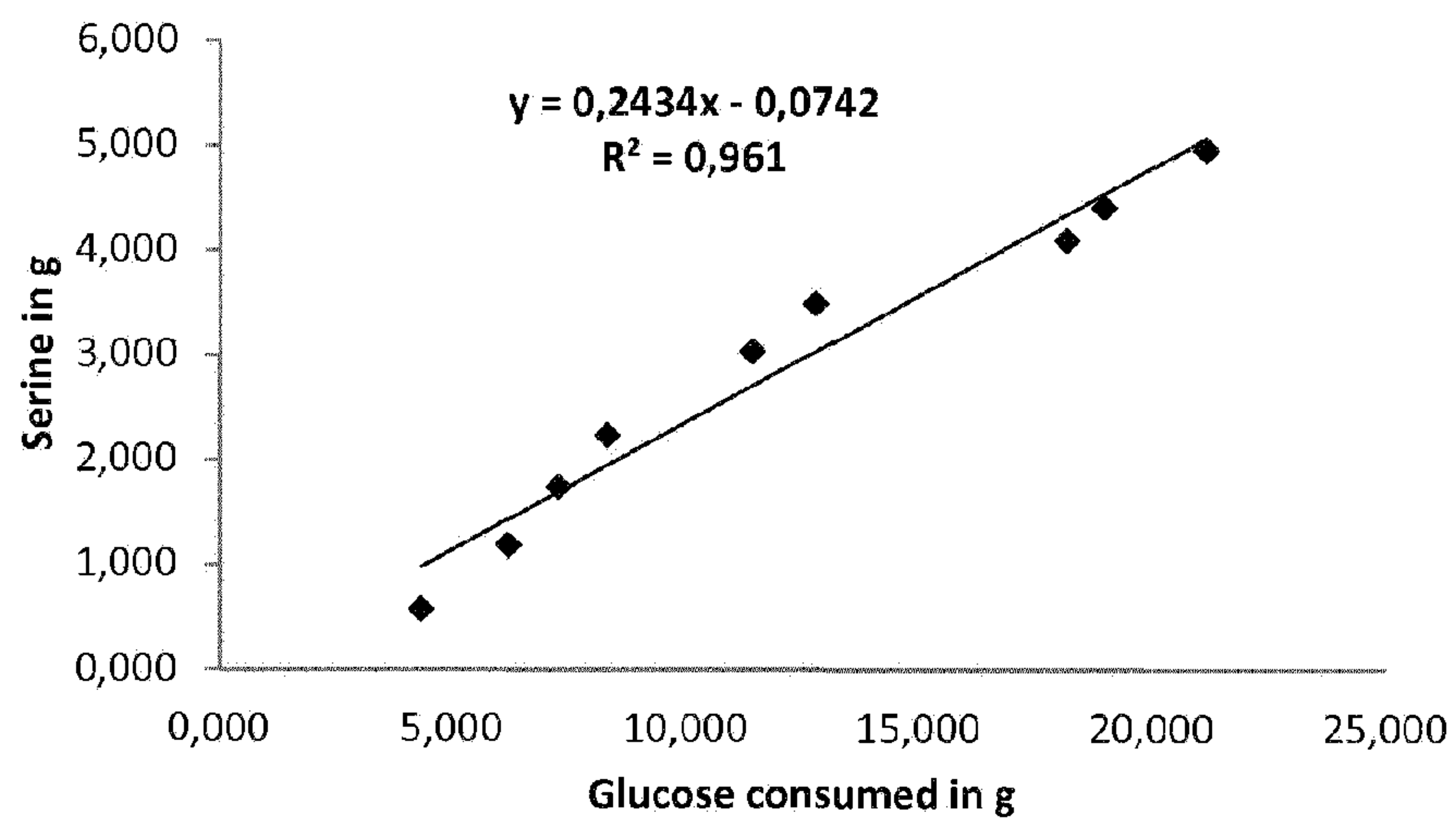
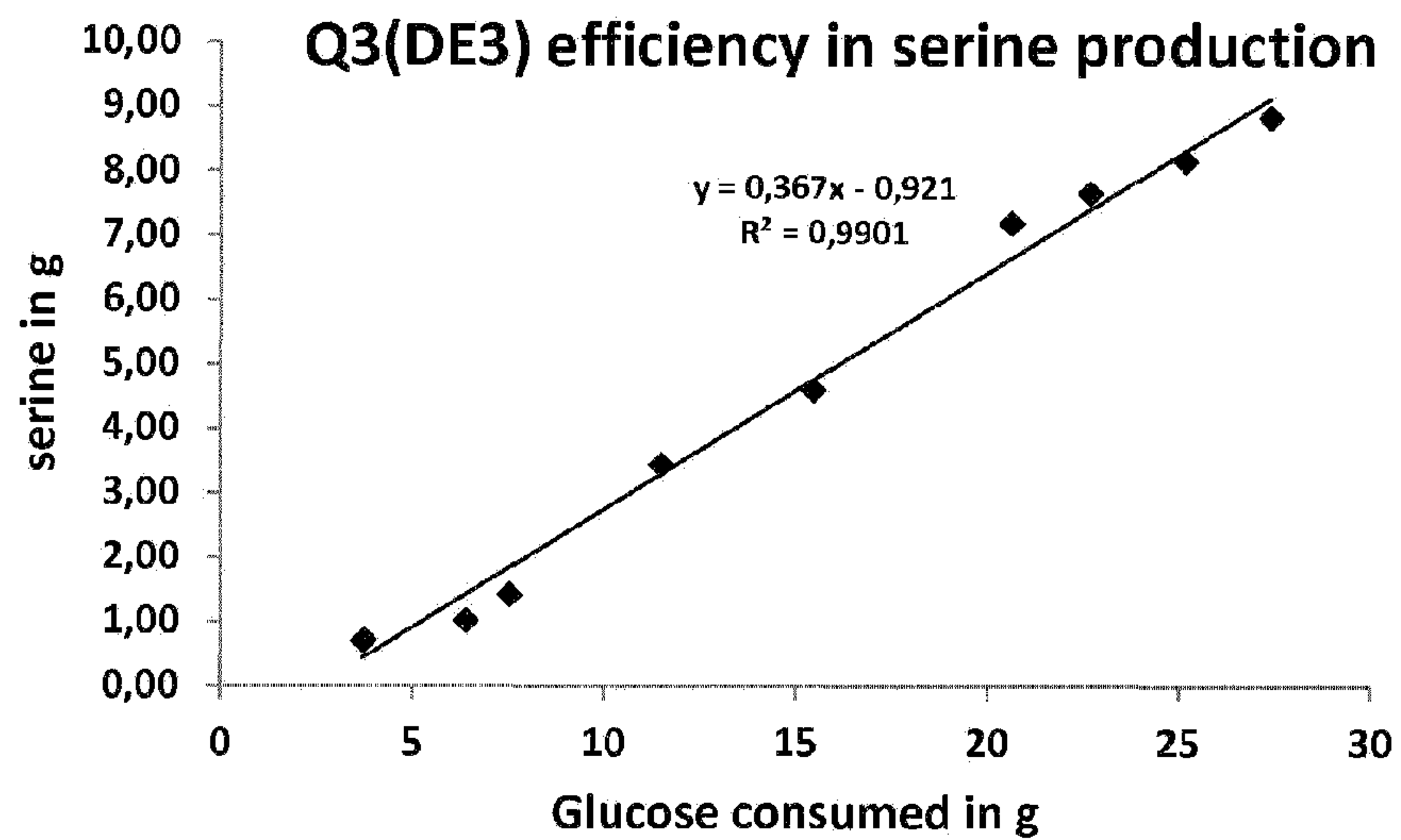
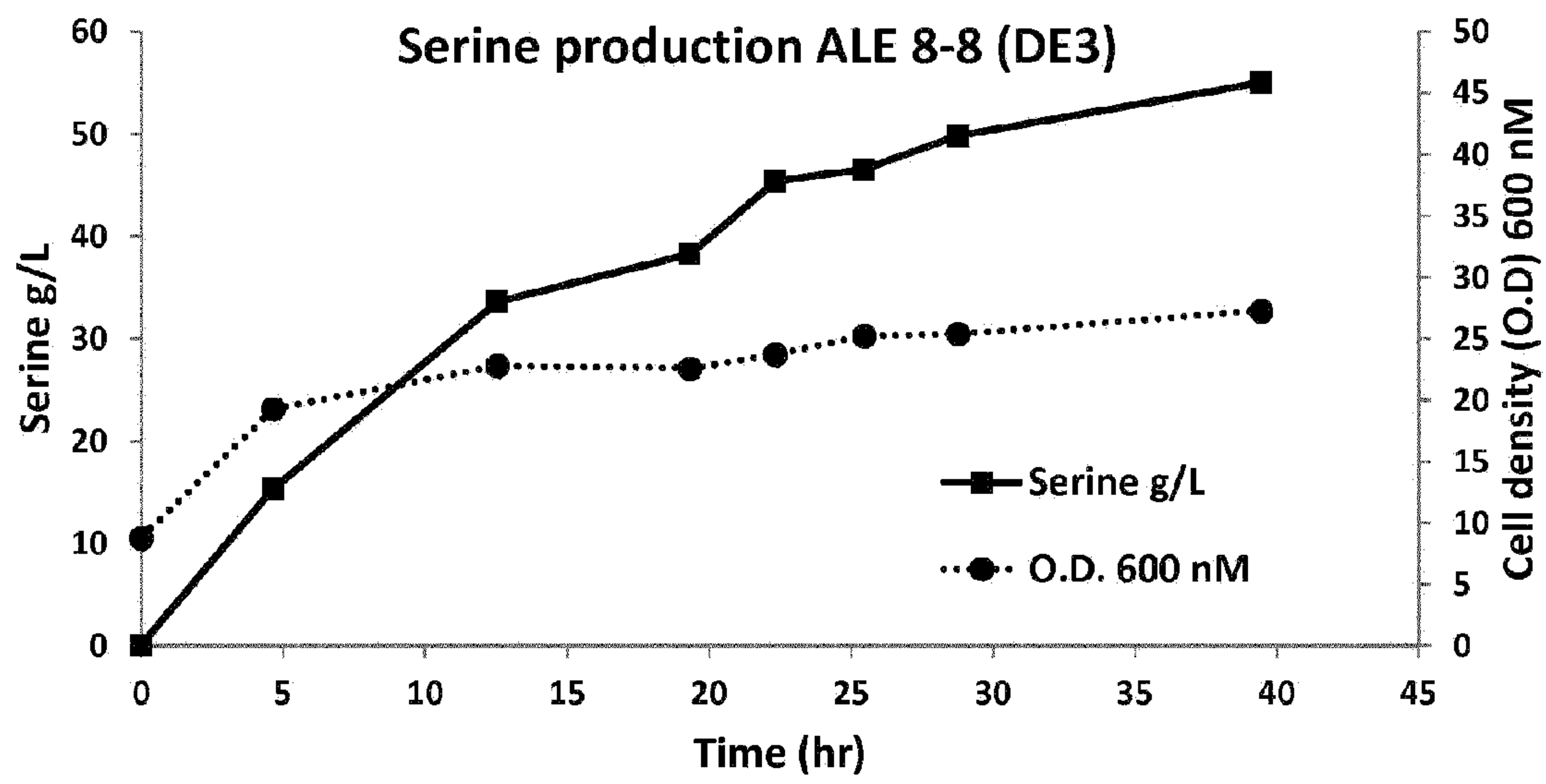


Figure 9

9B.

Q1(DE3) efficiency in serine production**Q3(DE3) efficiency in serine production****Figure 9**

10A.



10B.

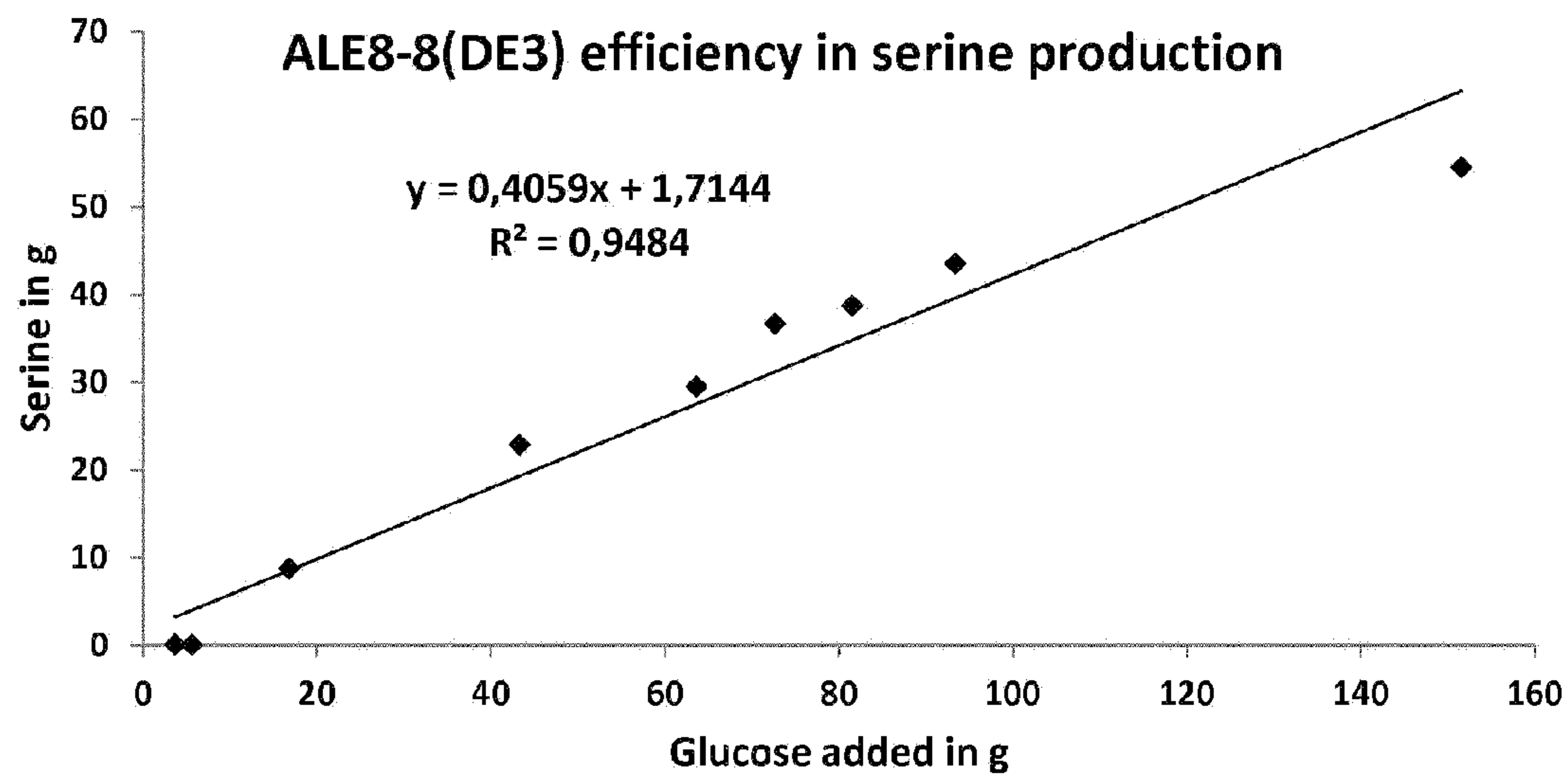
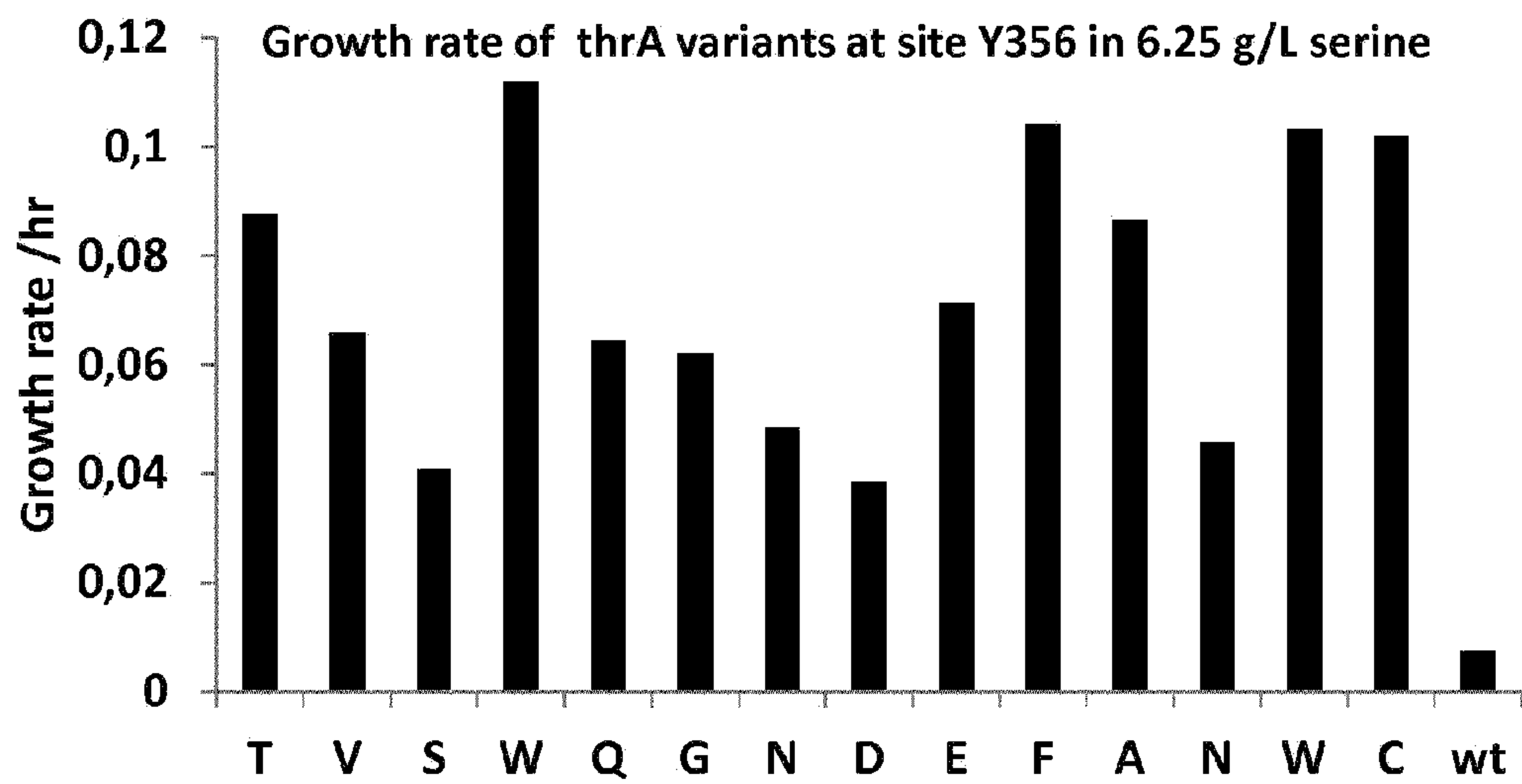


Figure 10

11A.



11B.

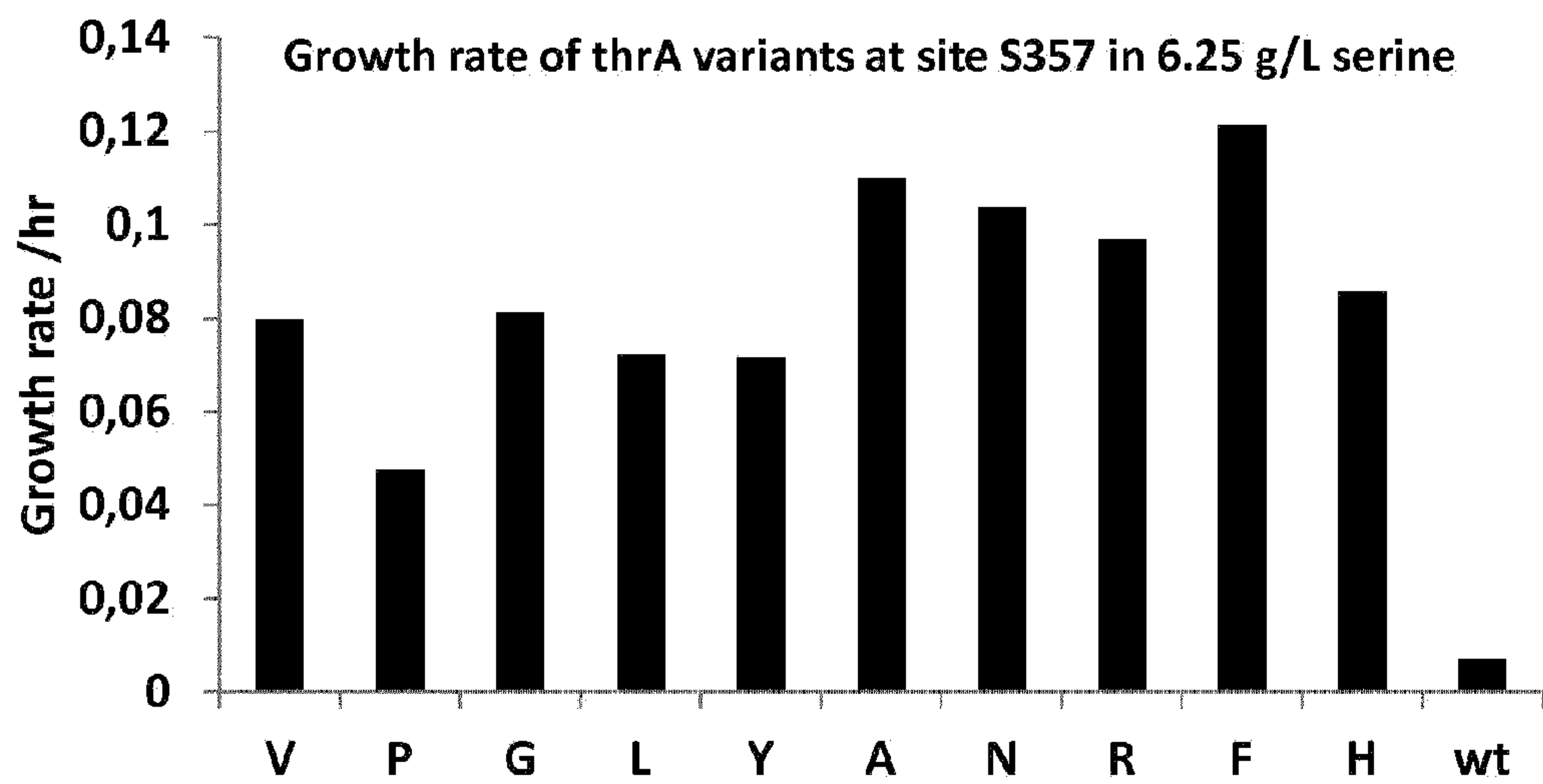


Figure 11

11C.

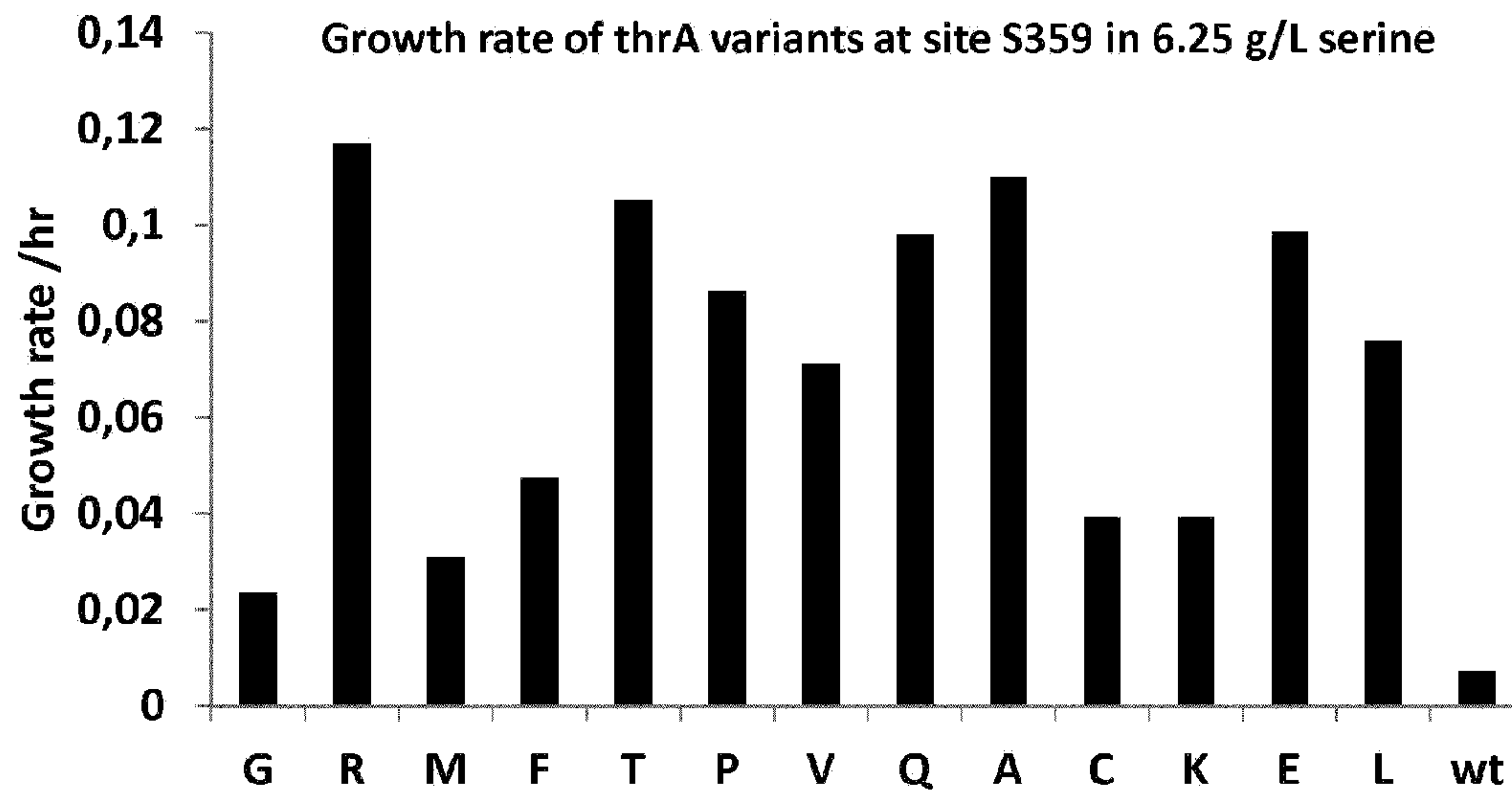


Figure 11