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(54) **MICROORGANISMS WITH EXTENDED SUBSTRATE UTILIZATION RANGE**
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Description

FIELD OF INVENTION

5 [0001] The present invention relates to new microorganisms having improved capability of utilizing monomeric sugars found in renewable resources, in particular lignocellulose. More specifically, the invention relates to genetically modified *Ralstonia* and *Cupriavidus* hosts and methods of using them.

BACKGROUND

10 [0002] Lignocellulose is the main renewable resource with several applications, including feedstock for bulk chemicals. The main components of lignocellulose are cellulose, hemicellulose and lignin. The cellulose fraction comprises polymers of the hexose sugar, glucose. The hemicellulose fraction is mainly comprised of polymers of the pentose sugars xylose, and arabinose, and the hexose sugars mannose, glucose and galactose. These are abundant in the lignocellulosic biomass of soft woods, whereas xylose occurs in both soft and hard woods.

15 [0003] There are numerous examples for efforts to utilize lignocelluloses more effectively by microorganisms and to extend the substrate utilization range of microorganisms towards low-cost and abundant carbon sources. For example, attempts have been made to broaden the utilization of unfermentable pentoses like xylose and arabinose occurring in lignocellulosic biomass in many microorganisms. Many of these investigations aimed at the extension of carbohydrate metabolism in ethanol producers, such as *Zymomonas mobilis* and *Saccharomyces cerevisiae* for ethanol fermentation bioprocess. WO 2009/081941, for example, describes genetical modification of ethanol producing bacterium belonging to the genus *Zymomonas* to utilize glucose, mannose, and xylose.

20 [0004] Bacteria belonging to genus *Ralstonia* and *Cupriavidus* are chemolithoautotrophs which are capable of accumulating large amounts of polyhydroxyalkanoates (PHAs) from renewable resources or from carbon dioxide. Strains of the genus *Ralstonia* and *Cupriavidus*, in particular, *R. eutropha* (new name *Cupriavidus necator*) have been shown to possess large biotechnological potential as these strains can be cultivated to high cell densities in large scale (Schlegel et al. 1961) and is suitable for large-scale industrial bioprocesses. Unfortunately, *Cupriavidus* and *Ralstonia* have a very narrow carbohydrate substrate range limited to fructose, N-acetylglucosamine and gluconate. Attempts to broaden the substrate utilization range of *R. eutropha* H16 have been described in the prior art (Pries et al. 1990, Schlegel and Gottschalk, 1965). A spontaneous glucose-utilizing mutant of *R. eutropha* H16 which presumably transported this sugar into the cell by a passive transporter has been reported to be isolated (Schlegel and Gottschalk 1965, König, et al. 1969). Furthermore, Pries et al. 1990 reported the introduction of β -galactosidase gene and the *gal operon* from *E. coli* into *R. eutropha* strain H16 and utilization of lactose and galactose by this bacterium.

30 [0005] Further, Buchholz et al. 1994 have described the construction of a genomic library of *Pseudomonas saccharophila* and its transfer to *Alcaligenes eutrophus* (*Ralstonia eutropha*). An insert containing *xyl* genes encoding xylose isomerase and xylulokinase made the host *A. eutrophus* (pGN3) to grow on xylose.

35 [0006] JP 2009 225662 A (KANEKA CORP) discloses a *Cupriavidus necator* (*Ralstonia eutropha*) H16 strain genetically modified to express a gene encoding glucose transporter protein obtained from *Zymomonas mobilis*, wherein the introduction of the gene allows the modified strain to grow on glucose.

40 [0007] In spite of the previous attempts to extend the substrate utilization range of certain microorganisms towards low-cost and abundant carbon sources, there is still a need for new methods and microorganisms for more efficient utilization of renewable resources, in particular lignocelluloses.

SUMMARY

45 [0008] One object of the present invention is to provide a process for effectively utilizing lignocelluloses, in particular monomeric sugars found in lignocelluloses.

[0009] Another object of the present invention is to provide new microorganisms capable of utilizing lignocelluloses, in particular monomeric sugars found in lignocellulose. To achieve these objects the invention is characterized by the features that are en-listed in the independent claims. Other claims represent the preferred embodiments of the invention.

50 [0010] A new microorganism, which is genetically modified to express genes broadening its capability of using different monomeric sugars which are present in lignocellulose is disclosed herein.

[0011] The present invention provides, in one aspect, a microorganism of the genus *Cupriavidus* or *Ralstonia*, which is capable of using mannose and optionally glucose as carbon source, said organism is genetically modified to express phosphomannose isomerase (EC 5.3.1.8) and facilitated diffusion protein for mannose uptake and optionally mannofructokinase (EC 2.7.1.4).

55 [0012] In another embodiment of the invention, the microorganism of the genus *Cupriavidus* or *Ralstonia* is further genetically modified to express xylose isomerase (EC 5.3.1.5), xylulokinase (EC 2.7.1.17) and a high affinity ABC-

transporter and/or xylose proton symporter E and capable of using xylose as carbon source.

[0013] In one embodiment the microorganism is genetically modified to express phosphomannose isomerase (EC 5.3.1.8) and facilitated diffusion protein for mannose uptake and/or xylose proton symporter E, and optionally mannofructokinase (EC 2.7.1.4). Typically, microorganisms of the genus *Cupriavidus* or *Ralstonia* are incapable of utilizing mannose without said modification, whereas the modified microorganisms are capable of utilizing mannose. The genes used for said genetic modification may be exogenous or endogenous, typically they are exogenous. In various embodiments, when the microorganism is genetically modified to express a gene encoding facilitated diffusion protein for mannose uptake, in particular gene *glt*, the microorganism host is surprisingly capable of utilizing also glucose.

[0014] In one embodiment the microorganism is genetically modified to express xylose isomerase (EC 5.3.1.5), xylulokinase (EC 2.7.1.17) and a high affinity ABC-transporter (Xyl G, EC 3.6.3.17, Xyl F, and/or Xyl H) and/or xylose proton symporter E. Typically, microorganisms of the genus *Cupriavidus* or *Ralstonia* are incapable of utilizing xylose without said modification, whereas the modified microorganisms are capable of utilizing xylose. The genes used for said genetic modification may be exogenous or endogenous, typically they are exogenous. Typically, said microorganisms are incapable of utilizing arabinose without said genes, whereas in various embodiments the modified microorganisms are surprisingly capable of utilizing arabinose as the carbon source. Furthermore, when the microorganisms are genetically modified to express high affinity ABC-transporter (in particular genes Xyl G, (EC 3.6.3.17), Xyl F and Xyl H), the microorganism hosts are surprisingly capable of utilizing glucose and galactose.

[0015] In one further embodiment of the invention the microorganism hosts are genetically modified to express glucokinase (EC 2.7.1.2). This modification makes the genetically modified microorganism to be also capable of utilizing glucose.

[0016] The microorganisms are thus capable of utilizing glucose as carbon source in addition to mannose, or in addition to mannose, xylose and arabinose.

[0017] In a preferred embodiment of the invention the microorganisms are capable of utilizing mannose, glucose, galactose, xylose and arabinose as carbon source.

[0018] In one further embodiment the microorganisms are genetically modified to express transaldolase (EC 2.2.1.2) and/or transketolase (EC 2.2.1.1). The genes encoding transaldolase and/or transketolase improve the fastness of growth of the modified microorganism in particular on pentoses, such as xylose and arabinose.

[0019] The microorganism may be any species or strains belonging to the genus *Cupriavidus* or *Ralstonia*. Preferably, the microorganism belongs to *C. necator* (earlier *R. eutropha* or *Alkaligenes eutrophus*) species.

[0020] In one aspect the invention provides a vector comprising a gene encoding phosphomannose isomerase (EC 5.3.1.8), and optionally a gene encoding facilitated diffusion protein for mannose uptake, a gene encoding xylose proton symporter E and/or a gene encoding mannofructokinase (EC 2.7.1.4), and regulatory elements for regulating expression of said genes in *Cupriavidus* or *Ralstonia*. Said genes may be in the same or separate vectors.

[0021] In one embodiment, the vector comprises a gene encoding xylose isomerase (EC 5.3.1.5), xylulokinase (EC 2.7.1.17), a high affinity ABC- transporter (Xyl G, EC 3.6.3.17, Xyl F, and/or Xyl H) and/or xylose proton symporter E, and regulatory elements for regulating expression of said genes in *Cupriavidus* or *Ralstonia*. Said genes may be in the same or separate vectors.

[0022] In one embodiment the vector may comprise a gene encoding transaldolase (EC 2.2.1.2) and/or transketolase (EC 2.2.1.1). Said genes may be in the same or separate vectors.

[0023] In one further embodiment the vector may comprise a gene encoding glucokinase (EC 2.7.1.2). Said gene may be in the same or separate vectors as the above listed genes.

In one still further embodiment a vector comprise any one or any combination of the above listed genes.

Any promoter that can be expressed in strains of the genera *Ralstonia* or *Cupriavidus* can be used in the vector.

Suitable promoters comprise for example *lac*-promoter, *neokanamycin*-promoter, glyceraldehyde-3-phosphate dehydrogenase promoter, P₂, hydrogenase (SH) promoter and P_L.

In one preferred embodiment the vector is a broad host range plasmid which can be used to extend the substrate utilization range of various different host strains of *Cupriavidus* or *Ralstonia*.

[0024] Disclosed herein is a method for modifying the microorganisms of the genera *Ralstonia* or *Cupriavidus*. The method comprises the hosts are genetically modified to express one or more nucleotide sequences selected from the group comprising a nucleotide sequence or sequences encoding phosphomannose isomerase (EC 5.3.1.8) and facilitated diffusion protein for mannose uptake and/or xylose proton symporter E, and optionally mannofructokinase (EC 2.7.1.4), wherein said microorganism is capable of growing on mannose and optionally also on glucose as the carbon source, and/or

a nucleotide sequence or sequences encoding xylose isomerase (EC 5.3.1.5), xylulokinase (EC 2.7.1.17) and a high affinity ABC- transporter and/or xylose proton symporter E, wherein said microorganism is capable of growing on xylose, or xylose and arabinose, and optionally also on glucose and galactose as the carbon source.

[0025] The method may further comprise that the microorganism is genetically modified to express transaldolase (EC 2.2.1.2) and/or transketolase (EC 2.2.1.1).

[0026] The method may further comprise that the microorganism is genetically modified to express glucokinase (EC 2.7.1.2).

[0027] In one still further aspect the invention provides a method for cultivating the modified microorganism of the present invention in a cultivation medium comprising mannose, glucose, galactose, xylose or arabinose, any of them, any combination of them, or all of them, as carbon source.

[0028] In one embodiment the cultivation medium may comprise mannose as carbon source and optionally glucose.

[0029] In another embodiment the cultivation medium may comprise either or both xylose and arabinose as carbon source, and optionally also glucose and/or galactose.

[0030] In one further embodiment the cultivation medium may comprise mannose and either or both xylose and arabinose as carbon source, and optionally also glucose and/or galactose.

[0031] In one further embodiment the method comprises that the microorganism is allowed to synthesize bulk chemicals, such as lipids, for example polyhydroxyalkanoates, fatty acids or fatty acid derivatives, and the chemical is recovered from the cells, or from the cultivation medium.

[0032] Considerable advantages are obtained by means of the present invention. By means of the invention new microorganisms belonging to genus *Cupriavidus* or *Ralstonia* are obtained, said microorganisms having an extended substrate utilization range. The modified microorganisms as described herein can utilize effectively low-cost carbohydrates, in particular lignocellulose comprising monomeric sugars. Xylose, mannose and arabinose are wood sugars present in a polymerized form in the hemicelluloses of plant cell walls. The microorganisms of the present invention are also able to use monomeric glucose, which is found in particular in sucrose and starch and also in cellulose and hemicellulose fractions in lignocellulosic materials. The modified microorganisms of the present invention are suitable for producing many bulk chemicals.

DESCRIPTION OF THE FIGURES

[0033]

Figure 1. Pathways of mannose and glucose utilization established in *R. eutropha* strain H16. GLF, glucose-facilitated diffusion transporter; KDPG, Entner-Doudoroff pathway; MAK, mannofructokinase; PMI, phosphomannose isomerase, GLK, glucokinase; PGI, phosphoglucose isomerase.

Figure 2. Physical maps of the constructed plasmids pBBR1MCS-3::glf, pBBR1MCS-3::glf::mak, pBBR1MCS-3::glf::pmi, pBBR1MCS-3::glf::mak::pmi, pBBR1MCS-2::P_{nk}::glf and pBBR1MCS-2::P_{nk}::glk::glf. The genes glf, glk, mak and pmi, respectively, were excised from hybrid plasmids of pJET1.2 or pCR2.1 after subcloning using the indicated restriction sites, and were then ligated in various arrays to the linearized expression vectors pBBR1 MCS-2 and pBBR1 MCS-3. Relevant cleavage sites and structural genes are indicated (Km^R, kanamycin resistance cassette, Tc^R, tetracycline resistance cassette; mob, mobilization site; rep, origin of replication; glf, gene encoding the glucose-facilitated diffusion transporter (GLF) from *Z. mobilis*; glk, gene encoding the glucokinase (GLK) from *E. coli*; mak, gene encoding a mannofructokinase (MAK) from *E. coli*; pmi, gene encoding a phosphomannose isomerase (PMI) from *E. coli*).

Figure 3. Transcription analysis of glf, mak and pmi in *R. eutropha* strain H16 harboring pBBR1 MCS-3::glf::mak::pmi and in the negative control harboring only the vector pBBR1 MCS-3. Expression of glf, mak and pmi was analyzed by RT-PCR in samples containing total RNA isolated from cells of the exponential growth phase of *R. eutropha* strain H16 harboring pBBR1MCS-3::glf::mak::pmi and of *R. eutropha* strain H16 harboring the vector pBBR1 MCS-3. Cells were grown in MSM containing 1 % (wt/vol) sodium gluconate as sole carbon source. The resulting PCR products were separated by agarose gel electrophoresis and stained with ethidium bromide. Lanes 1, 3 and 5 represent the RT-PCR assay for glf, mak and pmi, respectively, for *R. eutropha* strain H16 harboring pBBR1MCS-3::glf::mak::pmi, whereas lanes 2, 4 and 6 represent the negative controls to detect whether there is any expression of glf, mak and pmi in the strain harboring only the vector pBBR1MCS-3. A λ IPstI DNA Marker (lanes M, Fermentas, Germany) served for size comparison.

Figure 4. Crude extracts and solubilized membrane proteins of recombinant *R. eutropha* strain H16 harboring pBBR1 MCS-2 or pBBR1MCS-2::P_{nk}::glk::glf. Cells were grown for 48h in MSM medium containing 1% (wt/vol) sodium glucose as carbon source. Proteins were separated in 12.5% (wt/vol) SDS-polyacrylamide gels and stained with Coomassie brilliant blue. A, crude extracts. Lane 1: *R. eutropha* strain H16 pBBR1 MCS-2, lane 2: *R. eutropha* strain H16 pBBR1MCS-2::P_{nk}::glf::glf; B, solubilized membrane proteins. Lane 3: *R. eutropha* strain H16 pBBR1MCS-2, lane 4: *R. eutropha* strain H16 pBBR1 MCS-2::P_{nk}::glk::glf. St, molecular weight standard. Arrows indicate detected recombinant proteins glucokinase (Glc) and glucose transporter (Glf).

Figure 5. Growth of recombinant *R. eutropha* strain H16. Recombinant strains of *R. eutropha* harboring different plasmids. Cells were cultivated in liquid MSM containing 1% (wt/vol) glucose as sole carbon source. □ pBBR1MCS-3::glf, ● □ □ pBBR1MCS-2::P_{nk}::glf, △ pBBR1MCS-2::P_{nk}::glk::glf, ▲ pBBR1MCS-3::glf::mak::pmi; ♦ control *R. eutropha* H16 strain G⁺1. Cultivations were done in Erlenmeyer flasks incubated on a horizontal rotary shaker at 110 rpm at 30 °C. The optical density was measured at 600 nm in a Klett photometer.

Figure 6. Recombinant strains of *R. eutropha* harboring plasmid pBBR1MCS-3::glf::mak::pmi. Cells were cultivated in liquid MSM containing tetracycline (12.5 µg/ml) and △ 0.5% (wt/vol) sodium gluconate, ▲ 0.5% (wt/vol) mannose, □ 1% (wt/vol) mannose, ♦ 2% (wt/vol) mannose, or ○ 4% (wt/vol) mannose as sole carbon source. Cultivations were done in Erlenmeyer flasks incubated on a horizontal rotary shaker at 130 rpm and at 30 °C. The optical density was measured at 600 nm in a Klett photometer.

Figure 7. The utilization pathway of D-xylose in bacteria (XI, xylose isomerase; XK, xylulokinase; PPP, pentose phosphate pathway)

Figure 8. Physical maps of the constructed plasmids pBBR1 MCS-3::xy/AB, pBBR1MCS-3::xy/ABE and pBBR1MCS-3::xy/ABFGH. The genes *xylA*, *xylB*, *xylE* and *xylFGH*, respectively, were excised from hybrid plasmids of pJET1.2 after subcloning using the indicated restriction sites, and were then ligated to the linearized expression vector pBBR1 MCS-3. Relevant cleavage sites and structural genes are indicated (Tc^R, tetracycline resistance cassette; mob, mobilization site; rep, origin of replication; *xylA*, gene encoding the xylose-isomerase (XylA); *xy/B*, gene encoding the xylulokinase (XylB); *xylE*, gene encoding the low affinity transporter (XylE); and *xylFGH*, genes encoding the ABC-transporter (XylFGH) from *E. coli*, respectively).

Figure 9. Growth of the recombinant plasmid pBBR1MCS-3::xy/ABFGH harboring strain of *R. eutropha* strain H16, and of *R. eutropha* strain H16, which were cultivated in fluid MSM containing different concentrations (0.5%, 1%, 2% [wt/vol]) of D-xylose, 1% (wt/vol) glucose and with 1% (wt/vol) Na-gluconate as sole carbon source, respectively. □ pBBR1MCS-3::xy/ABFGH, 0.5% (wt/vol) D-xylose; ■ pBBR1MCS-3::xy/ABFGH, 1.0% (wt/vol) D-xylose; △ pBBR1MCS-3::xy/ABFGH, 2.0% (wt/vol) D-xylose; ▲ pBBR1MCS-3::xy/ABFGH, 4.0% (wt/vol) D-xylose; ◇ pBBR1MCS-3::xy/ABFGH, 1.0% (wt/vol) Na-gluconate; ♦ pBBR1MCS-3::xy/ABFGH, 1.0% (wt/vol) glucose; + control *R. eutropha* H16, 1.0% (wt/vol) Na-gluconate. Cultivations were done in Erlenmeyer flasks incubated on a horizontal rotary shaker at 110 rpm at 30 °C. The optical density was measured at 600 nm in a Klett photometer.

DETAILED DESCRIPTION OF THE INVENTION

[0034] "A genetically modified microorganism" refers here to a microorganism whose genetic material has been altered using genetic engineering techniques. These techniques involve the use of nucleic acid molecules from different sources, for example from another microorganism or they may be at least partly synthetic. Nucleic acid molecules from different sources may be combined to one or more molecules. These constructions may be transferred into a microorganism, giving it modified or novel genetic constructions.

[0035] "A gene" refers here typically to a nucleotide sequence encoding a specific product, here usually an enzyme. The coding region is typically operationally linked into regulatory regions, in particular into a promoter, which is able to function in the host cell. Into a host cell is here typically introduced a genetic construct, which consist essentially of a coding region of a gene and a regulatory region, in particular a promoter, functional in the host cell. A suitable vector may be used to introduce the desired genetic construct into a host cell. "A gene" may refer to one or more genes.

[0036] Genetical modification may mean that one or more exogenous genes are introduced into and expressed in a microorganism host, or that one or more of the expressed genes are endogenous and one or more are exogenous, or that the expression of one or more endogenous genes is increased in a host compared to the parent host.

[0037] Microorganism hosts in various embodiments of the invention are bacteria belonging to the genus *Cupriavidus* or *Ralstonia*.

[0038] A microorganism refers here typically to an isolated microorganism, i.e. a microorganism is used as a pure culture.

[0039] In one embodiment the species may be any *Ralstonia* species including, but not limited to *Ralstonia insidiosa*, *R. mannitolitica*, *R. pickettii*, *R. solanacearum* and *R. syzygii*. In another embodiment the species may be any *Cupriavidus* species including, but not limited to *Cupriavidus basilensis*, *C. campinensis*, *C. gilardii*, *C. laharis*, *C. metallidurans*, *C. necator*, *C. oxalaticus*, *C. pauculus*, *C. pinatubonensis*, *C. respiraculi* and *C. taiwanensis* (Bacterial Nomenclature up-to-date (approved lists, validation list), May 2010, compiled by DSMZ - Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH). Preferred species are *R. eutropha* and *C. metallidurans*. Most preferred species is *R. eutropha* (present name *C. necator*, earlier names *Alcaligenes eutrophus* and *Hydrogenomonas eutropha*). *Cu-priavidus* and

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Ralstonia strains are available to the public from microbial culture collections, such as ATCC, DSMZ and NCIB.

[0040] The invention has been exemplified by using *R. eutropha* strain H 16 (present name *C. necator*). This strain is a wild type strain, which is available from Deutsche Sammlung von Mikroorganismen und Zellkulturen, DSMZ, under accession number DSM428, from American Type Culture Collection under accession number ATCC17699, and from National Collection of Industrial and Marine Bacteria, NCIBM, under Accession number NCIB10442. All these strains are available to the public.

[0041] Deposited *R. eutropha* H16 strains, which can be used in the invention are *Ralstonia eutropha* H16, DSMZ Accession No.: DSM428, DSM 15443 (mutant of DSM 428)

[0042] Taxonomy ID: 381666; Inherited blast name: b-proteobacteria

Other names:

synonyms: *Cupriavidus necator* H16, *Alcaligenes eutropha* H16, *Wautersia eutropha* H16, *Cupriavidus necator* ATCC 17699, *Ralstonia eutropha* ATCC 17699;

equivalent names: *Ralstonia eutropha* strain H16, *Ralstonia eutropha* str. H16

Other *R. eutropha* strains which can be used in the invention are:

Ralstonia eutropha H850

DSMZ Accession No.: DSM 13439

Taxonomy ID: 53482; Inherited blast name: b-proteobacteria

Other names:

synonyms: *Wautersia eutropha* H850, *Cupriavidus necator* H850, *Alcaligenes eutrophus* H850

Ralstonia eutropha JMP134

DSMZ Accession No.: DSM 4058

Taxonomy ID: 264198; Inherited blast name: b-proteobacteria

Other names:

synonyms: *Cupriavidus pinatubonensis* JMP134, *Cupriavidus necator* JMP134, *Wautersia eutropha* JMP134, *Ralstonia* sp. JMP134;

equivalent names: *Ralstonia eutropha* str. JMP134 and *Ralstonia eutropha* strain JMP134

[0043] In addition, also the following other *Ralstonia eutropha* strains can be used in the invention:

Ralstonia eutropha 335 (DSMZ Accession No.: DSM 531, ATCC 17697)

Ralstonia eutropha N9A (DSMZ Accession No.: DSM 518;

Ralstonia eutropha B19 (DSMZ Accession No.: DSM 515);

Ralstonia eutropha G27 (DSMZ Accession No.: DSM 516);

Ralstonia eutropha G29 (DSMZ Accession No.: DSM 517);

Ralstonia eutropha 336 (DSMZ Accession No.: DSM 529, ATCC 17698)

Ralstonia eutropha 338 (DSMZ Accession No.: DSM 530, ATCC 17700);

Ralstonia eutropha TA06 (DSMZ Accession No.: DSM 4182);

Ralstonia eutropha H850 (DSMZ Accession No.: DSM 5536; NRRL B-15940)

Ralstonia eutropha KTO₂ (DSMZ Accession No.: DSM 6519);

Ralstonia eutropha Tfa17 (DSMZ Accession No.: DSM 11098)

Ralstonia eutropha N-1 (DSMZ Accession No.: DSM 13513; ATCC 43291)

Ralstonia eutropha NRRL B-2804 (DSMZ Accession No.: DSM 30029, ATCC 25207, NRRL B-2804);

[0044] "Lignocellulosic biomass" refers here to plant biomass that comprises mainly cellulose, hemicelluloses and lignin. Lignocellulosic biomass is for example agricultural residues (e.g. wheat, barley or rice straw, husk (chaff), corn stover, sugar cane bagasse), energy crops, wood or plant materials or residues and municipal waste, e.g. paper waste.

[0045] "Bulk chemical" refers to any useful chemicals, which may be produced from inexpensive raw materials in high amounts. Examples of useful bulk chemicals are lipids, for example polyhydroxyalkanoates, in particular polyhydroxy-

butyrate (PHB), or other lipids, such as fatty acids and/or fatty acid derivatives, for example acylglycerols.

[0046] The term "lipid" refers to a fatty substance, whose molecule generally contains, as a part, an aliphatic hydrocarbon chain, which dissolves in nonpolar organic solvents but is poorly soluble in water. Lipids are an essential group of large molecules in living cells. Lipids are, for example, fats, oils, waxes, wax esters, sterols, terpenoids, isoprenoids, carotenoids, polyhydroxyalkanoates, nucleic acids, fatty acids, fatty alcohols, fatty acid esters, phospholipids, glycolipids, sphingolipids and acylglycerols. The terms lipids and oils are used in this description synonymously.

[0047] The term "acylglycerol" refers to an ester of glycerol and fatty acids. Acylglycerols occur naturally as fats and fatty oils. Examples of acylglycerols include triacylglycerols (TAGs, triglycerides) diacylglycerols (diglycerides) and monoacylglycerols (monoglycerides).

[0048] "Raw materials" or "starting materials" are materials which form or are included to the cultivation medium of a *Cupriavidus* or *Ralstonia* host and which the microorganism can use for its growth and/or production of desired chemicals. Advantageously the materials are inexpensive and available in large amounts. Suitable starting materials are for example lignocellulosic biomass (e.g. wood or plant materials, their fractions or residues) and agricultural waste(s) or residue(s), micro- or macroalgae and any materials, which comprise carbohydrates, such as glucose, galactose, mannose, xylose, or arabinose or combinations thereof. Some starting materials may comprise mainly xylose (hemicelluloses), some may comprise mainly glucose (cellulose or starch), and some may comprise mainly mannose (galactoglucomannan based hemicelluloses).

[0049] Sugars, mannose, glucose, xylose, arabinose, and galactose need to be provided to microorganisms as monomeric sugars. The raw materials containing these sugars in polymeric form, e.g. lignocelluloses, can be treated with any known method to hydrolyse the sugar polymers into monomeric sugars that are utilizable by microorganisms according to the present invention.

[0050] In the cultivation of *Cupriavidus* or *Ralstonia* can be used any cultivation medium comprising components suitable for *Cupriavidus* or *Ralstonia* growth.

[0051] In industrial applications the cultivation medium typically comprises monomeric sugars originating from lignocellulosic materials as carbon source, and in addition usually nutrients and salts or components thereof as micro- and macronutrients, and water. A cultivation medium may comprise mannose, glucose, galactose, xylose, or arabinose or any combination thereof as carbon source, typically as the main carbon source, in some embodiments as the sole carbon source.

[0052] In the cultivation of *Cupriavidus* or *Ralstonia* can be used cultivation conditions suitable for cultivation of *Cupriavidus* or *Ralstonia*. Such cultivation conditions are well known to a person skilled in the art. In the cultivation can be used liquid or solid media. Liquid cultures may be agitated, suitable agitation 50 - 1000 rpm, typically 100 - 600 rpm. The cultivation temperature may be 15 to 45 °C, preferably 25 to 37 °C, typically 30 °C. The incubation time may be 1 day to 14 days, typically 2 to 7 days, preferably 24 to 72 hours. The cultivation may be a batch, fed-batch or continued cultivation.

[0053] The term "endogenous gene" refers here to a gene which is natural to the *Cupriavidus* or *Ralstonia* host.

[0054] The term "exogenous gene" refers here to a gene which is not natural to the *Cupriavidus* or *Ralstonia* host.

[0055] The genes introduced into the *Ralstonia* or *Cupriavidus* host may be endogenous or exogenous, typically they are exogenous, if/since the host does not possess the genes naturally. In some embodiments the endogenous genes naturally produced by the host may be overexpressed in the host for example by expressing the coding region under a strong promoter.

[0056] A gene encoding "phosphomannose isomerase" may be any gene which encodes an enzyme having phosphomannose isomerase activity. According to Enzyme Classification the enzyme has EC number 5.3.1.8. In a specific embodiment of the invention the gene may be a gene encoding phosphomannose isomerase (PMI) or mannose-6-phosphate isomerase from *Escherichia coli* strain K12 (EC 5.3.1.8) (ACCESSION No. P00946) (gene name: *pmi*, origin: *Escherichia coli* strain K12) (SEQ ID NO:1).

[0057] A gene encoding "facilitated diffusion protein for mannose uptake" may be any gene which encodes facilitated diffusion protein for mannose uptake activity or a gene encoding xylose proton symporter E. In a specific embodiment of the invention the gene may be a gene encoding glucose-facilitated diffusion transporter (GLF) from *Zymomonas mobilis* (ACCESSION No. P21906) (gene name: *glf*, origin: *Zymomonas mobilis*) (SEQ ID NO:2). Surprisingly, a *Ralstonia* or *Cupriavidus* host introduced to express a gene encoding facilitated diffusion protein for mannose uptake activity, in particular *glf*, can use glucose as carbon source.

[0058] A gene encoding "mannofructokinase" may be any gene which encodes mannofructokinase (EC 2.7.1.4) activity. In a specific embodiment of the invention the gene may be a gene encoding mannofructokinase (MAK) (EC 2.7.1.4) from *Escherichia coli*, in particular from strain K12 (ACCESSION No. P23917) (gene name: *mak*, origin: *Escherichia coli* strain K12) (SEQ ID NO:3).

[0059] A gene encoding "xylose isomerase" may be any gene, which encodes xylose isomerase (EC 5.3.1.5) activity. In a specific embodiment of the invention the gene may be a gene encoding D-xylose isomerase (XylA) from *Escherichia coli* strain K12 (ACCESSION No. P00944) (EC 5.3.1.5) (gene name: *xylA*, origin: *Escherichia coli* strain K12) (SEQ ID

NO:4).

[0060] A gene encoding "xylulokinase" may be any gene, which encodes xylulokinase activity (EC 2.7.1.17). In a specific embodiment of the invention the gene may be a gene encoding xylulose kinase (XylB) from *Escherichia coli* strain K12 (ACCESSION No. P09099) (EC 2.7.1.17) (gene name: *xylB*, origin: *Escherichia coli* strain K12) (SEQ ID NO:5).

[0061] A gene encoding "xylose proton symporter E" may be any gene, which encodes xylose proton symporter E. In a specific embodiment of the invention the gene may be a gene encoding D-xylose-proton symporter (XylE) from *Escherichia coli* strain K12 (gene name: *xylE*, origin: *Escherichia coli* strain K12) (ACCESSION No. P0AGF4) (SEQ ID NO:6).

[0062] A gene encoding "high affinity ABC- transporter" may be any gene, which encodes high affinity ABC- transporter. In a specific embodiment of the invention the gene may be a gene encoding D-xylose-binding periplasmic protein (**XylF**) from *Escherichia coli* strain K12 (gene name: *xylF*, origin: *Escherichia coli* strain K12) (ACCESSION No. P37387) (SEQ ID NO:7), or a gene encoding xylose import ATP-binding protein (**XylG**) from *Escherichia coli* strain K12 (gene name: *xylG*, origin: *Escherichia coli* strain K12) (ACCESSION No. P37388) (EC 3.6.3.17) (SEQ ID NO:8), or a gene encoding xylose transport system permease protein (**XylH**) from *Escherichia coli* strain K12 (gene name: *xylH*, origin: *Escherichia coli* strain K12) (ACCESSION No. P0AGI4) (SEQ ID NO:9). Surprisingly, a *Ralstonia* or *Cupriavidus* host introduced to express genes, which encode high affinity ABC-transporter, in particular genes encoding Xyl F, Xyl G and Xyl H, can use glucose and galactose as carbon source.

[0063] A gene encoding "transaldolase" may be any gene, which encodes transaldolase (EC 2.2.1.2). In a specific embodiment of the invention the gene may be a gene encoding transaldolase B (TalB) from *Escherichia coli* strain K12 (gene name: *talB*, origin: *Escherichia coli* strain K12) (ACCESSION No. BAB96586) (EC 2.2.1.2) (SEQ ID NO:10).

[0064] A gene encoding "ketolase" may be any gene, which encodes transketolase (EC 2.2.1.1). In a specific embodiment of the invention the gene may be a gene encoding transketolase 1 (TktA) from *Escherichia coli* strain K12 (gene name: *tktA*, origin: *Escherichia coli* strain K12) (ACCESSION No. P27302) (EC 2.2.1.1) (SEQ ID NO: 11).

[0065] A gene encoding "glucokinase" may be any gene, which encodes glucokinase (EC 2.7.1.2). In a specific embodiment of the invention the gene may be a gene encoding glucokinase (glk) from *Escherichia coli* strain K12 (gene name: *glk*, origin: *Escherichia coli* strain K12) (ACCESSION No. POA6V8.1) (EC 2.7.1.2) GLK_ECOLI RecName: Full=Glucokinase; AltName: Full=Glucose kinase [Escherichia coli K-12] (SEQ ID NO: 12).

[0066] A *Ralstonia* or *Cupriavidus* host introduced to express glucokinase is capable of utilizing glucose.

[0067] Any gene, which gene product is known to catalyze the reaction of interest, may be used. Within the scope of the present invention are also genes, which encode the same or equivalent function and are highly homologous to the genes of interest. This means, for example, that the proteins encoded by the genes have high identity % when compared to each other.

[0068] For example the following genes may be used:

The respective genes from different *Escherichia* strains (e.g. *Escherichia albertii* [phosphomannose isomerase ZP_02903372.1, mannose-6-phosphate isomerase, class I [Escherichia albertii TW07627] (SEQ ID NO:13); mannofructokinase ZP_02902328.1, manno kinase [Escherichia albertii TW07627] (SEQ ID NO:14); *Escherichia fergusonii* [phosphomannose isomerase YP_002382576.1, mannose-6-phosphate isomerase [Escherichia fergusonii ATCC 35469] (SEQ ID NO:15); xylose isomerase YP_002384641.1] xylose isomerase [Escherichia fergusonii ATCC 35469] (SEQ ID NO:16); *Shigella* strains (e.g. *Shigella flexneri* [phosphomannose isomerase NP_837299.1], mannose-6-phosphate isomerase [Shigella flexneri 2a str. 2457T] (SEQ ID NO:17); *Shigella sonnei* [phosphomannose isomerase YP_310481.1, mannose-6-phosphate isomerase [Shigella sonnei Ss046] (SEQ ID NO:18); xylose isomerase YP_312597.1], xylose isomerase [Shigella sonnei Ss046] (SEQ ID NO:19); *Shigella dysenteriae* [phosphomannose isomerase ZP_03064125.1, mannose-6-phosphate isomerase, class I [Shigella dysenteriae 1012] (SEQ ID NO:20); mannofructokinase ZP_03064822.1, manno(fructo)kinase [Shigella dysenteriae 1012] (SEQ ID NO:21); xylose isomerase ZP_03063610.1]), xylose isomerase [Shigella dysenteriae 1012] (SEQ ID NO:22); *Citrobacter* strains (e.g. *Citrobacter koseri* [phosphomannose isomerase P_001453192.1, hypothetical protein CKO_01624 [Citrobacter koseri ATCC BAA-895] (SEQ ID NO:23); xylose isomerase YP_001456500.1], xylose isomerase [Citrobacter koseri ATCC BAA-895] (SEQ ID NO:24); *Citrobacter rodentium* [phosphomannose isomerase YP_003365034, mannose-6-phosphate isomerase [Citrobacter rodentium ICC168]. (SEQ ID NO:25); mannofructokinase YP_003364074.1, probable manno(fructo)kinase [Citrobacter rodentium ICC168] (SEQ ID NO:26); xylose isomerase YP_003367655.1], D-xylose isomerase [Citrobacter rodentium ICC168] (SEQ ID NO:27); *Citrobacter youngae* [phosphomannose isomerase ZP_06352970.2, mannose-6-phosphate isomerase, class I [Citrobacter youngae ATCC 29220] (SEQ ID NO:28); xylose isomerase ZP_06571492.1]), xylose isomerase [Citrobacter youngae ATCC 29220] (SEQ ID NO:29); *Salmonella* strains (e.g. *Salmonella enterica* [phosphomannose isomerase YP_002146578.1, mannose-6-phosphate isomerase, class I [Salmonella enterica subsp. enterica serovar Agona str. SL483] (SEQ ID NO:30); xylose isomerase ZP_02342859.1], xylose isomerase [Salmonella enterica subsp. enterica serovar Saintpaul str. SARA29] (SEQ ID NO:31); *Salmonella typhimurium* [phosphomannose isomerase

CAA40399.1)), phosphomannose isomerase [*Salmonella typhimurium*] (SEQ ID NO:32); *Enterobacter* strains (e.g. *Enterobacter cloacae* [phosphomannose isomerase ABF71065.1, mannose-6-phosphate isomerase [Enterobacter cloacae] (SEQ ID NO:33); xylose isomerase YP_003610718.1], xylose isomerase [Enterobacter cloacae subsp. cloacae ATCC 13047] (SEQ ID NO:34); *Enterobacter cancerogenus* [phosphomannose isomerase ZP_05967481.1]), mannose-6-phosphate isomerase, class I [Enterobacter cancerogenus ATCC 35316] (SEQ ID NO:35); *Cronobacter* strains (e.g. *Cronobacter turicensis* [phosphomannose isomerase YP_003210382.1], mannose-6-phosphate isomerase [Cronobacter turicensis z3032] (SEQ ID NO:36); *Cronobacter sakazakii* [phosphomannose isomerase YP_001438057.1]), hypothetical protein ESA_01968 [Cronobacter sakazakii ATCC BAA-894] (SEQ ID NO:37); *Klebsiella* strains (e.g. *Klebsiella pneumoniae* [phosphomannose isomerase YP_002238770.1, mannose-6-phosphate isomerase, class I [Klebsiella pneumoniae 342] (SEQ ID NO:38); mannofructokinase YP_002240149.1]), manno(fructo)kinase [Klebsiella pneumoniae 342] (SEQ ID NO:39); *Serratia* strains (e.g. *Serratia odorifera* [phosphomannose isomerase ZP_06640499.1]), mannose-6-phosphate isomerase [Serratia odorifera DSM 4582] (SEQ ID NO:40).

[0069] Furthermore, the following genes may be used:

D-xylose transporter XylE [*Escherichia fergusonii* ATCC 35469] YP_002385136.1 (SEQ ID NO: 41);
 D-xylose transporter XylE [*Shigella flexneri* 2a str. 301] NP_709888.1 (SEQ ID NO: 42);
 D-xylose-proton symporter (D-xylose transporter) [*Salmonella enterica* subsp. enterica serovar Schwarzengrund str. SL480], Xyl E, ZP_02664392.1 (SEQ ID NO: 43);
 sugar transporter family protein [*Citrobacter youngae* ATCC 29220], Xyl E, ZP_06356420.1 (SEQ ID NO: 44);
 D-xylose:proton symporter [*Acidobacterium capsulatum* ATCC 51196], Xyl E, YP_002753901.1 (SEQ ID NO: 45);
 xylulokinase [*Shigella flexneri* 2a str. 301] NP_709351.1 (SEQ ID NO: 46); xylulose kinase [*Citrobacter rodentium* ICC168] YP_003367656.1 (SEQ ID NO: 47);
 D-xylose transporter subunit XylF [*Shigella flexneri* 5 str. 8401] YP_691285.1 (SEQ ID NO: 48);
 D-xylose transporter subunit XylF [*Citrobacter koseri* ATCC BAA-895] YP_001456501.1 (SEQ ID NO: 49);
 D-xylose ABC transporter, ATP-binding protein [*Shigella boydii* CDC 3083-94] YP_001882237.1, Xyl G, (SEQ ID NO: 50);
 D-xylose ABC transporter, periplasmic D-xylose-binding protein [*Klebsiella pneumoniae* 342], Xyl G, YP_002236058.1 (SEQ ID NO: 51);
 xylose transporter ATP-binding subunit [*Citrobacter koseri* ATCC BAA-895] YP_001456502.1, Xyl G, (SEQ ID NO: 52);
 D-xylose ABC transporter, permease protein [*Shigella dysenteriae* 1012] ZP_03063638.1, Xyl H, (SEQ ID NO: 53);
 inner-membrane translocator [*Enterobacter cloacae* subsp. cloacae ATCC 13047] YP_003610715.1, Xyl H, (SEQ ID NO: 54);
 glucokinase [*Shigella dysenteriae* 1012] ZP_03065183.1 (SEQ ID NO: 55);
 glucokinase [*Escherichia fergusonii* ATCC 35469] YP_002381954.1 (SEQ ID NO: 56);
 transaldolase [*Shigella dysenteriae* 1012] ZP_03066451.1 (SEQ ID NO: 57);
 transaldolase B [*Escherichia fergusonii* ATCC 35469] YP_002381239.1 (SEQ ID NO: 58);
 transketolase [*Shigella dysenteriae* 1012] ZP_03063878.1 (SEQ ID NO: 59);
 transketolase [*Escherichia fergusonii* ATCC 35469] YP_002383968.1 (SEQ ID NO: 60) can be used in the invention.

[0070] Alternative sources for various genes encoding the desired enzyme activities are for example :

Alternative sources for glucokinases:

glucokinase [*Shigella boydii* Sb227] YP_408803.1
 glucokinase [*Escherichia albertii* TW07627] ZP_02901664.1
 glucokinase [*Salmonella enterica* subsp. enterica serovar Typhimurium str. LT2] NP_461344.1
 glucokinase [*Citrobacter youngae* ATCC 29220] ZP_06351950.1
 Glucokinase [*Citrobacter rodentium* ICC168] YP_003366335.1
 glucokinase [*Enterobacter cloacae* subsp. cloacae NCTC 9394] CBK87115.1
 glucokinase [*Citrobacter koseri* ATCC BAA-895] YP_001452005.1
 glucokinase [*Cronobacter sakazakii* ATCC BAA-894] YP_001436959.1
 glucokinase [*Enterobacter cancerogenus* ATCC 35316] ZP_05968594.1
 glucokinase [*Klebsiella pneumoniae* subsp. pneumoniae MGH 78578] YP_001336384.1
 glucokinase [*Serratia odorifera* DSM 4582] ZP_06639362.1
 Glucokinase [*Erwinia billingiae* Eb661] CAX60782.1

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Glucokinase [Erwinia amylovora CFBP1430] YP_003531826.1

Alternative sources for mannofructokinases are for example:

fructokinase [Shigella sonnei Ss046] YP_309383.2
hypothetical protein CKO_02777 [Citrobacter koseri ATCC BAA-895] YP_001454319.1
fructokinase [Enterobacter sp. 638] YP_001175599.1
fructokinase [Escherichia fergusonii ATCC 35469] YP_002383736.1
fructokinase [Enterobacter cloacae subsp. cloacae ATCC 13047] YP_003611661.1

Alternative sources for facilitated diffusion transporter, GLF and XylE are for example:

D-xylose transporter XylE [Shigella sonnei Ss046] YP_312943.1 xylose-proton symport [Citrobacter sp. 30_2] ZP_04558597.1 sugar transporter family protein [Citrobacter youngae ATCC 29220] ZP_06356420.1
D-xylose transporter XylE [Salmonella enterica subsp. arizonae serovar 62:z4,z23:--] YP_001572426.1
D-xylose-proton symporter (D-xylose transporter) [Citrobacter rodentium ICC168] YP_003367154.1
D-xylose transporter XylE [Mannheimia succiniciproducens MBEL55E] YP_089566.1
D-xylose transporter XylE [Actinobacillus succinogenes 130Z] YP_001343806.1
D-xylose transporter XylE [Bacillus cereus ATCC 10987] NP_978526.1 xylose permease [Bacillus megaterium DSM319] YP_003597063.1

[0071] Due to high homology between facilitated diffusion protein for mannose uptake, in particular GLF, and xylose proton symporter E, xyl E, genes encoding xylose proton symporter E, xyl E, may be used instead of genes encoding facilitated diffusion protein for mannose uptake, and vice versa.

Alternative sources for xylulokinases are for example:

xylulokinase [Shigella sp. D9] ZP_05434777.1
xylulokinase [Escherichia fergusonii ATCC 35469] YP_002384640.1
xylulokinase [Citrobacter sp. 30_2] ZP_04559359.1
xylulokinase [Shigella boydii Sb227] YP_409882.1
xylulose kinase [Salmonella enterica subsp. enterica serovar Paratyphi C strain RKS4594] YP_002639258.1
hypothetical protein CKO_05020 [Citrobacter koseri ATCC BAA-895] YP_001456499.1
xylulokinase [Shigella sonnei Ss046] YP_312598.1
xylulokinase [Citrobacter youngae ATCC 29220] ZP_06355683.1
xylulokinase [Klebsiella sp. 1_1_55] ZP_06551221.1
hypothetical protein ECL_00202 [Enterobacter cloacae subsp. cloacae ATCC 13047] YP_003610719.1
xylulokinase [Klebsiella pneumoniae 342] YP_002236061.1
xylulokinase [Klebsiella variicola At-22] YP_003437118.1
Xylulose kinase [Cronobacter turicensis z3032] YP_003212435.1
Xylulose kinase [Yersinia intermedia ATCC 29909] ZP_04636833.1
Xylulokinase [Erwinia billingiae Eb661] CAX60830.1
xylulokinase [Serratia odorifera DSM 4582] ZP_06638097.1

Alternative sources for XylIF are for example:

D-xylose transporter subunit XylIF [Shigella boydii Sb227] YP_409884.1
D-xylose transporter subunit XylIF [Enterobacter sp. 638] YP_001174896.1
D-xylose transporter subunit XylIF [Cronobacter sakazakii ATCC BAA-894] YP_001440178.1
D-xylose-binding periplasmic protein [Cronobacter turicensis z3032] YP_003212438.1
D-xylose transporter subunit XylIF [Enterobacter cloacae subsp. cloacae ATCC 13047] YP_003610717.1
hypothetical protein ENTAN_08293 [Enterobacter cancerogenus ATCC 35316] ZP_05969667.2
D-xylose ABC transporter, periplasmic substrate-binding protein [Erwinia billingiae Eb661] CAX60828.1
xylose ABC superfamily ATP binding cassette transporter, binding protein [Serratia odorifera DSM 4582] ZP_06638100.1
D-xylose transporter subunit XylIF [Actinobacillus succinogenes 130Z] YP_001343809.1

Alternative sources for XylG are for example:

xylose transporter ATP-binding subunit [Shigella flexneri 5 str. 8401] YP_691284.1 D-xylose ABC transporter, ATP-binding protein [Shigella dysenteriae 1012] ZP_03063359.1
 5 xylose transporter ATP-binding subunit [Enterobacter cloacae subsp. cloacae ATCC 13047] YP_003610716.1 D-xylose ABC transporter, ATP-binding protein [Klebsiella pneumoniae 342] YP_002236058.1
 hypothetical protein ENTCAN_08292 [Enterobacter cancerogenus ATCC 35316] ZP_05969666.1
 xylose transporter ATP-binding subunit [Cronobacter sakazakii ATCC BAA-894] YP_001440177.1
 Xylose import ATP-binding protein xylG [Cronobacter turicensis z3032] YP_003212439.1
 10 D-xylose ABC transporter, ATPase subunit [Erwinia billingiae Eb661] CAX60827.1 xylose ABC superfamily ATP binding cassette transporter, ABC protein [Serratia odorifera DSM 4582] ZP_06638101.1
 COG1129: ABC-type sugar transport system, ATPase component [Actinobacillus pleuropneumoniae serovar 1 str. 4074] ZP_00134918.2

Alternative sources for XylH are for example:

hypothetical protein CKO_05024 [Citrobacter koseri ATCC BAA-895]YP_001456503.1
 xylose ABC transporter membrane protein [Enterobacter cloacae subsp. cloacae NCTC 9394] CBK87790.1
 hypothetical protein ENTCAN_08291 [Enterobacter cancerogenus ATCC 35316] ZP_05969665.1
 20 inner-membrane translocator [Enterobacter sp. 638] YP_001174894.1 hypothetical protein ESA_04159 [Cronobacter sakazakii ATCC BAA-894]YP_001440176.1
 D-xylose ABC transporter, permease protein [Klebsiella pneumoniae 342] YP_002236057.1
 Xylose transport system permease protein xylH [Cronobacter turicensis z3032] YP_003212440.1
 sugar transport system permease protein [Yersinia enterocolitica subsp. enterocolitica 8081] YP_001008391.1
 25 D-xylose ABC transporter, permease protein [Erwinia billingiae Eb661] CAX60826.1
 monosaccharide-transporting ATPase [Serratia proteamaculans 568] YP_001476339.1
 xylose ABC superfamily ATP binding cassette transporter, membrane protein [Serratia odorifera DSM 4582] ZP_06638102.1
 monosaccharide-transporting ATPase [Actinobacillus succinogenes 130Z] YP_001343807.1

Alternative sources for transaldolase are for example

transaldolase B [Shigella boydii Sb227] YP_406573.1
 transaldolase [Citrobacter youngae ATCC 29220] ZP_06354813.1
 35 transaldolase B [Citrobacter koseri ATCC BAA-895] YP_001454897.1
 transaldolase B [Salmonella enterica subsp. arizonae serovar 62:z4,z23:-YP_001571972.1
 transaldolase B [Citrobacter rodentium ICC168] YP_003363651.1
 transaldolase B [Enterobacter sp. 638] YP_001175308.1
 hypothetical protein ENTCAN_05194 [Enterobacter cancerogenus ATCC 35316] ZP_05966848.1
 40 transaldolase B [Citrobacter sp. 30_2] ZP_04559788.1
 transaldolase [Enterobacter cloacae subsp. cloacae NCTC 9394] CBK87626.1
 transaldolase B [Klebsiella pneumoniae 342] YP_002240539.1
 transaldolase B [Enterobacter cloacae subsp. cloacae ATCC 13047]YP_003611333.1
 transaldolase B [Cronobacter sakazakii ATCC BAA-894] YP_001439387.1
 45 transaldolase B [Cronobacter turicensis z3032] YP_003208998.1
 transaldolase [Escherichia albertii TW07627] ZP_02902842.1
 transaldolase [Yersinia kristensenii ATCC 33638] ZP_04625574.1
 transaldolase A [Serratia odorifera DSM 4582] ZP_06637249.1
 transaldolase B [Erwinia amylovora CFBP1430] YP_003532302.1
 50 transaldolase B [Erwinia amylovora ATCC 49946] YP_003537736.1
 transaldolase B [Erwinia pyrifoliae DSM 12163] CAY73102.1

Alternative transketolases are for example:

transketolase [Shigella flexneri 2a str. 2457T] NP_838419.1
 transketolase [Shigella boydii CDC 3083-94] YP_001881708.1
 transketolase [Escherichia albertii TW07627] ZP_02900973.1
 transketolase [Citrobacter koseri ATCC BAA-895] YP_001455802.1

transketolase [Citrobacter sp. 30_2] ZP_04560349.1
transketolase [Citrobacter youngae ATCC 29220] ZP_06355044.1
transketolase [Enterobacter cancerogenus ATCC 35316] ZP_05970189.1
transketolase [Enterobacter cloacae subsp. cloacae NCTC 9394] CBK86553.1
5 transketolase [Klebsiella pneumoniae subsp. pneumoniae MGH 78578] YP_001336984.1
transketolase 1 [Citrobacter rodentium ICC168] YP_003368408.1
transketolase [Klebsiella variicola At-22] YP_003440166.1
transketolase [Klebsiella pneumoniae 342] YP_002239249.1
Transketolase 1 [Cronobacter turicensis z3032] YP_003211835.1
10 transketolase [Cronobacter sakazakii ATCC BAA-894] YP_001436540.1
transketolase [Enterobacter cloacae subsp. cloacae ATCC 13047] YP_003614730.1
transketolase [Erwinia tasmaniensis Et1/99] YP_001908733.1
transketolase [Serratia odorifera DSM 4582] ZP_06641778.1

[0072] The accession numbers are derived from NCBI database (National Center for Biotechnology Information, U.S. National Library of Medicine, 8600 Rockville Pike, Bethesda MD, 20894 USA). The date of providing the accession numbers was 2nd June 2010.

[0073] Within the scope of the present invention are proteins encoded by any of the above listed genes, in particular those comprising the amino acid sequences SEQ ID NO: 1 to 60, preferably sequences SEQ ID NO: 1 to 12.

[0074] It is evident that small variations in the nucleotide sequence of a gene do not significantly change the catalytic properties of the encoded protein. For example many changes in the nucleotide sequence do not change the amino acid sequence of the encoded protein. Also an amino acid sequence may have variations, which do not change the functional properties of a protein, in particular they do not prevent an enzyme from carrying out its catalytic function. As it is known to a person skilled in the art, variations can exist or can be made to regions which are outside of the region critical to the function of the enzyme and still result in an active polypeptide. Such variations in the nucleotide sequence or DNA molecules or in an amino acid sequence are known as "functional equivalents", because they do not significantly change the function of the gene to encode a protein with a particular function, e.g. catalyzing a particular reaction or, respectively, change the particular function of the protein. Disclosed are functional equivalents, including fragments or derivatives, or closest homologues of the listed proteins, in particular functional equivalents of sequences SEQ ID NO: 1 to 60, preferably sequences SEQ ID NO: 1 to 12; for example genes encoding amino acid sequences showing at least 50%, preferably at least 60 % identity, preferably at least 65 %, preferably at least 70 %, preferably at least 75 %, preferably at least 80 %, preferably at least 85 %, more preferably at least 90 %, still more preferably at least 95 %, more preferably at least 98 % identity to any of the amino acid sequences of the above listed proteins, in particular sequences SEQ ID NO: 1 to 60, preferably sequences SEQ ID NO: 1 to 12; such as genes encoding proteins comprising any of the amino acid sequences SEQ ID NO: 1 to 60, preferably sequences SEQ ID NO: 1 to 12; for example also genes encoding amino acid sequences showing at least 50%, preferably at least 60 % identity, preferably at least 65 %, preferably at least 70 %, preferably at least 75 %, preferably at least 80 %, preferably at least 85 %, more preferably at least 90 %, still more preferably at least 95 %, more and more preferably at least 98 % identity to any of the amino acid sequences SEQ ID NO: 1 to 60, preferably sequences SEQ ID NO: 1 to 12. The term "identity" refers to the identity between two nucleic acid or amino acid sequences, respectively compared to each other from the first nucleic acid to the last nucleic acid or from the first amino acid encoded by the corresponding gene to the last amino acid. The identity of the full-length sequences can be measured by using BLAST program (Altschul, S. F., T.L. Madden, A. A. Schaffer, J. Zhang, Z. Zhang, W. Miller und D. J. Lipman 1997. Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. Nucleic Acids Res. 25:3389-3402). In the comparison is preferably used the mature sequences of the proteins.

[0075] Bacteria belonging to genus *Cupriavidus* or *Ralstonia* and capable of expressing genes encoding phosphomannose isomerase and facilitated diffusion protein for mannose uptake or xylose proton symporter E and optionally mannofructokinase are capable of growing on mannose as the carbon source. A *Cupriavidus* host introduced to express a gene encoding facilitated diffusion protein for mannose uptake activity, in particular *glf*, can use also glucose as carbon source.

[0076] Mannose can be found in wood and plant materials, especially in hemicelluloses of soft woods (e.g. pine or spruce) consisting of galactoglucomannane polymer, in some fruits or berries (e.g. cranberry), and in cell structures of various microorganisms such as fungi (yeasts and filamentous fungi) and microalgae (e.g. *Chlorella*, *Nannochloropsis* and *Phaeodactylum*).

[0077] Bacteria belonging to genus *Cupriavidus* or *Ralstonia* and capable of expressing genes encoding xylose isomerase and xylulokinase and a high affinity ABC- transporter or xylose proton symporter E are capable of growing on xylose or xylose and arabinose as the carbon source. A *Cupriavidus* host introduced to express genes, which encode high affinity ABC- transporter, in particular genes encoding Xyl F, Xyl G and Xyl H, are able to use also glucose and

galactose as carbon source.

[0078] In addition the microorganism may be genetically modified to express transaldolase and/or transketolase making the growth of the microorganism faster.

[0079] As is described herein a microorganism introduced to express a gene encoding facilitated diffusion protein for mannose uptake is capable of using also glucose. This is achieved in particular by gene *glf*.

[0080] The microorganism host is capable of utilizing glucose also, if to the host is introduced to express a gene encoding glucokinase EC 2.7.1.2).

[0081] The microorganism host is capable of utilizing mannose. In one embodiment a microorganism according to this disclosure is capable of utilizing one or more of mannose, glucose, galactose, xylose and/or arabinose as carbon source.

[0082] In one embodiment a microorganism according to this disclosure is capable of utilizing mannose and optionally glucose as carbon source.

[0083] In one further embodiment a microorganism according to this disclosure is capable of utilizing xylose, or xylose and arabinose, and optionally glucose and/or galactose as carbon source.

[0084] A microorganism introduced to express xylose isomerase, xylulokinase and a high affinity ABC- transporter or xylose protein symporter E, is capable of utilizing xylose. Surprisingly, the microorganism host is capable of utilizing also arabinose. To the best knowledge of the present inventors, this is the first time a *Ralstonia* or *Cupriavidus* host has obtained the capability to use arabinose. In the prior art Buchholz et al. (1994) transferred an *Alcaligenes eutrophus* host by a 28-kb insert, 16.4 of which comprised the minimal information required for xylose utilization by *A. eutrophus*. Buchholz et al. assumed that the *xyl* genes encoding xylose isomerase and xylulokinase were located within this region, since they were induced during growth on xylose. Buchholz et al. obtained three transconjugants which grew on xylose as sole source of carbon and energy, but ribose or arabinose-utilizing strains were not obtained. As herein disclosed the microorganism host has been introduced to express inserts which consist essentially of nucleotide sequences needed for xylose utilization. Surprisingly the constructions make the host capable of utilizing also arabinose.

[0085] A microorganism introduced to express a high affinity ABC- transporter, in particular genes encoding Xyl F, Xyl G and Xyl H, is surprisingly capable of using also glucose and galactose as carbon source.

[0086] The activity of MAK (mannofructokinase), PMI (phosphomannose isomerase), GLK (glucokinase) can be assayed by methods well known in the art. Soluble protein fractions of recombinant host strain can be applied for MAK, PMI and GLK activity measurements at 30°C using a Nicolet Evolution 100 UV/VIS spectrophotometer (Thermo Electron Corporation, Cambridge, UK). Activity of MAK can be assayed with an NADH-coupled system as described by Sebastian and Asensio (1972) with some modifications as described in the examples.

[0087] PMI activity can be measured with an NADP-coupled assay (Kang and Markovitz 1967) with some modifications as described in the examples.

[0088] GLK activity can be measured with an NADPH-coupled system according to the method of Gottschalk (Gottschalk et al. 1964) with modifications as described in the examples.

[0089] XylA and XylB activity can be assayed by methods well known in the art. Soluble protein fractions of recombinant host strain can be applied for XylA and XylB activity measurements at 30 °C using a Nicolet Evolution 100 UV/VIS spectrophotometer (Thermo Electron Corporation, Cambridge, UK). Activity of XylA (Gao et al. 2002, Mejnen et al. 2008) or XylB (Shamanna and Sangerson 1979, Eliasson et al. 2000) can be assayed with an NADH-coupled system with some modifications, using sorbitol dehydrogenase or pyruvate kinase and lactate dehydrogenase, respectively, as auxiliary enzymes as described in the examples

[0090] "Regulatory elements" refer here to regulatory elements which can regulate the expression of a foreign gene introduced into a host cell, here in particular into *Cupriavidus* or *Ralstonia*. Regulatory elements include promoters, terminators, enhancers and signal sequences.

[0091] "Vector" refers here to a nucleic acid molecule, typically a DNA molecule, which can be used to transfer foreign genetic material into a host cell. A vector may be a plasmid, bacteriophage, virus or cosmid, here it is typically a plasmid. Preferably the vector is a broad host range vector. In one embodiment the vector may be an episomal vector not incorporated to the genome of the host cell. In another embodiment the vector may be integrated into the genome of the host cell.

[0092] Broad-host-range plasmids permit replication in diverse bacterial species and are not restricted to bacteria of a certain genus or species.

[0093] In one embodiment of the invention a vector may comprise a gene encoding phosphomannose isomerase, a gene encoding facilitated diffusion protein for mannose uptake, a gene encoding xylose proton symporter E and/or a gene encoding mannofructokinase. The genes may be in the same or in separate vectors.

[0094] In another embodiment of the invention a vector may comprise a gene encoding xylose isomerase, xylulokinase, xylose proton symporter E, and/or a high affinity ABC- transporter A. The genes may be in the same or in separate vectors.

[0095] In one embodiment of the invention the vector may be a broad host range vector. This provides the advantage that the extension of the substrate range can be achieved with the broad host range vectors in any species or strains

of *Cupriavidus* or *Ralstonia*.

[0096] Preferably the vector is a high copy number vector.

[0097] Furthermore, the vector comprises regulatory elements, in particular a promoter, for regulating expression of said genes in *Cupriavidus* or *Ralstonia*. Preferably the vector comprises a strong promoter for expression of the desired genes in *Cupriavidus* or *Ralstonia*.

[0098] In one embodiment regulatory elements in the vector may comprise *neokanamycin*-promoter, *lac*-promoter, or glyceraldehyde-3-phosphate dehydrogenase promoter. In addition, the following promoters can be used: promoter P2, typically from *Pseudomonas syringae* (Solaiman and Swingle 2010), in particular of plasmid pBS29-P2 (Solaiman et al. 2010), the hydrogenase (SH) promoter, typically from *R. eutropha* (Porthun et al. 2002, Schwartz et al. 1998), and the promoter P_L, typically from the *cbb* operon (Jeffke et al. 1999, Schäferjohann et al. 1996, Windhövel and Bowien 1990). Preferably the promoters are constitutive.

[0099] Suitable promoters are here in particular a constitutive *lac*-promoter (P_{lac}) (Siedow et al. 1999), *neokanamycin*-promoter (P_{nk}), glyceraldehyde-3-phosphate dehydrogenase (GAPDH) promoter (P_{GAPDH}).

[0100] Vectors, suitable for use in the invention are for example pBBR1 MCS and its derivatives pBBR1 MCS-2, pBBR1 MCS-3, pBBR1 MCS-4 and pBBR1 MCS-5 (Kovach et al. 1995).

[0101] Furthermore, the shuttle plasmid pBHR1 can be used (MoBiTec GmbH, Göttingen, Germany) (Aneja et al. 2009) in the invention.

[0102] Plasmid pKT230 (Park et al. 1995), which is derived from the broad host range plasmid RSF1010 (Franklin et al. 1981) can also be used in the invention.

[0103] Also plasmid pBS29 (Swingle et al. 2008) or pBS29-P2 (Solaiman et al. 2010), which are derived from plasmid pUCP26 capable of replicating in *E. coli* and *Pseudomonas* species (West et al. 1994), can be used in this invention.

[0104] Within the scope of the present invention is also the production of desired chemicals by using the modified microorganisms according to the invention. The desired chemicals may be products naturally produced by the host, such as polyhydroxyalkanoates (Reinecke and Steinbuechel 2009 and Verlinden et al. 2007) or the host may be introduced to express the desired chemicals, for example lipids. Genes which can be used to genetically modify microorganism hosts to express various lipids has been disclosed in WO 2007/136762. The capability of a *Cupriavidus* or *Ralstonia* host to produce lipids was exemplified by introducing a *Cupriavidus* or *Ralstonia* host to express fatty acid ethyl esters (FAEE). This was carried out by expressing in a polyhydroxy butyrate (PHB) -negative mutant of *R. eutropha* H16 host genes encoding wax ester synthase/diacylglycerol acyltransferase (*attA*), pyruvate decarboxylase (*pdh*) and alcohol dehydrogenase B (*adhB*).

[0105] The benefit of this invention is an extended substrate utilization range of *Cupriavidus* or *Ralstonia* towards abundant and low-cost carbohydrates. The extension of substrate range was achieved by using broad host range plasmids, which make it possible to extend the substrate range in any species or strain of the *Cupriavidus* or *Ralstonia* host. By chemical methods it is possible to introduce a mutation only in a specific strain. Furthermore, in chemically induced mutants there is the risk of additional mutations that might affect the productivity and/or may lead to unfavourable side effects.

[0106] The present invention was exemplified by constructing two different recombinant strains of *Ralstonia*, more specifically two *R. eutropha* H16 strains. The strains were developed to be capable of utilizing mannose/glucose, and xylose/arabinose respectively, by metabolic engineering. The induction of different gene in *R. eutropha*, necessary for either glucose/mannose uptake and utilization of mannose, or for xylose uptake and utilization, respectively, complemented the lacking parts of either glucose/mannose or xylose/arabinose catabolism, respectively. The recombinant strains of *R. eutropha* with the extended substrate utilization range synthesized poly(3-hydroxybutyrate) PHB from glucose, mannose, xylose and arabinose in similar amounts than from gluconate.

[0107] In one embodiment of the invention in *Cupriavidus* or *Ralstonia* may be expressed genes, which are responsive of sugar uptake and conversion. Hexokinases from other prokaryotes, such as *Leuconostoc mesenteroides* or *Streptomyces* sp., which also exhibit an activity for mannose (Anderson and Sapico 1975, Coulombel et al. 1982), may be expressed in *Cupriavidus* or *Ralstonia* to improve sugar conversion in the host. An overexpression of the chromosomally encoded hexokinase with its side activity for mannose may be used to enhance mannose utilization in *Ralstonia*. The pBBR plasmids used in the examples can be replaced for example with high copy number vectors and/or with stronger promoters (Kovach et al. 1995, Siedow et al. 1999), or other vectors more suitable for expression of the desired genes for mannose utilization. The *lac*-promoter may be replaced with a stronger constitutive promoter, for example the *neokanamycin*-promoter as described herein and which give elevated expression levels. The desired genes may also be integrated into the genome of *Cupriavidus* or *Ralstonia* giving advantages, such as stability. Furthermore, mutants containing marker-free integrations in their genomes are not subjected to any selection, which save costs especially for large scale cultivations.

[0108] According to one further embodiment the present invention provides a method, which comprises that the microorganism is allowed to synthesize bulk chemicals, such as lipids, for example polyhydroxyalkanoates, fatty acids or fatty acid derivatives, and the chemical is recovered from the cultivation medium or from the cells.

[0109] In the cultivation of *Cupriavidus* or *Ralstonia* can be used any cultivation medium comprising components suitable for *Cupriavidus* or *Ralstonia* growth. Typically they include a carbon and/or a nutrient source, salts, typically mineral salts, and water. Selection markers, such as antibiotic resistance genes may be needed to confirm the presence of desired genes in the host cells.

[0110] The substrate utilization of *Ralstonia* has been here exemplified by cultivating *Cupriavidus* or *Ralstonia* in a mineral salts medium (MSM) described by Schlegel et al. (1961). In industrial applications the cultivation medium typically comprises monomeric sugars originating from lignocellulosic materials, as carbon source, and in addition possibly nutrients and salts or components thereof as micro- and macronutrients, and water.

[0111] In the cultivation of *Cupriavidus* or *Ralstonia* can be used cultivation conditions suitable for cultivation of *Cupriavidus* or *Ralstonia*. Such cultivation conditions are well known to a person skilled in the art. In the cultivation can be used liquid or solid media. Liquid cultures may be agitated, suitable agitation 50 -1000 rpm, typically 100 - 600 rpm. The cultivation temperature is typically 15 to 45, preferably 25 to 37 °C, typically 30 °C. The incubation time may be 1 day to 14 days, typically 2 to 7 days, preferably 24 to 72 hours. The cultivation may be a batch, fed-batch or continued cultivation.

[0112] *Escherichia coli* and *Zymomonas mobilis* were cultivated by methods well known to a person skilled in the art and described in the examples.

[0113] As herein described recombinant *Ralstonia* strains and wild type strains were cultivated on different carbon sources. Cells were incubated on MSM agar plates containing 1 % (wt/vol) of the respective carbon source for 5 days at 30 °C. Visible colony growth was phenotypically examined. Characters: (++) growth after an incubation time of 24-72 h, (+) growth after more than 72 h of incubation, (-) no visible growth detectable. The use of the following carbon sources were examined: L-Arabinose, D- fructose, D-Galactose, D- Gluconate, D-Glucose, D-glycerol, D-maltose- mannitol, D-mannose, N-acetyl-D-glucosamine, pyruvate, D, ribose, D-trehalose and D-xylose.

[0114] The activity of MAK (mannofructokinase), PMI (phosphomannose isomerase), GLK (glucokinase) can be assayed by methods well known in the art. Soluble protein fractions of recombinant host strain can be applied for MAK, PMI and GLK activity measurements at 30°C using a Nicolet Evolution 100 UV/VIS spectrophotometer (Thermo Electron Corporation, Cambridge, UK). Activity of MAK can be assayed with an NADH-coupled system as described by Sebastian and Asensio (1972) with some modifications as described in the examples.

[0115] PMI activity can be measured with an NADP-coupled assay (Kang and Markovitz 1967) with some modifications as described in the examples.

[0116] GLK activity can be measured with an NADPH-coupled system according to the method of Gottschalk (Gottschalk et al. 1964) with modifications as described in the examples.

[0117] The present invention has been exemplified by generating recombinants of *R. eutropha* strain H16 which, in contrast to the wild type, were able to utilize mannose and/or glucose as sole carbon sources. Up to four genes (*glf*, *glk*, *mak*, *pmi*) were introduced episomally in *R. eutropha* strain H16 under control of a constitutive *neokanamycin*- or *lac*-promoter, enabling uptake and conversion of glucose and mannose into intermediates of the KDPG pathway.

[0118] Bacteria belonging to the genera *Ralstonia* and *Cupriavidus* typically have a very narrow substrate utilization spectrum and are only limited amount of sugars. E.g. the capability of *Ralstonia eutropha* H 16 to use carbohydrates and related compounds is limited to fructose, N-acetylglucosamine and Gluconate. To broaden the substrate utilization range of *Ralstonia* and *Cupriavidus* strains, the strains were engineered to use mannose as sole carbon source for growth. The genes for a facilitated diffusion protein (*glf*) from *Zymomonas mobilis* and for mannofructokinase (*mak*), phosphomannose isomerase (*pmi*) and glucokinase (*glk*) from *E. coli* either alone or in combination constitutively expressed in *R. eutropha* under control of the *lac*-promoter, using an episomal broad host range vector. Recombinant strains harboring pBBR1 MCS-3::*glf*::*mak*::*pmi* or pBBR1MCS-3::*glf*::*pmi* grew on mannose, whereas pBBR1 MCS-3::*glf*::*mak* and pBBR1 MCS-3::*glf* did not confer the ability to utilize mannose as carbon source to *R. eutropha*. The recombinant strains harboring pBBR1MCS-3::*glf*::*mak*::*pmi* exhibited faster growth than pBBR1 MCS-3::*glf*::*pmi* on mannose. These data indicated that phosphomannose isomerase is required to convert mannose-6-phosphate into fructose-6-phosphate for subsequent catabolism via Entner-Doudoroff pathway. In addition, surprisingly all plasmids conferred to *Ralstonia* also the ability to grow in the presence of glucose.

[0119] Furthermore, expression of the respective enzymes was demonstrated at the transcriptional level and by measuring the activities of mannofructokinase (0.14 U mg⁻¹ protein), and phosphomannose isomerase (0.25 U mg⁻¹ protein).

[0120] To identify genes in the genome of *R. eutropha* H16 coding for functional enzymes required for uptake and catabolism of D-xylose, a protein-protein-BLAST-analysis (Altschul et al. 1990) using an amino acid sequence database of *R. eutropha* H16 (Pohlmann et al. 2006) was performed. No genes encoding a transporter specific for D-xylose could be identified (Pohlmann et al. 2006). Also no genes encoding enzymes catabolizing D-xylose, e.g. a xylose isomerase or xylulose kinase, were identified. Indeed, *R. eutropha* H16 possesses genes for a fructokinase and a glucokinase (Pohlmann et al. 2006). All other genes, encoding enzymes of the non-oxidative pentose phosphate pathway for further catabolism of D-xylulose-5-phosphate are present in *R. eutropha* strain H16.

[0121] The construction of *Ralstonia* hosts to use xylose as sole carbon source for growth was exemplified by using

R. eutropha strains. The genes for a low-affinity D-xylose-proton-symporter (xylE), a high-affinity ABC-transporter (xylIFGH), a xylose isomerase (xylA), and a xylulokinase (xylB), from *E. coli* were in combination constitutively expressed in *Ralstonia* hosts under control of a lac-promoter and glyceraldehyde-3-phosphate dehydrogenase (GADPH) promoter, respectively, using an episomal broad host range vector. Recombinant strains harboring pBBR1 MCS-3::xylABFGH

grew on xylose conferring the ability to utilize xylose as carbon source to *Ralstonia*. In addition, surprisingly, plasmids conferring xylose-utilization to *Ralstonia* conferred also the ability to grow in the presence of arabinose.

[0122] To exemplify the ability of recombinant *R. eutropha* strains to produce PHB from mannose or glucose, *Ralstonia* cells were cultivated under conditions permissive for PHB accumulation in MSM containing 1% (wt/vol) mannose, glucose or sodium gluconate as sole carbon source containing tetracycline or kanamycin, respectively, for selection and 0.05% (wt/vol) NH₄Cl. The PHB contents of the cells were analyzed gas chromatographically as described in the materials and methods section. Cultures containing gluconate as a sole carbon source served as a reference for the recombinant strain, and samples were taken in the early stationary growth phase. GC analysis of the cells revealed a PHB content of 63% (wt/wt) of cell dry mass. Cells grown in presence of 1% (wt/vol) glucose or mannose were also examined in the stationary growth phase, and PHB contents of 65 and 67% (wt/wt) of cell dry mass, respectively, were determined.

[0123] To exemplify the ability of recombinant *R. eutropha* strains to produce PHB from xylose and arabinose, cells were cultivated under conditions permissive for PHB accumulation in MSM containing 1% (wt/vol) D-xylose, L-arabinose or sodium gluconate as sole carbon source containing tetracycline, respectively, for selection and 0.05% (wt/vol) NH₄Cl. The PHB contents of the cells were analyzed gas chromatographically as described in the Examples in the materials and methods section. Cultures containing gluconate as a sole carbon source served as a reference for the recombinant strain, and samples were taken in the early stationary growth phase. GC analysis of the cells revealed that the cells were able to produce PHB. Cells grown in presence of 1% (wt/vol) L-arabinose or D-xylose were also examined in the stationary growth phase, and production of PHB was shown.

[0124] The recombinant strains of *R. eutropha* with the extended substrate utilization range were thus able to synthesize poly(3-hydroxybutyrate) PHB from glucose, mannose, xylose and arabinose in similar amounts than from gluconate.

Examples

Example 1

Materials and methods

[0125] Bacterial strains, plasmids, oligonucleotides and cultivation conditions. All bacteria, plasmids and primers used in this application are listed in Table 1. Cells of *R. eutropha* strain H16 were cultivated in mineral salts medium (MSM) as described by Schlegel et al. (1961). Carbon sources were added to liquid MSM as indicated in the text. Liquid cultures in Erlenmeyer flasks were incubated on a horizontal rotary shaker at an agitation of 110 rpm. Solid media were prepared by addition of 1.5% (wt/vol) agar-agar. Cells of *Escherichia coli* were cultivated at 37 °C in Lysogeny Broth (LB) [Sambrook et al. 1989]. Cells of *Zymomonas mobilis* were cultivated in a medium containing 10 g/L Bacto peptone, 10 g/L yeast extract, and 20 g/L glucose. Antibiotics were applied according to Sambrook et al. (1989) and as indicated in the text.

Table 1. Bacterial strains, plasmids and oligonucleotides

Strain, plasmid or primer	Relevant characteristics	Source or reference
Strains		
<i>E. coli</i> K-12	Wild type	DSM 426
<i>E. coli</i> TOP10	F ⁻ <i>mcrA</i> , Δ (<i>mrr-hsdRMS-mcrBC</i>) <i>f80lacZ</i> Δ M15, Δ <i>lacX74</i> , <i>deoR</i> , <i>recA1</i> , <i>araD139</i> , Δ (<i>ara-leu</i>)7697, <i>galU</i> , <i>galK</i> , <i>rpsL</i> , <i>endA1</i> , <i>nupG</i>	Invitrogen
<i>E. coli</i> S17-1	<i>recA1</i> , <i>thi1</i> , <i>hsdR17</i> (<i>r_K⁻</i> , <i>m_K⁺</i>), <i>proA</i> , <i>tra</i> -genes of RP4 plasmid chromosomally integrated (Mobilization strain)	Simon et al. 1983
<i>E. coli</i> JE5511	Hfr, <i>manA4</i> , <i>lpp-1</i> , <i>pps-6</i>	<i>E. coli</i> Genetic Stock Center (Hirota et al. 1977)
<i>R. eutropha</i> strain H16	Wild type	DSM 428
<i>Z. mobilis</i> subsp. <i>mobilis</i>	Wild type	DSM 424 ATCC 10988
<i>Z. mobilis</i> subsp. <i>mobilis</i>	Wild type	DSM3580 ATCC 9191
Plasmids		
pJET1.2/blunt	Ap ^r	Fermentas
pCR2.1	Ap ^r , Km ^r , <i>LacZ</i> - α	Invitrogen
pJET1.2:: <i>glf</i>	with <i>glf</i> as blunt end PCR product	in the examples
pJET1.2:: <i>mak</i>	with <i>mak</i> as blunt end PCR product	in the examples
pJET1.2:: <i>pmi</i>	with <i>pmi</i> as blunt end PCR product	in the examples
pCR2.1::P _{nk}	with <i>neokanamycin</i> promoter as <i>XhoI/NdeI</i> fragment	in the examples
pCR2.1::P _{nk} :: <i>gk</i>	with <i>neokanamycin</i> promoter and <i>gk</i> as <i>XhoI/EcoRI</i> fragment	in the examples
pBBR1MCS-3	Tc ^r , <i>LacZ</i> - α , <i>mob</i> , <i>rep</i>	Kovach et al. 1995
pBBR1MCS-2::P _{nk} :: <i>glf</i>	with <i>neokanamycin</i> promoter and <i>glf</i> as <i>XhoI/SacI</i> fragment	in the examples
pBBR1MCS-2::P _{nk} :: <i>gk</i> :: <i>gk</i>	with <i>neokanamycin</i> promoter, <i>gk</i> and <i>glf</i> as <i>XhoI/SacI</i> fragment	in the examples
pBBR1MCS-3:: <i>glf</i>	with <i>glf</i> as <i>KpnI/PstI</i> fragment	in the examples
pBBR1MCS-3:: <i>gk</i> :: <i>mak</i>	with <i>glf</i> and <i>mak</i> as <i>KpnI/XbaI</i> fragment	in the examples
pBBR1MCS-3:: <i>gk</i> :: <i>pmi</i>	with <i>glf</i> as <i>KpnI/PstI</i> fragment and <i>pmi</i> <i>XbaI/SacI</i> fragment	in the examples

(continued)

Strain, plasmid or primer	Relevant characteristics	Source or reference in the examples
pBBR1MCS-3:: <i>glf::mak::pmi</i>	with <i>glf</i> , <i>mak</i> and <i>pmi</i> as <i>KpnI</i> / <i>SacI</i> fragment	
Oligonucleotides		
<i>Pglf_KpnI_f</i>	5'GGTACCAAGGAAGGACTGATCATG AGTTCTGAAAGTAGTCAGGGTCTA GTC3' (SEQ ID NO:61)	
<i>Pglf_PstI_r</i>	5'CTGCAGCTACTTCTCTGGGAGCGCCACATC3' (SEQ ID NO: 62)	
<i>Pmak_PstI_f</i>	5'CTGCAGAAGGAAGGACTGATC GTGCGTATAGGTATCGATTTAGGCG3' (SEQ ID NO: 63)	
<i>Pmak_XbaI_r</i>	5'TCTAGATTACTCTTGTGGCCATAACCCACGC3' (SEQ ID NO: 64)	
<i>Ppmi_XbaI_f</i>	5'TCTAGAAAGGAAGGTCGACTC ATGCAAAAACCTCACTAACTCAGTG CAAAAC3' (SEQ ID NO:65)	
<i>Ppmi_SacI_r</i>	5'GAGCTCTTACAGCTTGTGTAACACGCGCTAAAC3' (SEQ ID NO: 66)	
<i>glf_RT_f</i>	5'GTTCTATCGATTGGGTTAATGCCCAGTGG3' (SEQ ID NO: 67)	
<i>glf_RT_r</i>	5'GGAACATCTGCGGTGCATAATACAGC3' (SEQ ID NO: 68)	
<i>mak_RT_f</i>	5'GCGAGGTTGCAGCGGGAAGTG3 (SEQ ID NO: 69)	
<i>mak_RT_r</i>	5'AATTCACCTGCCCTTCAGCGCATGTCC3' (SEQ ID NO: 70)	
<i>pmi_RT_f</i>	5'CGCTGACGCCTTTCCTTGCGAT3' (SEQ ID NO: 71)	
<i>pmi_RT_r</i>	5'GCGGTGTTTCAGCGAACAGGAACA3' (SEQ ID NO: 72)	
pJET1.2 forward sequencing primer, 23-mer	5'CGACTCACTATAGGGAGAGCGGC3' (SEQ ID NO: 73)	Fermentas
pJET1.2 reverse sequencing primer, 24-mer	5'AAGAACATCGATTTTCCATGGCAG3' (SEQ ID NO: 74)	Fermentas
M13/pUC-forward, 17-mer	5'GTAAACGACGCGCCAGT3' (SEQ ID NO: 75)	Jena Bioscience
F_Pnk_XhoI	5'CTCGAGCCGGAATTGCCAGCTGGGG3' (SEQ ID NO: 76)	
R_Pnk_NdeI	5'CATAATGAACGATCCTCATCCTGTCTTG3' (SEQ ID NO: 77)	
F_glk_NdeI	5'CATATGACAAAAGTATGCATTAGTCGGTGATGTGGG3' (SEQ ID NO: 78)	
R-glk_SalI	5'GTCGACTTACAGAATGTGACCTAAGGCTGGCGTAAATGTGC3' (SEQ ID NO: 79)	

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(continued)

Strain, plasmid or primer	Relevant characteristics	Source or reference
F_glf_BamHI	5'GGATCCATGAGGATCGTTTTCGCATGAGTTCTGAAAGTAGTCAGGGTCTAGT3' (SEQ ID NO: 80)	
R_glf_SacI	5'GAGCTCCTACTTCTGGGAGCGCCACATCTCCTCG3' (SEQ ID NO: 81)	

Table 2. Growth of recombinant *R. eutropha* strain H16 and of the wild type (negative control) on different carbon sources. Cells were incubated on MSM agar plates containing 1% (wt/vol) of the respective carbon source for 5 days at 30 °C. Visible colony growth was phenotypically examined. Characters: (++) growth after an incubation time of 24-72 h, (+) growth after more than 72 h of incubation, (-) no visible growth detectable.

Carbon source	<i>R. eutropha</i> strain H16 wild type	<i>R. eutropha</i> harboring pBBR1MCS-3:: <i>glf</i> :: <i>mak</i> :: <i>pmi</i>	<i>R. eutropha</i> harboring pBBR1MCS-2:: <i>P_{nk}</i> :: <i>glk</i> :: <i>glf</i>
L-Arabinose	-	-	-
D-Fructose	++	++	++
D-Galactose	-	-	-
D-Gluconate	++	++	++
D-Glucose	-	+	++
D-Glycerol	+	+	+
D-Maltose	-	-	-
D-Mannitol	-	-	-
D-Mannose	-	+	-
N-Acetyl-D-glucosamine	++	++	++
Pyruvate	++	++	++
D-Ribose	-	-	-
D-Trehalose	-	-	-
D-Xylose	-	-	-

[0126] Isolation, analysis and modification of DNA. Plasmid DNA was prepared from crude lysates by the alkaline extraction method (Birnboim and Doly 1979). Total DNA of *Z. mobilis* DSM 424 or DSM3580 and of *E. coli* strain K-12 was prepared using the Qiagen DNeasy Blood & Tissue Kit (Qiagen, Hilden, Germany) according to the manufacturer's protocol. DNA was restricted with restriction endonucleases (Gibco/BRL, Gaithersburg, USA) under conditions recommended by the manufacturer. All other genetic procedures and manipulations were conducted as described by Sambrook et al. (1989).

[0127] Constructions of plasmids and its transfer into *R. eutropha* strain H16. The coding regions of *glf* from *Z. mobilis mobilis* DSM424 and DSM3580, and of *glk*, *mak* and *pmi* from *E. coli*, respectively, were amplified by PCR using oligonucleotides *Pglf_KpnI_f*, *Pglf_PstI_r*, *F_glf_BamHI* and *R_glf_SacI* for amplification of *glf*, *F_Pnk_XhoI* and *R_Pnk_NdeI* for *P_{nk}*, *F_glk_NdeI* and *R_glk_SalI* for *glk*, *Pmak_PstI_f* and *Pmak_XbaI_r* for amplification of *mak*, or *Ppmi_XbaI_f* and *Ppmi_SacI_r* for amplification of *pmi*, respectively (Table 1). For PCR, KOD Hot Start DNA Polymerase (Merck, Darmstadt, Germany) was used according to the manufacturer's instructions. PCR products were then cloned into the pJET1.2/blunt cloning vector (Fermentas, Germany) using T4 DNA ligase (Gibco BRL, Gaithersburg, USA) or into cloning vector pCR2.1 (Invitrogen, Carlsbad, Germany) and transferred into *E. coli* strain TOP10. Plasmids were isolated from ampicillin resistant clones, and the cloned fragments were excised by restriction with respective suitable restriction enzymes for further cloning, extracted from gel after separation using the E.Z.N.A gel extraction kit (Omega Bio-tec, Bangalore, India). For expression experiments in *R. eutropha* strain H16, the broad host range vectors pBBR1MCS-2 and pBBR1MCS-3 were used for cloning of *glf*, *glk*, *mak* and *pmi*. Vector pBBR1MCS-2 conferred kanamycin resistance, whereas pBBR1 MCS-3 conferred tetracycline resistance for selection to *E. coli* and *R. eutropha* strain H16. The coding region of *glf* was excised by restriction with *KpnI* and *PstI* and ligated to *KpnI/PstI* linearized plasmid pBBR1 MCS-3, yielding plasmids pBBR1MCS-3::*glf* (Fig.2). The coding sequence of the neokanamycin-promoter *P_{nk}* was amplified using primers *F_Pnk_XhoI* and *R_Pnk_NdeI* using isolated vector pBBR1 MCS-2 and further ligated into the vector pCR2.1 (Invitrogen, Carlsbad, USA) according to the manufacturer's instructions yielding pCR2.1::*P_{nk}*. The *glk*-gene was amplified by tailored PCR from total DNA of *E. coli* K12 using oligonucleotides *F_glk_NdeI* and *R_glk_SalI* as primers. The resulting PCR product was cloned as *NdeI/SalI* fragment into pCR2.1::*P_{nk}*, yielding pCR2.1::*P_{nk}*::*glk*. The *glf*-gene was amplified by tailored PCR from total DNA of *Z. mobilis* DSM3580 using oligonucleotides *F_glf_BamHI* and *R_glf_SacI* as primers. The resulting PCR product was cloned as *BamHI/SacI* fragment into pBBR1 MCS-2, yielding pBBR1MCS-2::*glf*. To obtain plasmid pBBR1MCS-2::*P_{nk}*::*glk*::*glf*, the coding region of *P_{nk}*::*glk* was excised by restriction with *XhoI* and *EcoRI* from plasmid pCR2.1::*P_{nk}*::*glk* and ligated into *XhoI/EcoRI* linearized pBBR1MCS-2::*glf*. The coding sequence of *glk* was further excised by restriction with *NdeI/SalI* from plasmid pBBR1MCS-2::*P_{nk}*::*glk*::*glf*, the linearized plasmid was blunted using T4 Polymerase (Gibco BRL, Gaithersburg, USA) and religated

using T4 DNA ligase (Gibco BRL, Gaithersburg, USA) yielding plasmid pBBR1MCS-2::P_{nk}::*glf*. To obtain plasmids pBBR1MCS-3::*glf*::*mak* and pBBR1MCS-3::*glf*::*pmi*, the coding regions of *mak* and *pmi* were excised by restriction with *Pst*I and *Xba*I or *Xba*I and *Sac*I, respectively, and ligated to *Pst*I/*Xba*I and *Xba*I/*Sac*I linearized plasmid pBBR1MCS-3::*glf*, respectively (Fig. 2). The excised coding regions of *mak* and *pmi* were also ligated together to *Pst*I/*Sac*I linearized plasmid pBBR1MCS-3::*glf* yielding pBBR1MCS-3::*glf*::*mak*::*pmi* (Fig. 2). All plasmids were transferred to *E. coli* strain S17-1 by transformation (Hanahan 1983).

[0128] Transfer of DNA by conjugation. Transfer of plasmids pBBR1 MCS-2, pBBR1MCS-2::P_{nk}::*glf*, pBBR1MCS-2::P_{nk}::*glk*::*glf*, pBBR1 MCS-3, pBBR1MCS-3::*glf*, pBBR1MCS-3::*glf*::*mak*, pBBR1MCS-3::*glf*::*pmi* and pBBR1MCS-3::*glf*::*mak*::*pmi* (Table 1) was performed by conjugation applying a previously described protocol (Friedrich et al. 1981), using *E. coli* S17-1 as donor and *R. eutropha* strain H16 as recipient.

[0129] DNA sequence analysis. DNA was sequenced in a 48-capillary 3730 DNA Analyzer electrophoresis system (Applied Biosystems, Foster City, CA). For sequencing of inserts in the pJET1.2/blunt cloning vector (Fermentas, Germany) the pJET1.2 forward sequencing primer, 23-mer and pJET1.2 reverse sequencing primer, 24-mer were used. The universal M13/pUC-forward primer and the M13/pUC-reverse primer were used for sequencing inserts in plasmids pBBR1 MCS-2 and pBBR1 MCS-3 (Kovach et al. 1995).

[0130] RT-PCR analysis of total RNA isolated from *R. eutropha* strain H16. DNA-free total RNA of *R. eutropha* strain H16 was prepared using the RNeasy RNA purification kit (Qiagen, Hilden, Germany) according to the manufacturer's protocol. For identification of *glf*-, *mak*- and *pmi*-derived mRNA, RT-PCR was applied using oligonucleotides *glf*_RT_f, *glf*_RT_r, *mak*_RT_f, *mak*_RT_r, *pmi*_RT_f and *pmi*_RT_r (Table 1). RT-PCR was carried out using a Qiagen OneStep RT-PCR kit (Qiagen, Hilden, Germany) according to the manufacturer's protocol and 0.5 ng RNA as template. To exclude any DNA contamination that could serve as template for PCR, template RNA was added in a control experiment, after inactivation of reverse transcriptase for 15 min at 95 °C in presence of *Taq*-polymerase. Absence of PCR products indicated that the RT-PCR products were not derived from contaminating DNA.

[0131] Preparation of soluble protein fractions from *R. eutropha* strain H16. Cells of recombinant strains of *R. eutropha* strain H16 were cultivated as described above in presence of 1% (wt/vol) sodium gluconate as sole carbon source. Cells were harvested by centrifugation for 15 min at 4 °C and 3,500 x *g*, washed with 100 mM MOPS buffer (pH 7.0) and resuspended in two volumes of the same buffer. Disruption was done by sonication employing a Sonifer 250 (Branson Sonic Power Company) with an amplitude of 16 µm (1 min/ml; 50% output control). During ultrasonication samples were cooled with an ice-NaCl mixture. Soluble membrane-free protein fractions were prepared by 60 min ultracentrifugation of the crude extracts at 100,000 x *g* and 4 °C.

[0132] Solubilisation of membrane proteins. Recombinant cells of *R. eutropha* H16 were cultivated as described above in presence of 1% (wt/vol) glucose as sole carbon source. After 48 h cells were harvested by 15 min centrifugation at 3,500 x *g* and 4 °C, washed once with 0.9% (wt/vol) NaCl and resuspended in one volume of 100 mM Tris/HCl buffer (pH 7.0). Cell disruption was carried out by a threefold passage through a precooled French Pressure Cell at 1.000 MPa. The obtained lysates were centrifuged as before in order to remove residual cells. The membrane fraction was prepared by 1 h centrifugation of the supernatant at 100,000 x *g* and 4 °C, resuspended in 2 mL of 20 mM Tris/HCl buffer (pH 7.4) containing 200 mM NaCl and 2% (vol/vol) TritonX-114 and incubated for 12 h on ice on a rotary shaker (W3, VWR international GmbH, Darmstadt, Germany). The lysate was centrifuged for 20 min at 3,500 x *g* and 4 °C. The supernatant was incubated for 5 min at 37 °C and centrifuged for 20 min at 3,500 x *g* and 37 °C to remove TritonX-114 according to Bordier (Bordier, 1981). The supernatant was further diluted with 2 volumes of the same buffer without TritonX-114 to decrease the detergent concentration.

[0133] One-dimensional PAGE. Protein samples were resuspended in gel loading buffer (0.6% (wt/vol) SDS; 1.25% (vol/vol) β-mercaptoethanol; 0.25 mM EDTA; 10% (vol/vol) glycerol; 0.001% (wt/vol) bromophenol blue; 12.5 mM Tris/HCl, pH 6.8) and were separated in 12.5% (wt/vol) SDS-polyacrylamide gels as described by Laemmli (1970). The proteins were stained with Coomassie brilliant blue R-250 (Weber and Osborn, 1969). Samples of crude extracts and solubilized membrane proteins were analyzed by this method.

[0134] MAK, PMI and GLK activity assays. Soluble protein fractions of recombinant *R. eutropha* strain H16 were applied for MAK, PMI and GLK activity measurements at 30 °C using a Nicolet Evolution 100 UV/VIS spectrophotometer (Thermo Electron Corporation, Cambridge, UK). Activity of MAK was assayed with an NADH-coupled system as described by Sebastian and Asensio (1972) with some modifications, using pyruvate kinase and lactate dehydrogenase as auxiliary enzymes. The buffered reaction mixture (100 mM MOPS, pH 7.0) contained 2 mM MgCl₂, 2 mM ATP, 0.2 NADH, 5 mM mannose, 0.2 mM PEP, 5 U ml⁻¹ pyruvate kinase, 5 U ml⁻¹ lactate dehydrogenase and 5 to 100 µl of soluble extract. PMI activity was measured with an NADP-coupled assay (Kang and Markovitz 1967) with some modifications, using phosphoglucose isomerase and glucose-6-phosphate dehydrogenase as auxiliary enzymes. The buffered reaction mixture (100 mM MOPS, pH 7.0) contained 5 mM MgCl₂, 1 mM NADP, 3 mM mannose-6-phosphate, 1 U ml⁻¹ phosphoglucose isomerase, 1 U ml⁻¹ glucose-6-phosphate dehydrogenase and 10 to 100 µl of soluble extract.

[0135] GLK activity was measured with an NADPH-coupled system according to the method of Gottschalk (Gottschalk et al. 1964) with modifications concerning the buffer. The buffer mixture (100 mM Tris/HCl, pH 7.6) contained 7 mM

MgCl₂, 0.9 mM NADP, 460 mM D-glucose, 0.7 mM ATP, 0.35 U ml⁻¹ glucose-6-phosphate-dehydrogenase and 10 to 50 µl of enzyme preparation.

[0136] Analysis of PHB content of recombinant *R. eutropha* cells by GC. Lyophilized cell material was subjected to methanolysis in presence of methanol and sulfuric acid (85%, vol/vol, MeOH and 15%, vol/vol, H₂SO₄) for 4 h at 100 °C, and the resulting methylesters of the PHA constituents were characterized by gas chromatography using an Agilent 6850 GC (Agilent Technologies, Waldbronn, Germany) equipped with a BP21 capillary column (50 m by 0.22 mm; film thickness, 250 nm; SGE, Darmstadt, Germany) and a flame ionization detector (Agilent Technologies). A 2 µl portion of the organic phase was analyzed after split injection (split ratio, 1:5), and a constant hydrogen flow of 0.6 ml min⁻¹ was used as carrier gas. The temperatures of the injector were 250 °C and 220 °C, respectively. The following temperature program was applied: 120 °C for 5 min, increase of 3 °C min⁻¹ to 180 °C and increase of 10 °C min⁻¹ to 220 °C and 220 °C for 31 min. Substances were identified by comparison of their retention times to those of standard fatty acid methyl ester.

[0137] The *Z. mobilis glf* gene plus the *E. coli mak* and *pmi* genes were episomally introduced and expressed in *R. eutropha* and characterized the recombinant strains. The engineered pathways of glucose and mannose catabolism in the recombinant *R. eutropha* strain are depicted in Fig. 1.

[0138] Construction of different pBBR1 expression vectors for *R. eutropha* strain H16. The coding regions of the *Z. mobilis glf* gene (1,449 bp) and the *E. coli* genes *mak* (936 bp), *pmi* (1,203 bp) and *glk* (966 bp) were amplified by PCR from genomic gDNA of *Z. mobilis* DSM 424 and *E. coli* strain K-12, respectively. The four PCR products comprised beside the coding regions also suitable ribosome binding sites for *R. eutropha* upstream of the respective start codon to enable expression in this host. These fragments were cloned via the pJET1.2/blunt or pCR2.1 vector into the broad-host range vectors pBBR1 MCS-2 and pBBR1 MCS-3 under the control of the *neokanamycin*- or *lac*-promoter, respectively, which allow constitutive expression of the cloned genes in *R. eutropha* H16, yielding plasmids pBBR1MCS-2::P_{nk}::*glf*, pBBR1MCS-2::P_{nk}::*glk*::*glf*, pBBR1MCS-3::*glf*, pBBR1MCS-3::*glf*::*mak*, pBBR1MCS-3::*glf*::*pmi* and pBBR1 MCS-3::*glf*::*mak*::*pmi* (Fig. 2). Accurate construction of these plasmids was confirmed by sequencing. Additionally, the functionality of the *pmi* gene in pBBR1MCS-3::*glf*::*pmi* and pBBR1MCS-3::*glf*::*mak*::*pmi* was confirmed by complementation of the *pmi* (*manA*) deficient *E. coli* mutant JE5511 (Hirota et al. 1977). The recombinant *E. coli manA* mutants exhibited good growth on solid M9 medium containing 0.5% (wt/vol) mannose as a sole carbon source after two days of incubation at 37 °C. In contrast, the pBBR1 MCS-3 vector did not confer growth to the *manA* mutant.

[0139] Transfer of plasmids to *R. eutropha* strain H16 and establishment of glucose and mannose utilization. Both plasmids pBBR1MCS-2::P_{nk}::*glf* and pBBR1MCS-2::P_{nk}::*glk*::*glf* were transferred via conjugation from *E. coli* S17-1 to *R. eutropha* strain H16. Transconjugants were selected on MSM agar plates containing 1% (wt/vol) sodium gluconate and kanamycin (300 µg/ml), and were from there transferred to MSM plates containing 1% (wt/vol) glucose as sole carbon source. Only recombinant strains harboring either pBBR1 MCS-2::P_{nk}::*glf* or pBBR1 MCS-2::P_{nk}::*glk*::*glf* exhibited significant growth after 5 days of incubation.

[0140] To investigate if all three genes are necessary for mannose utilization, all constructed plasmids and plasmid pBBR1 MCS-3 were mobilized from *E. coli* strain S17-1 to *R. eutropha* strain H16. The transconjugants were selected on MSM agar plates containing 1% (wt/vol) sodium gluconate as a sole carbon source and tetracycline (12.5 µg/ml). They were then transferred to MSM agar plates containing mannose as a sole carbon source at various concentrations (0.2, 0.5, 1.0, 2.0, 4.0% [wt/vol]). Only recombinant strains harboring pBBR1MCS-3::*glf*::*pmi* or pBBR1MCS-3::*glf*::*mak*::*pmi* exhibited significant growth in presence of any tested mannose concentration. Whereas cells harboring plasmid pBBR1MCS-3::*glf*::*mak*::*pmi* exhibited distinct growth on mannose after 3-4 days of incubation, cells with pBBR1 MCS-3::*glf*::*pmi* only showed growth after an incubation time of 5-6 days. In contrast, recombinant strains harboring plasmid pBBR1MCS-3::*glf* or pBBR1 MCS-3::*glf*::*mak*, respectively, exhibited barely no growth even after an incubation period of 30 days. Only after prior exposure of the latter recombinant strains on MSM plates containing fructose instead of gluconate, slight growth occurred after a prolonged time of incubation.

[0141] Control of expression of *glf*, *glk*, *mak* and *pmi*. To confirm the presence of *glf*, *mak*, and *pmi* derived mRNA, transcriptional analyses of the recombinant strains were performed. For this, cells of the recombinant strains of *R. eutropha* harboring pBBR1 MCS-3::*glf*::*mak*::*pmi* or pBBR1 MCS-3 were cultivated in MSM containing 1% (wt/vol) sodium gluconate as a sole carbon source. Since P_{lac} is a constitutive promoter in *R. eutropha* strain H16 (Siedow et al. 1999), all three genes should be expressed constitutively without further induction. Transcriptional analyses were done by one-step RT-PCR. Total RNA from cells of late logarithmic growth phase served for RT-PCR using primers *glf*_RT_f, *glf*_RT_r, *mak*_RT_f, *mak*_RT_r, *pmi*_RT_f and *pmi*_RT_r specific for *glf*, *mak* and *pmi* (Table 1). PCR products of the expected size of 351 bp for *glf*, *mak* and *pmi*, respectively, were obtained if RNA isolated from recombinant pBBR1MCS-3::*glf*::*mak*::*pmi* harboring cells of strain H16 was analyzed. This clearly demonstrated that *glf*, *mak* and *pmi* were only transcribed in cells of the recombinant strain of *R. eutropha* strain H16 harboring pBBR1MCS-3::*glf*::*mak*::*pmi* but not in the negative control harboring only the vector pBBR1 MCS-3. The absence of PCR products in the control without any activity of reverse transcriptase indicated that the RT-PCR product was not derived from contaminating DNA (Fig. 3).

[0142] To further confirm the transcription of *glk* and *glf* in recombinant strains harboring plasmid pBBR1MCS-2::P_{nk}::*glk*::*glf* at the protein level, an analysis by SDS-PAGE was performed. As Glf is a transmembrane protein (Parker

et al. 1995), membrane proteins of both *R. eutropha* strain H16 harboring vector pBBR1 MCS-2 and *R. eutropha* strain H16 harboring plasmid pBBR1MCS-2::P_{nk}::glk::glf were solubilized and separated by PAGE. The electropherogram showed a distinct protein band with an apparent mass of 50 kDa representing Glf, that was absent in the control. Similarly, analysis of the soluble cell fraction showed a distinct protein band at 35 kDa, representing Glk (Fig. 4).

[0143] The presence of functional active MAK, PMI and GLK in the recombinant strains was confirmed by enzymatic analyses. Coupled enzyme assays employing the soluble protein fractions obtained from cells of the recombinant strain of H16 harboring pBBR1MCS-3::glf::mak::pmi, or pBBR1MCS-2::P_{nk}::glk::glf cultivated in MSM containing 1% (wt/vol) sodium gluconate as sole carbon source, demonstrated the presence of active PMI (0.25 U mg⁻¹), MAK (0.14 U mg⁻¹) and also GLK (0.52 U mg⁻¹), respectively, whereas these activities except GLK (0.02 U mg⁻¹) were absent in the soluble protein fractions of the negative control of strain H16 harboring plasmids pBBR1MCS-2 or pBBR1MCS-3, respectively, thus indicating the absence of PMI and MAK in the negative control strain.

[0144] Investigations on utilization of several carbon sources by the recombinant strains of *R. eutropha* H16.

In comparison to the other recombinant strains, *R. eutropha* strain H16 harboring plasmids pBBR1MCS-2::P_{nk}::glk::glf or pBBR1MCS-3::glf::mak::pmi exhibited the fastest growth on glucose or mannose of all tested substrates, respectively, as a sole carbon source. These strains were therefore further investigated with regard to the utilization of several other carbon sources employing MSM agar plates each containing 1% (wt/vol) of L-arabinose, D-fructose, D-galactose, D-gluconate, D-glucose, glycerol, maltose, D-mannitol, D-mannose, N-acetyl-D-glucosamine, pyruvate, D-ribose, trehalose, or D-xylose as sole carbon source. The wild type of *R. eutropha* served as negative control. The results are summarized in Table 2. Both, the wild type strain and the recombinant strains, were able to grow in presence of D-fructose, D-gluconate, glycerol, N-acetylglucosamine, and pyruvate (Table 2). All other carbon sources mentioned above were not utilized for growth, except mannose by the recombinant harboring pBBR1MCS-3::glf::mak::pmi and glucose by the recombinant strains harboring pBBR1MCS-2::P_{nk}::glk::glf or pBBR1MCS-3::glf::mak::pmi (Table 2).

[0145] Growth of the recombinant *R. eutropha* with glucose as sole carbon source. Recombinant strains of *R. eutropha* strain H16 were cultivated in liquid MSM containing 1% (wt/vol) glucose. Although all strains exhibited growth on glucose, recombinant strain harboring pBBR1MCS-2::P_{nk}::glk::glf showed fastest growth ($\mu = 0.32 \text{ h}^{-1}$) in comparison to the recombinant strain harboring pBBR1MCS-3::glf::mak::pmi ($\mu = 0.12 \text{ h}^{-1}$) (Fig. 5). This was in the same order as the positive control strain G⁺1 ($\mu = 0.30 \text{ h}^{-1}$). Interestingly, recombinant cells harboring plasmids carrying only the glf gene either under the control of the neokanamycin- or lac-promoter exhibited a strongly diminished growth ($\mu = 0.085$ and 0.073 , respectively) when compared to recombinant cells harboring plasmids with also the other genes (Fig. 5).

[0146] Growth of the recombinant *R. eutropha* with mannose or gluconate as sole carbon source. Recombinant cells of *R. eutropha* strain H16 harboring plasmid pBBR1MCS-3::glf::mak::pmi were cultivated in liquid MSM containing different concentrations of mannose as sole carbon source to evaluate optimal concentration for maximal growth. Furthermore, it was examined if the introduced genes are affecting growth of the recombinant strain in comparison to wild type strain H16 in MSM containing 0.5% (wt/vol) sodium gluconate as sole carbon source. According to our expectations, the recombinant strain H16 harboring pBBR1MCS-3::glf::mak::pmi exhibited faster growth on gluconate ($\mu = 0.33 \text{ h}^{-1}$) than on mannose ($\mu = 0.078 \text{ h}^{-1}$). Maximal growth on mannose occurred at 1% (wt/vol) mannose, whereas lower and higher concentrations of this sugar yielded slightly slower growth (Fig. 6). With gluconate as sole carbon source growth of the recombinant strain was only slightly diminished in comparison to the wild type.

Example 2

[0147] Bacterial strains, plasmids, oligonucleotides and cultivation conditions. All bacteria, plasmids and primers used in this study are listed in Table 3 and 4, respectively. Cells of *R. eutropha* strain H16 were cultivated in nutrient broth, or mineral salts medium (MSM) as described by Schlegel et al. (1961). Carbon sources were added to liquid MSM as indicated in the text. Liquid cultures in Erlenmeyer flasks were incubated on a horizontal rotary shaker at an agitation of 110 rpm. Solid media were prepared by addition of 1.5% (wt/vol) agar-agar. Cells of *Escherichia coli* were cultivated at 37 °C in Lysogeny Broth (LB, [Sambrook et al. 1989]), or M9 mineral salts medium (Sambrook et al. 1989) with D-xylose as sole carbon source. Antibiotics were applied according to Sambrook et al. (1989) and as indicated in the text.

[0148] Isolation, analysis and modification of DNA. Plasmid DNA was prepared from crude lysates by the alkaline extraction method (Birnboim and Doly 1979). Total DNA of *E. coli* strain K-12 was prepared using the Qiagen DNeasy Blood & Tissue Kit (Qiagen, Hilden, Germany) according to the manufacturer's protocol. DNA was restricted with restriction endonucleases (Gibco/BRL, Gaithersburg, USA) under conditions recommended by the manufacturer. All other genetic procedures and manipulations were conducted as described by Sambrook et al. (1989).

Table 3. Bacterial strains, plasmids and oligonucleotides used in this study

Strain, plasmid or primer	Relevant characteristics	Source or reference
Strains		
<i>E. coli</i> K-12	Wild type	DSM 426
<i>E. coli</i> TOP10	F ⁻ <i>mcrA</i> , $\Delta(mrr-hsdRMS-mcrBC)f80lacZ \Delta M15, \Delta lacX74, deoR, recA1, araD139, \Delta(ara-leu)7697, galU, galk, rpsL, endA1, nupG$	Invitrogen
<i>E. coli</i> S17-1	<i>recA1, thi1, hsdR17</i> (r _k ⁻ , m _k ⁺), <i>proA</i> , <i>tragenes</i> of RP4 plasmid chromosomally integrated (Mobilization strain)	Simon et al. 1983
<i>E. coli</i> HB101	<i>hsdS, recA, proA, lacy, 9aIK, xyl-5, mtl</i>	<i>E. coli</i> Genetic Stock Center (Hirota et al. 1977)
<i>E. coli</i> JWL255	<i>xy191pR</i>	<i>E. coli</i> Genetic Stock Center (Hirota et al. 1977)
<i>E. coli</i> LR2-168	<i>thi, arg, met, his, gal, xyl-7</i>	<i>E. coli</i> Genetic Stock Center (Hirota et al. 1977)
<i>R. eutropha</i> strain H16	Wild type	DSM 428
Plasmids		
pJET1.2/blunt	Ap ^r	Fermentas
pJET1.2:: <i>xyIA</i>	with <i>xyIA</i> as blunt end PCR product	in the examples
pJET1.2:: <i>xyIB</i>	with <i>xyIB</i> as blunt end PCR product	in the examples
pJET1.2:: <i>xyIE</i>	with <i>xyIE</i> as blunt end PCR product	in the examples
pBBR1MCS-3	Tc ^r , <i>LacZ-α, mob, rep</i>	Kovach et al. 1995
pBBR1MCS-3:: <i>xyIFGH</i>	with <i>xyIFGH</i> as <i>SpeI/SacII</i> fragment	in the examples
pBBR1MCS-3:: <i>xyIAB</i>	with <i>xyIAB</i> as <i>Apal/SpeI</i> fragment	in the examples
pBBR1MCS-3:: <i>xyIABE</i>	with <i>xyIABE</i> as <i>Apal/SacII</i> fragment	in the examples
pBBR1MCS-3:: <i>xyIABFGH</i>	with <i>xyIABE</i> as <i>Apal/SacII</i> fragment	in the examples

Table 4. PCR and sequencing primers used in this study

Oligonucleotides	Sequence (5'→ 3')	Function
P_ <i>xyIA</i> _Apal_f	ATT AGG GCC CAA GGA GGT TAC AGC ATG CAA GCC TAT TTT GAC CAG (SEQ ID NO: 82)	5'-primer for the amplification of the coding region of <i>xyIA</i> . The primer contains the restriction site for <i>Apal</i> (underlined).
P_ <i>xyIA</i> _SmaI_r	ATT <u>GCC CGG</u> GTT ATT TGT CGA ACA GAT AAT G (SEQ ID NO: 83)	3'-primer for the amplification of the coding region of <i>xyIA</i> . The primer contains the restriction site for <i>SmaI</i> (underlined).

(continued)

Oligonucleotides	Sequence (5'→ 3')	Function
5 P_xy/B_SmaI_f	ATT <u>GCC CGG GAA</u> GGA GGT TAC AGC ATG TAT ATC GGG ATA GAT CTT (SEQ ID NO: 84)	5'-primer for the amplification of the coding region of xy/B. The primer contains the restriction site for SmaI (underlined).
10 P_xy/B_SpeI_r	CCC <u>GAC TAG</u> TTT ACG CCA TTA ATG GCA GAA G (SEQ ID NO: 85)	3'- primer for the amplification of the coding region of xy/B. The primer contains the restriction site for SpeI (underlined).
15 P_xy/E_SpeI_f	CCC <u>GAC TAG</u> TAA GGA GGT TAC AGC ATG AAT ACC CAG TAT AAT TC (SEQ ID NO: 86)	5'-primer for the amplification of the coding region of xy/E. The primer contains the restriction site for SpeI (underlined).
20 P_xy/E_SacII_r	ATT <u>GCC GCG</u> GTT ACA GCG TAG CAG TTT GTT G (SEQ ID NO: 87)	3'- primer for the amplification of the coding region of xy/E. The primer contains the restriction site for SacII (underlined).
25 P_xy/FGH_SpeI_f	CCC <u>GAC TAG</u> TAA GGA GGC TAC AGC ATG AAA ATA AAG AAC ATT CTA C (SEQ ID NO: 88)	5'-primer for the amplification of the coding region of xy/FGH: The primer contains the restriction site for SpeI (underlined).
30 P_xy/FGH_SacII_r	ATT <u>GCC GCG</u> GTC AAG AAC GGC GTT TGG TTG (SEQ ID NO: 89)	3'- primer for the amplification of the coding region xy/FGH. The primer contains the restriction site for SacII (underlined).
35	(SEQ ID NO: 89) AAAACCGCGGCGGCTCGGA AGTCG (SEQ ID NO: 90)	5'-primer for the amplification of the promoter P _{GAPDH} region: The primer contains the restriction site for SacII (underlined).
40 pJET1.2 forward	CGACTCACTATAGGGAGAG CGGC (SEQ ID NO: 91)	Fermentas (sequencing primer, 23-mer)
45 pJET1.2 reverse	AAGAACATCGATTTTCCATG GCAG (SEQ ID NO: 92)	Fermentas (sequencing primer, 24-mer)
M13/pUC-forward	GTAAACGACGGCCAGT (SEQ ID NO: 93)	Jena Bioscience (17-mer)

[0149] Constructions of plasmids and its transfer into *R. eutropha* strain H16. For amplification of the coding regions of *xyIA*, *xyIB*, *xyle*, *xyIFGH*, *talB* and *tktA* from *E. coli* strain K12 by PCR, oligonucleotides listed in Table 4 were used. The oligonucleotides P_xyIA_ApaI_f and P_xyIA_SmaI_r were used for amplification of *xyIA*, P_xy/B_SmaI_f and P_xy/B_SpeI_r for *xyIB*, P_xy/E_SpeI_f and P_xy/E_SacII_r for *xyle*, and P_xy/FGH_SpeI_f and P_xy/FGH_SacII_r for *xyIFGH*, respectively. Primers contained a ribosomal binding site in order to permit RNA translation in the target bacterium *R. eutropha* H16. KOD Hot Start DNA Polymerase (Merck, Darmstadt, Germany) was used for PCR amplification according to the manufacturer's instructions. PCR products were ligated to the pJET1.2/blunt cloning vector (Fermentas, Germany) using T4 DNA ligase (Gibco BRL, Gaithersburg, USA) or to plasmid pBBR1 MCS-3 (Kovach et al. 1995), and transferred into *E. coli* strain TOP10. Plasmids were isolated from ampicillin or tetracycline resistant clones, respectively,

and the cloned fragments were excised by restriction with the respective suitable restriction enzymes for further cloning, extracted from gel after separation using the E.Z.N.A gel extraction kit (Omega Biotec, Bangalore, India). For expression experiments in *R. eutropha* strain H16, the broad host range vector pBBR1MCS-3 was used for cloning of *xylA*, *xylB*, *xylE*, and *xylFGH*. Vector pBBR1 MCS-3 conferred tetracycline resistance for selection in *E. coli* and *R. eutropha* strain H16. The coding regions of *xylA* or *xylB* were excised by restriction with *Apal* and *SmaI* or *SmaI* and *SpeI*, respectively, ligated to *Apal* and *SpeI* linearized plasmid pBBR1 MCS-3, yielding plasmids pBBR1MCS-3::*xylAB* (Fig. 8). To obtain plasmids pBBR1MCS-3::*xylABE* and pBBR1MCS-3::*xylABFGH*, the coding regions of *xylE* and *xylFGH* were excised by restriction with *SpeI* and *SacII*, respectively, and ligated to *SpeI* and *SacII* linearized plasmid pBBR1MCS-3::*xylAB* yielding plasmids pBBR1MCS-3::*xylABE* and pBBR1MCS-3::*xylABFGH*, respectively (Fig. 8). All plasmids were transferred to *E. coli* strain S17-1 by transformation (Hanahan 1983).

[0150] Transfer of DNA by conjugation. Transfer of plasmids pBBR1MCS-3, pBBR1MCS-3::*xylABE*, pBBR1MCS-3::*xylABFGH* (Table 3) was performed by conjugation applying a previously described protocol (Friedrich et al. 1981), using *E. coli* S17-1 as donor and *R. eutropha* strain H16 as recipient.

[0151] DNA sequence analysis. DNA was sequenced in a 48-capillary 3730 DNA Ana-lyzer electrophoresis system (Applied Biosystems, Foster City, CA). For sequencing of inserts in the pJET1.2/blunt cloning vector (Fermentas, Germany) the pJET1.2 forward sequencing primer, 23-mer and the pJET1.2 reverse sequencing primer, 24-mer were used. The universal M13/pUC-forward primer and the M13/pUC-reverse primer were used for sequencing inserts in plasmid pBBR1 MCS-3 (Kovach et al. 1995).

[0152] Preparation of soluble protein fractions from *R. eutropha* strain H16. Cells of recombinant strains of *R. eutropha* strain H16 were cultivated in nutrient broth medium. Cells were harvested by centrifugation for 15 min at 4 °C and 3,500 x g, washed with 50 mM Tris/HCl buffer (pH 7.5) and resuspended in two volumes of the same buffer. Disruption was done by sonication employing a Sonifer 250 (Branson Sonic Power Company) with an amplitude of 16 µm (1 min/ml; 50% output control). During ultrasonication samples were cooled with an ice-NaCl mixture. Soluble membrane-free protein fractions were prepared by 60 min ultracentrifugation of the crude extracts at 100,000 x g and 4 °C.

[0153] XylA and XylB activity assays. Soluble protein fractions of recombinant *R. eutropha* strain H16 were applied for XylA and XylB activity measurements at 30 °C using a Nicolet Evolution 100 UV/VIS spectrophotometer (Thermo Electron Corporation, Cambridge, UK). Activity of XylA (Gao et al. 2002, Meijnen et al. 2008) or XylB (Shamanna and Sangerson 1979, Eliasson et al. 2000) was assayed with an NADH-coupled system with some modifications, using sorbitol dehydrogenase or pyruvate kinase and lactate dehydrogenase, respectively, as auxiliary enzymes. The buffered reaction mixture (50 mM Tris/HCl, pH 7.5) for XylA activity measurement contained 10 mM MgSO₄, 1 mM triethanolamine, 0.2 mM NADH, 50 mM D-xylose, 0.5 U ml⁻¹ sorbitol dehydrogenase, and 5 to 100 µl of soluble extract. The buffered reaction mixture (50 mM Tris/HCl, pH 7.5) for XylB activity measurement contained 2 mM MgCl₂, 2 mM ATP, 0.2 mM NADH, 10 mM xylulose, 0.2 mM PEP, 10 U ml⁻¹ pyruvate kinase, 10 U ml⁻¹ lactate dehydrogenase and 5 to 100 µl of soluble extract.

[0154] D-xylose utilization in *R. eutropha*. After D-xylose is taken up by the cell, the *xyIA* gene encoding the enzyme D-xylose isomerase (XylI or XylA; EC 5.3.1.5) catalyzes the reversible conversion of the aldose D-xylose into its keto-form D-xylulose. Subsequently, the D-xylulose is phosphorylated to D-xylulose-5-phosphate, utilizing ATP as energy and phosphate source. This step is catalyzed by a xylulose kinase (XylK or XylB; EC 2.7.1.17). D-Xylulose-5-phosphate can be utilized further as an intermediate of the non-oxidative pentose phosphate pathway. As *E. coli* strain K12 is one of the most extensively studied organisms utilizing D-xylose, the genes *xylA*, encoding xylose-isomerase, *xylB* encoding xylulokinase, *xylE* encoding the low affinity transporter and *xylFGH* encoding the ABC-transporter were amplified using genomic DNA of *E. coli* K12, episomally introduced and expressed in *R. eutropha* H16. The engineered pathway of xylose catabolism in the recombinant *R. eutropha* strain is depicted in Fig. 7.

[0155] Construction of different pBBR1 expression vectors for *R. eutropha* strain H16. The coding regions of the genes *xylA* (1,323 bp), *xylB* (1,455 bp), *xylE* (1,476 bp) and *xylFGH* (3,771 bp) were amplified by PCR from genomic gDNA of *E. coli* strain K12. The three PCR products comprised beside the coding regions also suitable ribosome binding sites for *R. eutropha* upstream of the respective start codon to enable expression in this host. These fragments were cloned via the pJET1.2/blunt vector into the broad-host range vector pBBR1 MCS-3 under the control of the *lac*-promoter, which allows constitutive expression of the cloned genes in *R. eutropha* H16 (Siedow et al. 1999), yielding plasmids pBBR1MCS-3::*xylAB*, pBBR1MCS-3::*xylABE* and pBBR1MCS-3::*xylABFGH* (Fig. 8). Accurate construction of these plasmids was confirmed by sequencing. Additionally, the functionality of the *xylA* or *xylB* genes in pBBR1MCS-3::*xylAB*, pBBR1 MCS-3::*xylABE* and pBBR1MCS-3::*xylABFGH* was confirmed by complementation of the *xylA* deficient *E. coli* mutant HB101, *xylB* deficient *E. coli* mutant JWL255, or *xylAB* deficient *E. coli* mutant LR2-168, respectively (Hirota et al. 1977). The recombinant *E. coli* mutants HB101, JWL255 or LR2-168 harboring pBBR1MCS-3::*xylAB*, pBBR1MCS-3::*xylABE* or pBBR1MCS-3::*xylABFGH*, respectively, exhibited good growth on solid M9 medium containing 0.5% (wt/vol) xylose as a sole carbon source after two days of incubation at 37 °C. In contrast, vector pBBR1 MCS-3 did not confer growth to the mutants HB101, JWL255 or LR2-168, respectively.

[0156] Transfer of plasmids to *R. eutropha* strain H16 and establishment of D-xylose utilization. Plasmids

pBBR1MCS-3::xylAB, pBBR1MCS-3::xylABE and pBBR1MCS-3::xylABFGH were transferred via conjugation from *E. coli* S17-1 to *R. eutropha* strain H16. Transconjugants were selected on MSM agar plates containing 1% (wt/vol) sodium gluconate and tetracycline (12.5 µg/ml), and were subsequently transferred to MSM plates containing 1% (wt/vol) D-xylose as sole carbon source. Recombinant strains harboring plasmid pBBR1MCS-3::xylABFGH exhibited weak growth after 5 days of incubation.

[0157] Evolutionary optimization of the plasmid pBBR1MCS-3::xylABFGH-harboring strain of *R. eutropha* H16. Although the recombinant plasmid pBBR1 MCS-3::xylABFGH-harboring strain of *R. eutropha* H16 was engineered for D-xylose utilization, this strain exhibited only weak growth after 5 days of incubation. A single colony of this recombinant strain was cultivated in fluid MSM containing 1% (wt/vol) D-xylose as a sole carbon source for 2 days at 30 °C. Dilutions were transferred on solid MSM containing 1% (wt/vol) D-xylose as a sole carbon source and incubated at 30 °C. A single colony which showed fastest growth was again cultivated in fluid MSM containing 1% (wt/vol) D-xylose as a sole carbon source, and a dilution was again transferred on solid MSM containing 1% (wt/vol) D-xylose as a sole carbon source. After repetition of this procedure for several times, a recombinant plasmid pBBR1MCS-3::xylABFGH-harboring strain of *R. eutropha* H16 was obtained exhibiting comparably good growth on solid MSM containing 1% (wt/vol) D-xylose as a sole carbon source after 2 days of incubation.

[0158] Investigations on utilization of several carbon sources by the recombinant plasmid pBBR1MCS-3::xylABFGH-harboring strain of *R. eutropha* H16 and of the respective optimized strain. Cells of the recombinant strain of H16 harboring pBBR1 MCS-3::xylABFGH and of the respective optimized recombinant strain of H16, of *R. eutropha* strain G+1, a glucose-utilizing mutant of *R. eutropha* strain H16 (Schlegel and Gottschalk 1965), and of *R. eutropha* strain H16 were cultivated on solid MSM agar plates containing different carbon sources (1% [wt/vol] L-arabinose, D-fructose, D-galactose, D-gluconate, D-glucose, D-maltose, D-mannose, N-acetylglucosamine, D-ribose, D-trehalose, D-xylose) to compare the growth of the strains on several carbon sources. The wild type of *R. eutropha* H16 served as negative control. All the strains were able to use D-fructose, D-gluconate, and N-acetylglucosamine as sole carbon sources. The plasmid pBBR1 MCS-3::xylABFGH containing strain of *R. eutropha* strain H16 was also able to use glucose as sole carbon source showing a similar growth as *R. eutropha* H16 strain G+1. The plasmid pBBR1 MCS-3::xylABFGH containing strain was also able to use D-xylose, L-arabinose and D-galactose as sole carbon sources.

[0159] Growth of the recombinant plasmid pBBR1MCS-3::xylABFGH-harboring strain of *R. eutropha* H16 with glucose, gluconate or xylose as sole carbon source. Cells of the recombinant plasmid pBBR1MCS-3::xylABFGH-harboring strain of *R. eutropha* H16 were cultivated in fluid MSM containing different concentrations (0.5%, 1%, 2%, and 4% [wt/vol]) of D-xylose, 1% (wt/vol) D-glucose and 1% (wt/vol) Na-gluconate as sole carbon source. Also *R. eutropha* strain H16 wild type was cultivated with 1% (wt/vol) Na-gluconate as sole carbon source to compare the growth rate. The recombinant strain showed a similar growth on 1% (wt/vol) Na-gluconate ($\mu = 0.47 \text{ h}^{-1}$) and also on 1% (wt/vol) glucose ($\mu = 0.55 \text{ h}^{-1}$) as sole carbon source in comparison to the wild type strain on 1% (wt/vol) Na-gluconate ($\mu = 0.47 \text{ h}^{-1}$) (Fig. 9). After almost 22 h, the stationary growth phase was reached. Growth of the recombinant strain on D-xylose as sole carbon source was slower. After almost 40 h, the stationary growth phase was reached for the cultures containing 0.5% (wt/vol) ($\mu = 0.20 \text{ h}^{-1}$) to 2% (wt/vol) ($\mu = 0.18 \text{ h}^{-1}$) of D-xylose as sole carbon source. The culture containing 4% (wt/vol) of D-xylose as sole carbon source reached the stationary growth phase after almost 50 h ($\mu = 0.17 \text{ h}^{-1}$) (Fig. 9).

[0160] Control of expression of xylA and xylB in the recombinant plasmid pBBR1MCS-3::xylABFGH-containing strain of *R. eutropha* H16. The presence of functional active XylA and XylB in the recombinant strain was confirmed by enzymatic analyses. Coupled enzyme assays employing the soluble protein fractions obtained from cells of the recombinant strain of H16 harboring pBBR1MCS-3::xylABFGH cultivated in MSM containing 1% (wt/vol) xylose as sole carbon source demonstrated the presence of active XylA (0.035 U mg⁻¹) and XylB (0.364 U mg⁻¹), respectively, whereas these activities were absent in the soluble protein fractions of the negative control of strain H16 harboring plasmid pBBR1MCS-3, thus indicating the absence of XylA and XylB in the negative control strain.

[0161] Analysis of PHB content of recombinant *R. eutropha* cells by GC. Lyophilized cell material was subjected to methanolysis in presence of methanol and sulfuric acid (85%, vol/vol, MeOH and 15%, vol/vol, H₂SO₄) for 4 h at 100 °C, and the resulting methylesters of the PHA constituents were characterized by gas chromatography using an Agilent 6850 GC (Agilent Technologies, Waldbronn, Germany) equipped with a BP21 capillary column (50 m by 0.22 mm; film thickness, 250 nm; SGE, Darmstadt, Germany) and a flame ionization detector (Agilent Technologies). A 2 µl portion of the organic phase was analyzed after split injection (split ratio, 1:5), and a constant hydrogen flow of 0.6 ml min⁻¹ was used as carrier gas. The temperatures of the injector were 250 °C and 220 °C, respectively. The following temperature program was applied: 120 °C for 5 min, increase of 3 °C min⁻¹ to 180 °C and increase of 10 °C min⁻¹ to 220 °C and 220 °C for 31 min. Substances were identified by comparison of their retention times to those of standard fatty acid methyl ester.

[0162] Construction of different vectors suitable for expression of transketolase (Tkt) and transaldolase (TalB) in strains of the genera *Ralstonia* and *Cupriavidus*. The coding regions of genes encoding a transketolase (Tkt) and transaldolase (TalB) homologous protein were amplified by PCR from genomic gDNA of the respective host strain. The PCR products can comprise beside the coding regions also suitable restriction sites for cloning, suitable regulatory elements as e.g. a promoter sequence, and ribosome binding sites for strains of the genera *Ralstonia* and *Cupriavidus*.

upstream of the respective start codon to enable expression in this host. These features can either be provided by synthetic oligonucleotides or by fusion PCR. These fragments can be cloned via cloning vectors into vectors suitable for expression in strains of the genera *Ralstonia* and *Cupriavidus* under the control of a suitable promoter, which allows expression of the cloned genes in the respective host strain. The coding regions of genes encoding a transketolase (Tkt) and transaldolase (TalB) homologous protein can either be cloned separately or in combination with different genes, e.g. *xylA*, *xylB*, *xylC*, and *xylFGH*. Accurate construction of these plasmids can be confirmed by sequencing. The constructed plasmids can be either transferred via conjugation from *E. coli* S17-1 or by electroporation to strains of the genera *Ralstonia* and *Cupriavidus*. Transconjugants or transformants, respectively, can be selected on MSM agar plates containing 1% (wt/vol) sodium gluconate and a suitable selection for plasmid stability. Recombinant strains of the genera *Ralstonia* and *Cupriavidus* can be characterized concerning their growth on different carbon sources.

Example 3

[0163] Bacterial strains, plasmids, oligonucleotides and cultivation conditions. All bacteria, plasmids and primers used in this application are listed in Table 5. Cells of *E. coli* were cultivated in Lysogeny Broth (LB) (Sambrook et al., 1989) or in mineral salts medium (MSM) supplemented with 1% (w/v) glucose according to Schlegel et al. (1961). Cells of *R. eutropha* were cultivated in Nutrient Broth (NB) (Sambrook et al., 1989) or in MSM supplemented with 1% (w/v) sodium gluconate and sodium oleate (0.4%, w/v) for FAEE synthesis. Liquid cultures in baffled Erlenmeyer flasks were incubated on a horizontal rotary shaker at an agitation of 110 rpm at 37°C (*E. coli*) or 30°C (*R. eutropha* strains). Antibiotics were applied according to Sambrook et al. (1989) and as indicated in the text.

Table 5. Bacterial strains, plasmids and oligonucleotides

Strain, plasmid or primer	Relevant characteristics	Source or reference
Strains		
<i>E. coli</i> TOP10	<i>F-mcrA</i> , $\Delta(mrr-hsdRMS-mcrBC)$ <i>f80lacZ</i> $\Delta M15$, $\Delta lacX74$, <i>deoR</i> , <i>recA1</i> , <i>araD139</i> , $\Delta(ara-leu)7697$, <i>galU</i> , <i>galK</i> , <i>rpsL</i> , <i>endA1</i> , <i>nupG</i>	Invitrogen
<i>E. coli</i> S17-1	<i>recA1</i> , <i>thi1</i> , <i>hsdR17</i> (rk-, mk+), <i>proA</i> , <i>tra</i> -genes of RP4 plasmid chromosomally integrated (mobilization strain)	Simon et al. 1983
<i>R. eutropha</i> $\Delta phaC1$	PHB-negative mutant of <i>R. eutropha</i> H16	Peplinski et al., 2010
Plasmids		
pBBR1 MCS-3	Tc ^R , <i>lacZ</i> α , <i>mob</i> , <i>rep</i>	Kovach et al., 1995
pMicrodiesel	Amp ^R , <i>atfA</i> , <i>adhB</i> , <i>pdC</i>	Kalscheuer et al., 2006
pBBR1 MCS-3:: <i>atfA/pdc/adhB</i>	with <i>atfA/pdc/adhB</i> as <i>XbaI/ApaI</i> fragment	in this example
Oligonucleotides		
M13fwd	5' GTAAACGACGGCCAGT 3' (SEQ ID NO:94)	
M13 rev	5' AACAGCTATGACCATG 3' (SEQ ID NO: 95)	

[0164] Isolation, analysis and modification of DNA. Plasmid DNA from *R. eutropha* strains was prepared from crude lysates by the alkaline extraction method (Birnboim and Doly, 1979). Plasmid DNA from *E. coli* was prepared with a commercial plasmid preparation kit (Roti Prep Plasmid Kit, Roth, Karlsruhe) applying the conditions recommended by the manufacturer. DNA was restricted with restriction endonucleases (Fermentas GmbH, St. Leon-Rot) under conditions recommended by the manufacturer. All other genetic procedures and manipulations were conducted as described by Sambrook et al. (1989).

[0165] Transfer of DNA by conjugation. Transfer of plasmids pBBR1MCS-3 and pBBR1MCS-3::*atfA/pdc/adhB* was

performed by conjugation applying the protocol described by Friedrich et al. (1981). *E. coli* S17-1 was used as donor strain and *R. eutropha* strains *AphaC1* as the recipient.

[0166] Preparation of soluble protein fractions from *R. eutropha* strains and *E. coli*. Cells of recombinant strains of *R. eutropha* or *E. coli* were cultivated as described above in presence of 1% (w/v) sodium gluconate or 1% (w/v) glucose, respectively. Cells were harvested by 15 min of centrifugation at 4°C and 3500 x g, washed in a suitable buffer as described below and resuspended in two volumes of the same buffer. The cells were disrupted by sonication employing a MS72 probe and a Sonoplus GM200 sonicator (Bandelin, Berlin) with an amplitude of 10 µm (2 min/ml; 50% output control). During ultrasonication the samples were cooled with ice. Protein concentrations in cell crude extracts were determined as described by Bradford (1976).

[0167] DNA sequence analysis. DNA was sequenced in a 48-capillary 3730 DNA Analyzer electrophoresis system (Applied Biosystems, Foster City, CA). For sequencing of inserts in plasmid pBBR1 MCS-3 (Kovach et al. 1995) the universal "M13 rev"- and "M13 fwd" primers were used.

[0168] Construction of the used plasmids. As the expression vector in *R. eutropha* H16 the vector pBBR1 MCS-3 was chosen allowing a stable expression of the containing genes in *R. eutropha*. The necessary genes for FAEE synthesis were cut from the plasmid pMicrodiesel (Kalscheuer et al., 2006) by restriction with *Xba*I and *Apa*I resulting in a 4.8 kbp fragment containing genes *atfA*, *pdc* and *adhB*. This fragment was ligated to the *Xba*I and *Apa*I restricted plasmid pBBR1 MCS-3 using T4 DNA ligase (Fermentas GmbH, St. Leon-Rot) resulting in the 9.9 kbp plasmid pBBR1MCS-3::*atfA/pdc/adhB*. The constructed plasmid was transferred by transformation (Hanahan 1983) to *E. coli* Top10 for verification and finally to *E. coli* strain S17-1 serving as the donor for conjugation experiments.

[0169] WS/DGAT, PDC and ADHB activity assays. Soluble protein fractions of recombinant *R. eutropha* strains were applied for WS/DGAT, PDC and ADHB activity measurements at 30°C using a Nicolet Evolution 100 UV/VIS spectrophotometer (Thermo Electron Corporation, Cambridge, UK). Activity of PDC was assayed with an NADH-coupled system using ADH as an auxiliary enzyme measuring NAD⁺ production by the decrease of absorption at 340 nm. The buffered reaction mixture (50 mM potassium phosphate (KP) buffer, pH 6.5) contained 5 mM MgSO₄, 0.1 mM thiamine pyrophosphate (TPP), 0.175 mM NADH, 17.5 mM pyruvate, 0.1 U/ml ADH and 100 µg/ml protein solution of the soluble extract.

[0170] ADH activity was measured directly by the decrease of NADH due to the reduction of acetic aldehyde. The same reaction mixture as for the PDC activity assay was used, though pyruvate was replaced by 0.1 mM acetic aldehyde and ADH was replaced by KP buffer.

[0171] WS/DGAT activity was measured radiometrically by the synthesis of labelled wax esters from hexadecanol or ethanol and 1-¹⁴C-palmitoyl coenzyme A. The buffered reaction mixture (125 mM sodium phosphate buffer, pH 7.4) contained 3.75 mM hexadecanol or ethanol, 12.5 µg/ml bovine serum albumine (BSA), 4.72 µM 1-¹⁴C-palmitoyl CoA and 200 µg/ml protein crude extract. Prior to use a double concentrated emulsion of hexadecanol, BSA and sodium-phosphate buffer was prepared by ultrasonication (1 min/ml). The reaction mixture was incubated for 30 min at 35°C and the reaction was stopped by the addition of one volume of chloroform-methanol (1:1; v/v). After vigorous vortexing for 1 min phases were separated by centrifugation for 2 min at 16000 x g. The organic phase was removed and applied to a thin layer chromatography (TLC-) plate (Silica gel 60, 20x20 cm aluminium sheets, Merck KGaA, Darmstadt) which was then developed with hexane:diethyl ether:acetic acid (90:7.5:1; (v/v/v) as the solvent. Radioactively marked wax ester spots were visualized by a PharosFX Phospho-Imager (Bio-Rad Laboratories GmbH, Munich) after exposing the developed TLC plate to a phosphor screen. As standard substances for TLC oleyloleate or ethyloleate were used which were visualized by iodine vapour staining.

[0172] Quantitative and qualitative determination of synthesized ethanol and FAEE. Ethanol amounts in cell free culture media were determined by high performance liquid chromatography (HPLC). HPLC analysis of 20 µl samples was carried out with a LaChrom Elite HPLC (VWR-Hitachi International GmbH, Darmstadt) equipped with a Metacarb 67H advanced C-column (length: 300 mm; inner diameter 6.5 mm; Varian, Palo Alto, USA) and a Type 2490 refractive index (RI) detector (VWR-Hitachi International GmbH, Darmstadt).

[0173] FAEE in lyophilised cells were analysed by TLC, gas chromatography (GC) and coupled gas chromatography mass spectrometry (GC/MS). For qualitative TLC analysis 100 mg cell dry matter were mixed with 1 ml chloroform:methanol (2:1; v/v) and broken up with a type MM301 bead mill (Retsch, Haan). After centrifugation for 10 min at 16000 x g the organic supernatant was separated by TLC as described for the WS/DGAT activity assays. Ethyloleate served as the standard substance. Spots were visualized by iodine vapour staining and spots of interest were scraped of the TLC plate, eluted with chloroform:methanol (2:1, v/v) and analyzed by GC or GC/MS. GC analyses of 10-100 mg cell extracts were performed with an Agilent 6850 capillary gas chromatograph (Agilent Technologies GmbH, Waldbronn) equipped with a BP21 capillary column (length: 50 m; inner diameter: 0.22 mm; film thickness 250 nm; SGE GmbH, Darmstadt) and a flame ionisation detector (FID). At a split ratio of 1:15 2 µl of sample were injected by an Agilent 6850 Series Auto Sampler (Agilent Technologies GmbH, Waldbronn). As the carrier gas H₂ (99.999 %) was used at a flow rate of 0.6 ml/min. Inlet and detector temperatures were 250°C and 275°C, respectively, the column temperature was kept at 120°C for 5 min, followed by an increase of 3°C/min to 180°C, a second increase of 10°C/min to 220°C and was finally kept at

220°C for 31 min.

[0174] GC/MS analyses were carried out on a Series 6890 GC System (Hewlett Packard, Waldbronn) equipped with a BPX35 capillary column (length: 50 m; inner diameter: 0.25 mm; film thickness 250 nm; SGE GmbH, Darmstadt). GC was coupled with a Series 5973 electron ionisation mass selective detector (EI-MSD) (Hewlett Packard, Waldbronn). Helium (99.999%) served as the carrier gas with a constant flow rate of 0.6 ml/min. 3 µl of sample were injected by a Series 7683 autoinjector (Hewlett Packard, Waldbronn) with a split ratio of 1:20. The inlet temperature was 250°C, that of the outlet was 240°C. The following temperature programme was applied: 5 min at 120°C, increase of 3°C/min to 180°C, followed by an increase of 5°C/min to 210°C and finally the temperature was kept for 29 min at 210°C. Data were analysed with the NIST mass spectral search programme (Stein et al., 1998)

[0175] Transfer of plasmids to *R. eutropha* Δ phaC1 and determination of enzyme activities. Both plasmids, pBBR1 MCS-3 as the control vector and pBBR1 MCS-3::atfA/pdc/adhB, were transferred via conjugation from *E. coli* S17-1 to *R. eutropha* Δ phaC1 resulting in the strains *R. eutropha* Δ phaC1 pBBR1MCS-3 and *R. eutropha* Δ phaC1 pBBR1MCS-3::atfA/pdc/adhB. Transconjugants could be selected on MSM agar plates containing 1% (wt/vol) sodium gluconate and tetracyclin (12.5 µg/ml) after 12 h of incubation at 30°C.

[0176] Confirmation of the presence of functional active WS/DGAT, PDC and ADHB. The presence of functional active WS/DGAT, PDC and ADHB in the recombinant strains was confirmed by enzymatic analyses.

[0177] Direct or coupled photometrical enzyme assays employing the soluble protein fractions obtained from cells of the recombinant strains of *R. eutropha* Δ phaC1 pBBR1MCS-3 and *R. eutropha* Δ phaC1 pBBR1MCS-3::atfA/pdc/adhB cultivated for 12 h at 30°C in MSM containing 1% (wt/vol) sodium gluconate as sole carbon source, demonstrated the presence of active PDC (18.73 U/mg) and ADH B (1.51 U/mg) in *R. eutropha* Δ phaC1 pBBR1MCS-3::atfA/pdc/adhB, in contrast to the control strain *R. eutropha* Δ phaC1 pBBR1MCS-3 (PDC: 3.52 U/mg and ADH B: 0.25 U/mg). The radiometrical enzyme assays for WS/DGAT revealed the synthesis of radiolabelled wax esters and ethyl esters from hexadecanol or ethanol, respectively, and 1-¹⁴C-palmitoyl CoA in the soluble protein fractions of *R. eutropha* Δ phaC1 pBBR1MCS-3::atfA/pdc/adhB but not of *R. eutropha* Δ phaC1 pBBR1MCS-3, thus indicating the absence of WS/DGAT in the negative control strain. For WS/DGAT activity no specific activities were determined.

[0178] Analysis of ethanol and FAEE biosynthesis by HPLC, GC, GC/MS and TLC. Ethanol and FAEE biosynthesis was analysed in the strains *R. eutropha* Δ phaC1 pBBR1MCS-3 and *R. eutropha* Δ phaC1 pBBR1MCS-3::atfA/pdc/adhB. The cells were grown in MSM-precultures containing 2% gluconate (w/v) and tetracycline or NB-medium with tetracycline for 12 h at 30°C and were used to inoculate main cultures of the same media in baffled 11 Erlenmeyer flasks with 2.5% of the culture volume of 200 ml. One additional MSM culture (400 ml) additionally contained 0.4% (w/v) Na-oleate. Every 4-8 h samples were withdrawn and centrifuged. The supernatant was used for ethanol determination by HPLC. The cell pellets were washed twice with sterile saline, lyophilized and used for ethylester determination by GC, GC/MS and TLC.

[0179] Without Na-oleate as the cosubstrate maximum ethanol amounts of 1.51 g/l were found after 22 h in *R. eutropha* Δ phaC1 pBBR1MCS-3::atfA/pdc/adhB grown in NB, while the control strain reached 1.16 g/l after 14 h. With MSM medium the maximum ethanol amounts reached 0.85 g/l for *R. eutropha* Δ phaC1 pBBR1 MCS-3::atfA/pdc/adhB after 32 h and 0.44 g/l for the control strain after 8 h. If Na-oleate was added to the MSM medium, the maximum ethanol concentration reached 4.38 g/l with *R. eutropha* Δ phaC1 pBBR1MCS-3::atfA/pdc/adhB after 36 h and 1.34 g/l with the control strain after 30 h. Thus, it could be shown that *R. eutropha* Δ phaC1 pBBR1MCS-3::atfA/pdc/adhB is able to synthesize three times higher ethanol concentrations as the control strain if grown in MSM with gluconate and Na-oleate.

[0180] Possibly FAEE occurrences were analyzed by TLC and GC from lyophilized cell mass. Without the addition of Na-oleate neither cells grown in NB nor those grown in MSM showed ethylester synthesis as revealed by GC and TLC analysis. If Na-oleate was added to the medium cell dry mass of *R. eutropha* Δ phaC1 pBBR1MCS-3::atfA/pdc/adhB showed ethylester concentrations of 0.45% (w/w) after 36 h and of 0.23% (w/w) after 48 h. The cell dry mass of the control strains gave no peak corresponding to ethylester in GC analyses. Comparable to the GC analyses, the TLC analyses of crude extracts of the respective cell dry masses re-vealed the occurrence of substances corresponding to ethylester only in the pBBR1MCS-3::atfA/pdc/adhB containing strain *R. eutropha* Δ phaC1.

[0181] While GC/MS analyses of the putative ethylester spot scraped from TLC plates gave no results, GC/MS analyses of the ethylester containing 36 h sample and the 48 h sample revealed the occurrence of ethylpalmitoleate in the cell dry mass. Other FAEE could not be detected. Thus, if supplemented with Na-oleate strain *R. eutropha* Δ phaC1 pBBR1MCS-3::atfA/pdc/adhB is able to synthesize FAEE.

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<150> EP10169808.2

<151> 2010-07-16

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<160> 95

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Gln Asn Ala Ala Gly Asp Ile Val Ser Leu Arg Asp Val Ile Glu Ser
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Asp Lys Ser Thr Leu Leu Gly Glu Ala Val Ala Lys Arg Phe Gly Glu
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Asn Ala Ala Gly Ile Pro Met Asp Ala Ala Glu Arg Asn Tyr Lys Asp
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Ala Glu Arg Leu Ser Glu Leu Phe Ala Ser Leu Leu Asn Met Gln Gly
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30	Lys	His	Ser	Leu	Asp	His	Gly	Arg	Lys	Thr	Gly	Gly	Arg	Leu	Leu	Met
				260					265					270		
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	385					390					395					400
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405

410

415

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	Met	Lys	Ile	Lys	Asn	Ile	Leu	Leu	Thr	Leu	Cys	Thr	Ser	Leu	Leu	Leu	
	1				5					10					15		
5	Thr	Asn	Val	Ala	Ala	His	Ala	Lys	Glu	Val	Lys	Ile	Gly	Met	Ala	Ile	
				20					25					30			
10	Asp	Asp	Leu	Arg	Leu	Glu	Arg	Trp	Gln	Lys	Asp	Arg	Asp	Ile	Phe	Val	
			35					40					45				
15	Lys	Lys	Ala	Glu	Ser	Leu	Gly	Ala	Lys	Val	Phe	Val	Gln	Ser	Ala	Asn	
		50					55					60					
20	Gly	Asn	Glu	Glu	Thr	Gln	Met	Ser	Gln	Ile	Glu	Asn	Met	Ile	Asn	Arg	
	65					70					75					80	
25	Gly	Val	Asp	Val	Leu	Val	Ile	Ile	Pro	Tyr	Asn	Gly	Gln	Val	Leu	Ser	
					85					90					95		
30	Asn	Val	Val	Lys	Glu	Ala	Lys	Gln	Glu	Gly	Ile	Lys	Val	Leu	Ala	Tyr	
				100					105					110			
35	Asp	Arg	Met	Ile	Asn	Asp	Ala	Asp	Ile	Asp	Phe	Tyr	Ile	Ser	Phe	Asp	
			115					120					125				
40	Asn	Glu	Lys	Val	Gly	Glu	Leu	Gln	Ala	Lys	Ala	Leu	Val	Asp	Ile	Val	
45																	
50																	
55																	

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	130		135			140											
5	Pro	Gln	Gly	Asn	Tyr	Phe	Leu	Met	Gly	Gly	Ser	Pro	Val	Asp	Asn	Asn	
	145					150					155					160	
	Ala	Lys	Leu	Phe	Arg	Ala	Gly	Gln	Met	Lys	Val	Leu	Lys	Pro	Tyr	Val	
10					165					170					175		
	Asp	Ser	Gly	Lys	Ile	Lys	Val	Val	Gly	Asp	Gln	Trp	Val	Asp	Gly	Trp	
				180					185					190			
15	Leu	Pro	Glu	Asn	Ala	Leu	Lys	Ile	Met	Glu	Asn	Ala	Leu	Thr	Ala	Asn	
			195					200					205				
20	Asn	Asn	Lys	Ile	Asp	Ala	Val	Val	Ala	Ser	Asn	Asp	Ala	Thr	Ala	Gly	
	210						215					220					
25	Gly	Ala	Ile	Gln	Ala	Leu	Ser	Ala	Gln	Gly	Leu	Ser	Gly	Lys	Val	Ala	
	225					230					235					240	
	Ile	Ser	Gly	Gln	Asp	Ala	Asp	Leu	Ala	Gly	Ile	Lys	Arg	Ile	Ala	Ala	
					245					250					255		
30	Gly	Thr	Gln	Thr	Met	Thr	Val	Tyr	Lys	Pro	Ile	Thr	Leu	Leu	Ala	Asn	
				260					265					270			
35	Thr	Ala	Ala	Glu	Ile	Ala	Val	Glu	Leu	Gly	Asn	Gly	Gln	Glu	Pro	Lys	
			275					280					285				
40	Ala	Asp	Thr	Thr	Leu	Asn	Asn	Gly	Leu	Lys	Asp	Val	Pro	Ser	Arg	Leu	
	290						295					300					
	Leu	Thr	Pro	Ile	Asp	Val	Asn	Lys	Asn	Asn	Ile	Lys	Asp	Thr	Val	Ile	
	305					310					315					320	
45	Lys	Asp	Gly	Phe	His	Lys	Glu	Ser	Glu	Leu							
					325					330							

<210> 8

50 <211> 513

<212> PRT

<213> Escherichia coli

<400> 8

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Met Pro Tyr Leu Leu Glu Met Lys Asn Ile Thr Lys Thr Phe Gly Ser
1 5 10 15

5 Val Lys Ala Ile Asp Asn Val Cys Leu Arg Leu Asn Ala Gly Glu Ile

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	20	25	30
5	Val Ser Leu Cys Gly Glu Asn Gly Ser Gly Lys Ser Thr Leu Met Lys 35 40 45		
10	Val Leu Cys Gly Ile Tyr Pro His Gly Ser Tyr Glu Gly Glu Ile Ile 50 55 60		
15	Phe Ala Gly Glu Glu Ile Gln Ala Ser His Ile Arg Asp Thr Glu Arg 65 70 75 80		
20	Lys Gly Ile Ala Ile Ile His Gln Glu Leu Ala Leu Val Lys Glu Leu 85 90 95		
25	Thr Val Leu Glu Asn Ile Phe Leu Gly Asn Glu Ile Thr His Asn Gly 100 105 110		
30	Ile Met Asp Tyr Asp Leu Met Thr Leu Arg Cys Gln Lys Leu Leu Ala 115 120 125		
35	Gln Val Ser Leu Ser Ile Ser Pro Asp Thr Arg Val Gly Asp Leu Gly 130 135 140		
40	Leu Gly Gln Gln Gln Leu Val Glu Ile Ala Lys Ala Leu Asn Lys Gln 145 150 155 160		
45	Val Arg Leu Leu Ile Leu Asp Glu Pro Thr Ala Ser Leu Thr Glu Gln 165 170 175		
50	Glu Thr Ser Ile Leu Leu Asp Ile Ile Arg Asp Leu Gln Gln His Gly 180 185 190		
55	Ile Ala Cys Ile Tyr Ile Ser His Lys Leu Asn Glu Val Lys Ala Ile 195 200 205		
	Ser Asp Thr Ile Cys Val Ile Arg Asp Gly Gln His Ile Gly Thr Arg 210 215 220		
	Asp Ala Ala Gly Met Ser Glu Asp Asp Ile Ile Thr Met Met Val Gly 225 230 235 240		
	Arg Glu Leu Thr Ala Leu Tyr Pro Asn Glu Pro His Thr Thr Gly Asp 245 250 255		
	Glu Ile Leu Arg Ile Glu His Leu Thr Ala Trp His Pro Val Asn Arg 260 265 270		

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	His	Ile	Lys	Arg	Val	Asn	Asp	Val	Ser	Phe	Ser	Leu	Lys	Arg	Gly	Glu	
			275					280					285				
5	Ile	Leu	Gly	Ile	Ala	Gly	Leu	Val	Gly	Ala	Gly	Arg	Thr	Glu	Thr	Ile	
		290					295					300					
10	Gln	Cys	Leu	Phe	Gly	Val	Trp	Pro	Gly	Gln	Trp	Glu	Gly	Lys	Ile	Tyr	
	305					310					315					320	
	Ile	Asp	Gly	Lys	Gln	Val	Asp	Ile	Arg	Asn	Cys	Gln	Gln	Ala	Ile	Ala	
15					325					330					335		
	Gln	Gly	Ile	Ala	Met	Val	Pro	Glu	Asp	Arg	Lys	Arg	Asp	Gly	Ile	Val	
				340					345					350			
20	Pro	Val	Met	Ala	Val	Gly	Lys	Asn	Ile	Thr	Leu	Ala	Ala	Leu	Asn	Lys	
			355					360					365				
	Phe	Thr	Gly	Gly	Ile	Ser	Gln	Leu	Asp	Asp	Ala	Ala	Glu	Gln	Lys	Cys	
25		370					375					380					
	Ile	Leu	Glu	Ser	Ile	Gln	Gln	Leu	Lys	Val	Lys	Thr	Ser	Ser	Pro	Asp	
	385					390					395					400	
30	Leu	Ala	Ile	Gly	Arg	Leu	Ser	Gly	Gly	Asn	Gln	Gln	Lys	Ala	Ile	Leu	
					405					410					415		
	Ala	Arg	Cys	Leu	Leu	Leu	Asn	Pro	Arg	Ile	Leu	Ile	Leu	Asp	Glu	Pro	
35				420					425					430			
	Thr	Arg	Gly	Ile	Asp	Ile	Gly	Ala	Lys	Tyr	Glu	Ile	Tyr	Lys	Leu	Ile	
40			435					440					445				
	Asn	Gln	Leu	Val	Gln	Gln	Gly	Ile	Ala	Val	Ile	Val	Ile	Ser	Ser	Glu	
		450					455					460					
45	Leu	Pro	Glu	Val	Leu	Gly	Leu	Ser	Asp	Arg	Val	Leu	Val	Met	His	Glu	
	465					470					475					480	
	Gly	Lys	Leu	Lys	Ala	Asn	Leu	Ile	Asn	His	Asn	Leu	Thr	Gln	Glu	Gln	
50					485					490					495		
	Val	Met	Glu	Ala	Ala	Leu	Arg	Ser	Glu	His	His	Val	Glu	Lys	Gln	Ser	
				500					505					510			
55	Val																

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<210> 9
 <211> 393
 <212> PRT
 <213> Escherichia coli

5

<400> 9

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

15

Gly Gly Phe Ser Gly Leu Lys Ser Leu Asn Leu Gln Val Phe Val Met
 20 25 30

20

Ile Ala Ala Ile Ile Ala Ile Met Leu Phe Phe Thr Trp Thr Thr Asp
 35 40 45

25

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

Ala Ile Thr Gly Ile Leu Ala Val Gly Met Val Phe Val Ile Ile Ser
 65 70 75 80

30

Ala Glu Ile Asp Leu Ser Val Gly Ser Met Met Gly Leu Leu Gly Gly
 85 90 95

35

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

Ile Ile Val Thr Leu Val Leu Gly Leu Leu Leu Gly Ala Trp Asn Gly
 115 120 125

40

Trp Trp Val Ala Tyr Arg Lys Val Pro Ser Phe Ile Val Thr Leu Ala
 130 135 140

Gly Met Leu Ala Phe Arg Gly Ile Leu Ile Gly Ile Thr Asn Gly Thr
 145 150 155 160

45

Thr Val Ser Pro Thr Ser Ala Ala Met Ser Gln Ile Gly Gln Ser Tyr
 165 170 175

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Leu Pro Ala Ser Thr Gly Phe Ile Ile Gly Ala Leu Gly Leu Met Ala
 180 185 190

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Phe Val Gly Trp Gln Trp Arg Gly Arg Met Arg Arg Gln Ala Leu Gly
 195 200 205

Leu Gln Ser Pro Ala Ser Thr Ala Val Val Gly Arg Gln Ala Leu Thr
 210 215 220

Ala Ile Ile Val Leu Gly Ala Ile Trp Leu Leu Asn Asp Tyr Arg Gly
225 230 235 240

Val Pro Thr Pro Val Leu Leu Leu Thr Leu Leu Leu Leu Gly Gly Met
245 250 255

Phe Met Ala Thr Arg Thr Ala Phe Gly Arg Arg Ile Tyr Ala Ile Gly
260 265 270

Gly Asn Leu Glu Ala Ala Arg Leu Ser Gly Ile Asn Val Glu Arg Thr
275 280 285

Lys Leu Ala Val Phe Ala Ile Asn Gly Leu Met Val Ala Ile Ala Gly
290 295 300

Leu Ile Leu Ser Ser Arg Leu Gly Ala Gly Ser Pro Ser Ala Gly Asn
305 310 315 320

Ile Ala Glu Leu Asp Ala Ile Ala Ala Cys Val Ile Gly Gly Thr Ser
325 330 335

Leu Ala Gly Gly Val Gly Ser Val Ala Gly Ala Val Met Gly Ala Phe
340 345 350

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

Phe Trp Gln Tyr Ile Val Lys Gly Ala Ile Leu Leu Leu Ala Val Trp
370 375 380

Met Asp Ser Ala Thr Lys Arg Arg Ser
385 390

<210> 10

<211> 317

<212> PRT

<213> Escherichia coli

<400> 10

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	Met	Thr	Asp	Lys	Leu	Thr	Ser	Leu	Arg	Gln	Tyr	Thr	Thr	Val	Val	Ala
	1				5					10					15	
5	Asp	Thr	Gly	Asp	Ile	Ala	Ala	Met	Lys	Leu	Tyr	Gln	Pro	Gln	Asp	Ala
				20					25					30		
10	Thr	Thr	Asn	Pro	Ser	Leu	Ile	Leu	Asn	Ala	Ala	Gln	Ile	Pro	Glu	Tyr
			35					40					45			
15																
20																
25																
30																
35																
40																
45																
50																
55																

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	Arg	Lys	Leu	Ile	Asp	Asp	Ala	Val	Ala	Trp	Ala	Lys	Gln	Gln	Ser	Asn	
	50						55					60					
5	Asp	Arg	Ala	Gln	Gln	Ile	Val	Asp	Ala	Thr	Asp	Lys	Leu	Ala	Val	Asn	
	65					70					75					80	
	Ile	Gly	Leu	Glu	Ile	Leu	Lys	Leu	Val	Pro	Gly	Arg	Ile	Ser	Thr	Glu	
10					85					90					95		
	Val	Asp	Ala	Arg	Leu	Ser	Tyr	Asp	Thr	Glu	Ala	Ser	Ile	Ala	Lys	Ala	
				100					105					110			
15	Lys	Arg	Leu	Ile	Lys	Leu	Tyr	Asn	Asp	Ala	Gly	Ile	Ser	Asn	Asp	Arg	
			115					120					125				
	Ile	Leu	Ile	Lys	Leu	Ala	Ser	Thr	Trp	Gln	Gly	Ile	Arg	Ala	Ala	Glu	
20		130					135					140					
	Gln	Leu	Glu	Lys	Glu	Gly	Ile	Asn	Cys	Asn	Leu	Thr	Leu	Leu	Phe	Ser	
25		145				150					155					160	
	Phe	Ala	Gln	Ala	Arg	Ala	Cys	Ala	Glu	Ala	Gly	Val	Phe	Leu	Ile	Ser	
					165					170					175		
30	Pro	Phe	Val	Gly	Arg	Ile	Leu	Asp	Trp	Tyr	Lys	Ala	Asn	Thr	Asp	Lys	
				180					185					190			
	Lys	Glu	Tyr	Ala	Pro	Ala	Glu	Asp	Pro	Gly	Val	Val	Ser	Val	Ser	Glu	
35			195					200					205				
	Ile	Tyr	Gln	Tyr	Tyr	Lys	Glu	His	Gly	Tyr	Glu	Thr	Val	Val	Met	Gly	
		210					215					220					
40	Ala	Ser	Phe	Arg	Asn	Ile	Gly	Glu	Ile	Leu	Glu	Leu	Ala	Gly	Cys	Asp	
	225					230					235					240	
	Arg	Leu	Thr	Ile	Ala	Pro	Ala	Leu	Leu	Lys	Glu	Leu	Ala	Glu	Ser	Glu	
45					245					250					255		
	Gly	Ala	Ile	Glu	Arg	Lys	Leu	Ser	Tyr	Thr	Gly	Glu	Val	Lys	Ala	Arg	
50				260					265					270			
	Pro	Ala	Arg	Ile	Thr	Glu	Ser	Glu	Phe	Leu	Trp	Gln	His	Asn	Gln	Asp	
		275						280					285				
55	Pro	Met	Ala	Val	Asp	Lys	Leu	Ala	Glu	Gly	Ile	Arg	Lys	Phe	Ala	Ile	
		290					295					300					

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<211> 663

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<400> 11

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	Met	Ser	Ser	Arg	Lys	Glu	Leu	Ala	Asn	Ala	Ile	Arg	Ala	Leu	Ser	Met	
	1				5					10					15		
5	Asp	Ala	Val	Gln	Lys	Ala	Lys	Ser	Gly	His	Pro	Gly	Ala	Pro	Met	Gly	
				20					25					30			
10	Met	Ala	Asp	Ile	Ala	Glu	Val	Leu	Trp	Arg	Asp	Phe	Leu	Lys	His	Asn	
			35					40					45				
15	Pro	Gln	Asn	Pro	Ser	Trp	Ala	Asp	Arg	Asp	Arg	Phe	Val	Leu	Ser	Asn	
		50					55					60					
20	Gly	His	Gly	Ser	Met	Leu	Ile	Tyr	Ser	Leu	Leu	His	Leu	Thr	Gly	Tyr	
	65					70					75					80	
25	Asp	Leu	Pro	Met	Glu	Glu	Leu	Lys	Asn	Phe	Arg	Gln	Leu	His	Ser	Lys	
					85				90						95		
30	Thr	Pro	Gly	His	Pro	Glu	Val	Gly	Tyr	Thr	Ala	Gly	Val	Glu	Thr	Thr	
				100					105					110			
35	Thr	Gly	Pro	Leu	Gly	Gln	Gly	Ile	Ala	Asn	Ala	Val	Gly	Met	Ala	Ile	
			115					120					125				
40	Ala	Glu	Lys	Thr	Leu	Ala	Ala	Gln	Phe	Asn	Arg	Pro	Gly	His	Asp	Ile	
		130					135					140					
45	Val	Asp	His	Tyr	Thr	Tyr	Ala	Phe	Met	Gly	Asp	Gly	Cys	Met	Met	Glu	
	145					150					155					160	
50	Gly	Ile	Ser	His	Glu	Val	Cys	Ser	Leu	Ala	Gly	Thr	Leu	Lys	Leu	Gly	
					165					170					175		
55	Lys	Leu	Ile	Ala	Phe	Tyr	Asp	Asp	Asn	Gly	Ile	Ser	Ile	Asp	Gly	His	
				180					185					190			
60	Val	Glu	Gly	Trp	Phe	Thr	Asp	Asp	Thr	Ala	Met	Arg	Phe	Glu	Ala	Tyr	
			195					200					205				

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	Gly	Trp	His	Val	Ile	Arg	Asp	Ile	Asp	Gly	His	Asp	Ala	Ala	Ser	Ile	
	210						215					220					
5	Lys	Arg	Ala	Val	Glu	Glu	Ala	Arg	Ala	Val	Thr	Asp	Lys	Pro	Ser	Leu	
	225					230					235					240	
10	Leu	Met	Cys	Lys	Thr	Ile	Ile	Gly	Phe	Gly	Ser	Pro	Asn	Lys	Ala	Gly	
					245					250					255		
	Thr	His	Asp	Ser	His	Gly	Ala	Pro	Leu	Gly	Asp	Ala	Glu	Ile	Ala	Leu	
				260					265					270			
15	Thr	Arg	Glu	Gln	Leu	Gly	Trp	Lys	Tyr	Ala	Pro	Phe	Glu	Ile	Pro	Ser	
			275					280					285				
20	Glu	Ile	Tyr	Ala	Gln	Trp	Asp	Ala	Lys	Glu	Ala	Gly	Gln	Ala	Lys	Glu	
	290						295					300					
25	Ser	Ala	Trp	Asn	Glu	Lys	Phe	Ala	Ala	Tyr	Ala	Lys	Ala	Tyr	Pro	Gln	
	305					310					315					320	
	Glu	Ala	Ala	Glu	Phe	Thr	Arg	Arg	Met	Lys	Gly	Glu	Met	Pro	Ser	Asp	
				325						330					335		
30	Phe	Asp	Ala	Lys	Ala	Lys	Glu	Phe	Ile	Ala	Lys	Leu	Gln	Ala	Asn	Pro	
				340					345					350			
35	Ala	Lys	Ile	Ala	Ser	Arg	Lys	Ala	Ser	Gln	Asn	Ala	Ile	Glu	Ala	Phe	
		355					360						365				
40	Gly	Pro	Leu	Leu	Pro	Glu	Phe	Leu	Gly	Gly	Ser	Ala	Asp	Leu	Ala	Pro	
	370					375						380					
	Ser	Asn	Leu	Thr	Leu	Trp	Ser	Gly	Ser	Lys	Ala	Ile	Asn	Glu	Asp	Ala	
	385					390					395					400	
45	Ala	Gly	Asn	Tyr	Ile	His	Tyr	Gly	Val	Arg	Glu	Phe	Gly	Met	Thr	Ala	
					405					410					415		
50	Ile	Ala	Asn	Gly	Ile	Ser	Leu	His	Gly	Gly	Phe	Leu	Pro	Tyr	Thr	Ser	
			420						425					430			
	Thr	Phe	Leu	Met	Phe	Val	Glu	Tyr	Ala	Arg	Asn	Ala	Val	Arg	Met	Ala	
		435					440					445					
55	Ala	Leu	Met	Lys	Gln	Arg	Gln	Val	Met	Val	Tyr	Thr	His	Asp	Ser	Ile	

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	450		455		460												
5	Gly	Leu	Gly	Glu	Asp	Gly	Pro	Thr	His	Gln	Pro	Val	Glu	Gln	Val	Ala	
	465					470					475					480	
	Ser	Leu	Arg	Val	Thr	Pro	Asn	Met	Ser	Thr	Trp	Arg	Pro	Cys	Asp	Gln	
10					485					490					495		
	Val	Glu	Ser	Ala	Val	Ala	Trp	Lys	Tyr	Gly	Val	Glu	Arg	Gln	Asp	Gly	
				500					505					510			
15	Pro	Thr	Ala	Leu	Ile	Leu	Ser	Arg	Gln	Asn	Leu	Ala	Gln	Gln	Glu	Arg	
			515					520					525				
	Thr	Glu	Glu	Gln	Leu	Ala	Asn	Ile	Ala	Arg	Gly	Gly	Tyr	Val	Leu	Lys	
20		530					535					540					
	Asp	Cys	Ala	Gly	Gln	Pro	Glu	Leu	Ile	Phe	Ile	Ala	Thr	Gly	Ser	Glu	
25	545					550					555					560	
	Val	Glu	Leu	Ala	Val	Ala	Ala	Tyr	Glu	Lys	Leu	Thr	Ala	Glu	Gly	Val	
					565					570					575		
30	Lys	Ala	Arg	Val	Val	Ser	Met	Pro	Ser	Thr	Asp	Ala	Phe	Asp	Lys	Gln	
				580					585					590			
	Asp	Ala	Ala	Tyr	Arg	Glu	Ser	Val	Leu	Pro	Lys	Ala	Val	Thr	Ala	Arg	
35			595					600					605				
	Val	Ala	Val	Glu	Ala	Gly	Ile	Ala	Asp	Tyr	Trp	Tyr	Lys	Tyr	Val	Gly	
40		610					615					620					
	Leu	Asn	Gly	Ala	Ile	Val	Gly	Met	Thr	Thr	Phe	Gly	Glu	Ser	Ala	Pro	
	625					630					635					640	
45	Ala	Glu	Leu	Leu	Phe	Glu	Glu	Phe	Gly	Phe	Thr	Val	Asp	Asn	Val	Val	
					645					650					655		
50	Ala	Lys	Ala	Lys	Glu	Leu	Leu										
					660												

<210> 12

<211> 321

<212> PRT

55 <213> Escherichia coli

<400> 12

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Met Thr Lys Tyr Ala Leu Val Gly Asp Val Gly Gly Thr Asn Ala Arg

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1	5	10	15
5	Leu Ala Leu Cys Asp Ile Ala Ser Gly Glu Ile Ser Gln Ala Lys Thr	20	25
10	Tyr Ser Gly Leu Asp Tyr Pro Ser Leu Glu Ala Val Ile Arg Val Tyr	35	40
15	Leu Glu Glu His Lys Val Glu Val Lys Asp Gly Cys Ile Ala Ile Ala	50	55
20	Cys Pro Ile Thr Gly Asp Trp Val Ala Met Thr Asn His Thr Trp Ala	65	70
25	Phe Ser Ile Ala Glu Met Lys Lys Asn Leu Gly Phe Ser His Leu Glu	85	90
30	Ile Ile Asn Asp Phe Thr Ala Val Ser Met Ala Ile Pro Met Leu Lys	100	105
35	Lys Glu His Leu Ile Gln Phe Gly Gly Ala Glu Pro Val Glu Gly Lys	115	120
40	Pro Ile Ala Val Tyr Gly Ala Gly Thr Gly Leu Gly Val Ala His Leu	130	135
45	Val His Val Asp Lys Arg Trp Val Ser Leu Pro Gly Glu Gly Gly His	145	150
50	Val Asp Phe Ala Pro Asn Ser Glu Glu Glu Ala Ile Ile Leu Glu Ile	165	170
55	Leu Arg Ala Glu Ile Gly His Val Ser Ala Glu Arg Val Leu Ser Gly	180	185
	Pro Gly Leu Val Asn Leu Tyr Arg Ala Ile Val Lys Ala Asp Asn Arg	195	200
	Leu Pro Glu Asn Leu Lys Pro Lys Asp Ile Thr Glu Arg Ala Leu Ala	210	215
	Asp Ser Cys Thr Asp Cys Arg Arg Ala Leu Ser Leu Phe Cys Val Ile	225	230
	Met Gly Arg Phe Gly Gly Asn Leu Ala Leu Asn Leu Gly Thr Phe Gly	245	250

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Gly Val Phe Ile Ala Gly Gly Ile Val Pro Arg Phe Leu Glu Phe Phe
260 265 270

5 Lys Ala Ser Gly Phe Arg Ala Ala Phe Glu Asp Lys Gly Arg Phe Lys
275 280 285

10 Glu Tyr Val His Asp Ile Pro Val Tyr Leu Ile Val His Asp Asn Pro
290 295 300

15 Gly Leu Leu Gly Ser Gly Ala His Leu Arg Gln Thr Leu Gly His Ile
305 310 315 320

Leu

<210> 13

20 <211> 391

<212> PRT

<213> Escherichia albertii

<400> 13

25 Met Gln Lys Leu Ile Asn Ser Val Gln Asn Tyr Ala Trp Gly Ser Lys
1 5 10 15

30 Thr Ala Leu Thr Glu Leu Tyr Gly Met Glu Asn Pro Ser Asn Gln Pro
20 25 30

35 Met Ala Glu Leu Trp Met Gly Ala His Pro Lys Ser Ser Ser Arg Val
35 40 45

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

40 Asn Lys Ser Thr Leu Leu Gly Glu Ala Val Ala Gln Arg Phe Gly Glu
65 70 75 80

45 Leu Pro Phe Leu Phe Lys Val Leu Cys Ala Ala Gln Pro Leu Ser Ile
85 90 95

50 Gln Val His Pro Asn Lys Arg Asn Ser Glu Ile Gly Phe Ala Lys Glu
100 105 110

Asn Ala Ala Gly Ile Pro Met Asp Ala Ala Glu Arg Asn Tyr Lys Asp
115 120 125

55 Pro Asn His Lys Pro Glu Leu Val Phe Ala Leu Thr Pro Phe Leu Ala
130 135 140

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	Met	Asn	Ala	Phe	Arg	Glu	Phe	Ser	Glu	Ile	Val	Ser	Leu	Leu	Gln	Pro	
	145					150					155					160	
5	Val	Ala	Asp	Ala	His	Pro	Ala	Ile	Thr	His	Phe	Leu	Gln	Gln	Pro	Asn	
					165					170					175		
	Ala	Glu	Arg	Leu	Ser	Glu	Leu	Phe	Ala	Ser	Leu	Leu	Asn	Met	Gln	Gly	
10				180					185					190			
	Glu	Glu	Lys	Ser	Arg	Ala	Leu	Ala	Ile	Leu	Lys	Ser	Ala	Leu	His	Ser	
			195					200					205				
15	Gln	Gln	Gly	Glu	Pro	Trp	Gln	Thr	Ile	Arg	Leu	Ile	Ser	Glu	Phe	Tyr	
	210						215					220					
	Pro	Asp	Asp	Ser	Gly	Leu	Phe	Ser	Pro	Leu	Leu	Leu	Asn	Val	Val	Lys	
20	225					230					235					240	
	Leu	Asn	Pro	Gly	Glu	Ala	Met	Phe	Leu	Phe	Ala	Glu	Thr	Pro	His	Ala	
25					245					250					255		
	Tyr	Leu	Gln	Gly	Val	Ala	Leu	Glu	Val	Met	Ala	Asn	Ser	Asp	Asn	Val	
				260					265					270			
30	Leu	Arg	Ala	Gly	Leu	Thr	Pro	Lys	Tyr	Ile	Asp	Ile	Pro	Glu	Leu	Val	
			275					280					285				
	Ala	Asn	Val	Lys	Phe	Glu	Ala	Lys	Pro	Ala	Asn	Gln	Leu	Leu	Thr	Gln	
35		290					295					300					
	Pro	Val	Lys	His	Gly	Ala	Glu	Leu	Asp	Phe	Pro	Ile	Pro	Val	Asp	Asp	
	305					310					315					320	
40	Phe	Ala	Phe	Ser	Leu	His	Asp	Leu	Asn	Asp	Lys	Glu	Cys	Thr	Ile	Gly	
					325					330					335		
	Gln	Gln	Ser	Ala	Ala	Ile	Leu	Phe	Cys	Val	Glu	Gly	Glu	Ala	Thr	Leu	
45				340					345					350			
	Asn	Lys	Asp	Ser	Gln	Gln	Leu	Leu	Leu	Lys	Pro	Gly	Glu	Ser	Ala	Phe	
50			355					360					365				
	Ile	Ala	Ala	Asn	Glu	Ser	Pro	Val	Lys	Val	Lys	Gly	His	Gly	Arg	Leu	
	370						375					380					
55	Ala	Arg	Val	Tyr	Asn	Lys	Leu										
	385					390											

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<210> 14
<211> 302
<212> PRT
<213> Escherichia blattae

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<400> 14

10	Met	Arg	Ile	Gly	Ile	Asp	Leu	Gly	Gly	Thr	Lys	Thr	Glu	Val	Ile	Ala	1	5	10	15
	Leu	Gly	Asp	Ala	Gly	Glu	Gln	Leu	Tyr	Arg	His	Arg	Leu	Pro	Thr	Pro	20	25	30	
15	Arg	Asp	Asp	Tyr	Arg	Gln	Thr	Ile	Glu	Thr	Ile	Ala	Thr	Leu	Val	Asp	35	40	45	
20	Met	Ala	Glu	Gln	Thr	Thr	Gly	Gln	Cys	Gly	Thr	Val	Gly	Ile	Gly	Ile	50	55	60	
25	Pro	Gly	Ser	Ile	Ser	Pro	Tyr	Thr	Gly	Val	Val	Lys	Asn	Ala	Asn	Ser	65	70	75	80
30	Thr	Trp	Leu	Asn	Gly	Gln	Pro	Phe	Asp	Lys	Asp	Leu	Ser	Ala	Arg	Leu	85	90	95	
	Gln	Arg	Glu	Val	Arg	Leu	Ala	Asn	Asp	Ala	Asn	Cys	Leu	Ala	Val	Ser	100	105	110	
35	Glu	Ala	Val	Asp	Gly	Ala	Ala	Ala	Gly	Ala	Lys	Thr	Val	Phe	Ala	Val	115	120	125	
40	Ile	Ile	Gly	Thr	Gly	Cys	Gly	Ala	Gly	Val	Ala	Phe	Asn	Gly	Arg	Ala	130	135	140	
	His	Ile	Gly	Gly	Asn	Gly	Thr	Ala	Gly	Glu	Trp	Gly	His	Asn	Pro	Leu	145	150	155	160
45	Pro	Trp	Met	Asp	Thr	Asp	Glu	Leu	Arg	Tyr	Arg	Glu	Glu	Val	Pro	Cys	165	170	175	
50	Tyr	Cys	Gly	Lys	Gln	Gly	Cys	Ile	Glu	Thr	Phe	Ile	Ser	Gly	Thr	Gly	180	185	190	
	Phe	Ala	Thr	Asp	Tyr	His	Arg	Leu	Ser	Gly	His	Val	Leu	Lys	Gly	Ser	195	200	205	
55	Glu	Ile	Ile	Arg	Leu	Val	Glu	Glu	Asn	Asp	Pro	Val	Ala	Glu	Leu	Ala	210	215	220	

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Leu Asp Arg Tyr Glu Arg Arg Leu Ala Lys Ser Leu Ala His Val Val
 225 230 235 240
 5 Asn Ile Leu Asp Pro Asp Val Ile Val Leu Gly Gly Gly Met Ser Asn
 245 250 255
 10 Val Glu Arg Leu Tyr Gln Thr Val Pro Pro Leu Ile Lys Arg Phe Val
 260 265 270
 Phe Gly Gly Glu Cys Glu Thr Pro Val Arg Lys Ala Lys His Gly Asp
 275 280 285
 15 Ser Ser Gly Val Arg Gly Ala Ala Trp Leu Trp Pro Gln Glu
 290 295 300
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 20 <211> 411
 <212> PRT
 <213> Escherichia fergusonii
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 25 Met Pro Glu Asn Ser Cys Phe Asn Asn Ala Asn Lys Leu His Met Lys
 1 5 10 15
 30 Gln Gly Leu Ile Met Gln Lys Leu Ile Asn Ser Val Gln Asn Tyr Ala
 20 25 30
 35 Trp Gly Ser Lys Thr Ala Leu Thr Asp Leu Tyr Gly Ile Ala Asn Pro
 35 40 45
 Ser Gln Gln Pro Met Ala Glu Leu Trp Met Gly Ala His Pro Lys Ser
 50 55 60
 40 Ser Ser Lys Val Glu Thr Ser Glu Gly Gln Ile Val Ser Leu Arg Asp
 65 70 75 80
 45 Val Ile Glu Asn Asp Lys Ser Ala Leu Leu Gly Asp Ala Val Ala Glu
 85 90 95
 Arg Phe Gly Glu Leu Pro Phe Leu Phe Lys Val Leu Cys Ala Ala Gln
 100 105 110
 50 Pro Leu Ser Ile Gln Val His Pro Asn Lys Arg Asn Ser Glu Ile Gly
 115 120 125
 55 Phe Ala Lys Glu Asn Ala Ala Gly Ile Pro Met Asp Ala Ala Glu Arg
 130 135 140

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	Asn	Tyr	Lys	Asp	Pro	Asn	His	Lys	Pro	Glu	Leu	Val	Phe	Ala	Leu	Thr	
	145					150					155					160	
5																	
	Pro	Phe	Leu	Ala	Met	Asn	Ala	Phe	Arg	Glu	Phe	Ser	Glu	Ile	Val	Ser	
					165					170					175		
10	Leu	Leu	Gln	Pro	Val	Ala	Gly	Ala	His	Pro	Ala	Ile	Ala	His	Phe	Leu	
				180					185					190			
	Gln	Gln	Pro	Asp	Ala	Glu	Arg	Leu	Ser	Glu	Leu	Phe	Ala	Ala	Leu	Leu	
			195					200					205				
15																	
	Asn	Met	Gln	Gly	Glu	Glu	Lys	Ser	Arg	Ala	Leu	Ala	Ile	Leu	Lys	Ser	
	210						215					220					
20	Ala	Leu	Asp	Thr	Gln	Gln	Gly	Glu	Pro	Trp	Gln	Thr	Ile	Arg	Val	Ile	
	225					230					235					240	
	Ala	Gln	Phe	Tyr	Pro	Asp	Asp	Ser	Gly	Leu	Phe	Ser	Pro	Leu	Leu	Leu	
25					245					250					255		
	Asn	Val	Val	Lys	Leu	Asn	Pro	Gly	Glu	Ala	Met	Phe	Leu	Phe	Ala	Glu	
				260					265					270			
30																	
	Thr	Pro	His	Ala	Tyr	Leu	Gln	Gly	Val	Ala	Leu	Glu	Val	Met	Ala	Asn	
			275					280					285				
35	Ser	Asp	Asn	Val	Leu	Arg	Ala	Gly	Leu	Thr	Pro	Lys	Tyr	Ile	Asp	Ile	
	290						295					300					
	Pro	Glu	Leu	Val	Ala	Asn	Val	Lys	Phe	Glu	Ala	Lys	Pro	Ala	Gly	Gln	
	305					310					315					320	
40																	
	Leu	Leu	Thr	Gln	Pro	Ile	Lys	Asn	Gly	His	Glu	Leu	Asp	Phe	Pro	Ile	
				325						330					335		
45	Pro	Val	Glu	Asp	Phe	Ala	Phe	Ala	Leu	His	Asp	Leu	Thr	Lys	Asp	Glu	
				340					345					350			
	Ala	Ala	Val	Asn	Gln	Gln	Ser	Ala	Ala	Ile	Leu	Phe	Cys	Val	Glu	Gly	
50			355					360					365				
	Glu	Ala	Cys	Leu	Arg	Lys	Gly	Asp	Gln	Gln	Leu	His	Leu	Lys	Pro	Gly	
	370						375					380					
55	Glu	Ser	Ala	Phe	Ile	Ala	Ala	Asn	Glu	Ser	Pro	Val	Leu	Val	Ser	Gly	
	385					390					395					400	

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Asn Gly Arg Leu Ala Arg Val Tyr Asn Lys Leu
405 410

5 <210> 16
<211> 440
<212> PRT
<213> Escherichia fergusonii

10 <400> 16

1	Met	Gln	Ala	Tyr	Phe	Asp	Gln	Leu	Asp	Arg	Val	Arg	Tyr	Glu	Gly	Pro
					5					10					15	
15	Lys	Thr	Thr	Asn	Pro	Leu	Ala	Phe	Arg	His	Tyr	Asn	Pro	Asp	Glu	Leu
				20					25					30		
20	Val	Leu	Gly	Lys	Arg	Met	Glu	Asp	His	Leu	Arg	Phe	Ala	Ala	Cys	Tyr
			35					40					45			
25	Trp	His	Thr	Phe	Cys	Trp	Asn	Gly	Ala	Asp	Met	Phe	Gly	Val	Gly	Ala
		50					55					60				
30	Phe	Asp	Arg	Pro	Trp	Gln	Gln	Pro	Gly	Glu	Ala	Leu	Met	Leu	Ala	Lys
	65					70					75					80
35	Arg	Lys	Ala	Asp	Val	Ala	Phe	Glu	Phe	Phe	His	Lys	Leu	Asn	Val	Pro
					85					90					95	
40	Phe	Tyr	Cys	Phe	His	Asp	Val	Asp	Val	Ser	Pro	Glu	Gly	Ala	Ser	Leu
				100					105					110		
45	Lys	Glu	Tyr	Lys	Asn	Asn	Phe	Ala	Glu	Met	Val	Asp	Val	Leu	Ala	Ala
			115					120					125			
50	Lys	Gln	Glu	Gln	Ser	Gly	Val	Lys	Leu	Leu	Trp	Gly	Thr	Ala	Asn	Cys
			130				135					140				
55	Phe	Thr	Asn	Pro	Arg	Tyr	Gly	Ala	Gly	Ala	Ala	Thr	Asn	Pro	Asp	Pro
	145					150					155				160	
60	Glu	Val	Phe	Ser	Trp	Ala	Ala	Thr	Gln	Val	Val	Thr	Ala	Met	Glu	Ala
					165					170					175	
65	Thr	Lys	Arg	Leu	Gly	Gly	Glu	Asn	Tyr	Val	Leu	Trp	Gly	Gly	Arg	Glu
				180					185					190		
70	Gly	Tyr	Glu	Thr	Leu	Leu	Asn	Thr	Asp	Leu	Arg	Gln	Glu	Arg	Glu	Gln
			195					200					205			

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	Leu	Gly	Arg	Phe	Met	Gln	Met	Val	Val	Glu	His	Lys	His	Lys	Ile	Gly	
	210						215					220					
5	Phe	Gln	Gly	Thr	Leu	Leu	Ile	Glu	Pro	Lys	Pro	Gln	Glu	Pro	Thr	Lys	
	225					230					235					240	
10	His	Gln	Tyr	Asp	Tyr	Asp	Ala	Ala	Thr	Val	Tyr	Gly	Phe	Leu	Lys	Gln	
					245					250					255		
15	Phe	Gly	Leu	Glu	Lys	Glu	Ile	Lys	Leu	Asn	Ile	Glu	Ala	Asn	His	Ala	
				260					265					270			
20	Thr	Leu	Ala	Gly	His	Ser	Phe	His	His	Glu	Ile	Ala	Thr	Ala	Ile	Ala	
				275				280					285				
25	Leu	Gly	Leu	Phe	Gly	Ser	Val	Asp	Ala	Asn	Arg	Gly	Asp	Ala	Gln	Leu	
	290						295					300					
30	Gly	Trp	Asp	Thr	Asp	Gln	Phe	Pro	Asn	Ser	Val	Glu	Glu	Asn	Ala	Leu	
	305					310					315					320	
35	Val	Met	Tyr	Glu	Ile	Leu	Lys	Ala	Gly	Gly	Phe	Thr	Thr	Gly	Gly	Leu	
					325					330					335		
40	Asn	Phe	Asp	Ala	Lys	Val	Arg	Arg	Gln	Ser	Thr	Asp	Lys	Tyr	Asp	Leu	
				340					345					350			
45	Phe	Tyr	Gly	His	Ile	Gly	Ala	Met	Asp	Thr	Met	Ala	Leu	Ala	Leu	Lys	
			355					360					365				
50	Val	Ala	Ala	Arg	Met	Ile	Glu	Asp	Gly	Glu	Leu	Asp	Lys	Arg	Val	Ala	
		370					375					380					
55	Gln	Arg	Tyr	Ser	Gly	Trp	Asn	Ser	Glu	Leu	Gly	Gln	Gln	Ile	Leu	Lys	
	385					390					395					400	
60	Gly	Gln	Met	Ser	Leu	Ser	Asp	Leu	Ala	Lys	Tyr	Ala	Gln	Asp	His	Asn	
					405					410					415		
65	Leu	Ser	Pro	Val	His	Gln	Ser	Gly	His	Gln	Glu	Leu	Leu	Glu	Ser	Leu	
				420					425					430			
70	Val	Asn	His	Tyr	Leu	Phe	Asp	Lys									
			435					440									

<210> 17
<211> 391

<212> PRT

<213> *Shigella flexneri*

<400> 17

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Met Gln Lys Leu Ile Asn Ser Val Gln Asn Tyr Ala Trp Gly Ser Lys
1 5 10 15

5 Thr Ala Leu Thr Glu Leu Tyr Gly Met Glu Asn Pro Ser Ser Gln Pro
20 25 30

10 Met Ala Glu Leu Trp Met Gly Ala His Pro Lys Ser Ser Ser Arg Val
35 40 45

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

15 Asp Lys Ser Thr Leu Leu Gly Glu Ala Val Ala Lys Arg Phe Gly Glu
65 70 75 80

20 Leu Pro Phe Leu Phe Lys Val Leu Cys Ala Ala Gln Pro Leu Ser Ile
85 90 95

25 Gln Val His Pro Asn Lys His Asn Ser Glu Ile Gly Phe Ala Lys Glu
100 105 110

Asn Ala Ala Gly Ile Pro Met Asp Ala Ala Glu Arg Asn Tyr Lys Asp
115 120 125

30 Pro Asn His Lys Pro Glu Leu Val Phe Ala Leu Thr Pro Phe Leu Ala
130 135 140

35 Met Asn Ala Phe Arg Glu Phe Ser Glu Ile Val Ser Leu Leu Gln Pro
145 150 155 160

40 Val Ala Gly Ala His Pro Ala Ile Ala His Phe Leu Gln Gln Pro His
165 170 175

Ala Glu Arg Leu Ser Glu Leu Phe Ala Asn Leu Leu Asn Met Gln Gly
180 185 190

45 Glu Glu Lys Ser Arg Ala Leu Ala Ile Leu Lys Ser Ala Leu Asp Ser
195 200 205

50 Gln Gln Gly Glu Pro Trp Gln Thr Ile Arg Leu Ile Ser Glu Phe Tyr
210 215 220

Pro Glu Asp Ser Gly Leu Phe Ser Pro Leu Leu Leu Asn Val Val Lys

55

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	225		230		235		240
5	Leu Asn Pro Gly	Glu Ala Met Phe Leu Phe Ala Glu Thr Pro His Ala	245	250	255		
10	Tyr Leu Gln Gly Val Ala Leu Glu Val Met Ala Asn Ser Asp Asn Val	260	265	270			
15	Leu Arg Ala Gly Leu Thr Pro Lys Tyr Ile Asp Ile Pro Glu Leu Val	275	280	285			
20	Ala Asn Val Lys Phe Glu Ala Lys Pro Ala Asn Gln Leu Leu Thr Gln	290	295	300			
25	Pro Val Lys Gln Gly Ala Glu Leu Asp Phe Pro Ile Pro Val Asp Asp	305	310	315			
30	Phe Ala Phe Ser Leu His Asp Leu Ser Asp Lys Glu Thr Thr Ile Ser	325	330	335			
35	Gln Gln Ser Ala Ala Ile Leu Phe Cys Val Glu Gly Asp Ala Thr Leu	340	345	350			
40	Trp Lys Gly Ser Gln Gln Leu Gln Leu Lys Pro Gly Glu Ser Ala Phe	355	360	365			
45	Ile Ala Ala Asn Glu Ser Pro Val Thr Val Lys Gly His Gly Arg Leu	370	375	380			
50	Ala Arg Val Tyr Asn Lys Leu	385	390				

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<400> 18

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Met Gln Lys Leu Ile Asn Ser Val Gln Asn Tyr Ala Trp Gly Ser Lys
1 5 10 15

5 Thr Ala Leu Thr Glu Leu Tyr Gly Met Glu Asn Pro Ser Ser Gln Pro
20 25 30

10 Met Ala Glu Leu Trp Met Gly Ala His Pro Lys Ser Ser Ser Arg Val
35 40 45

Gln Asn Ala Ala Gly Asp Ile Val Ser Leu Arg Asp Val Ile Glu Ser

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	50	55	60
5	Asp Lys Ser Thr Leu Leu Gly Glu Ala Val Ala Lys Arg Phe Gly Glu 65 70 75 80		
10	Leu Pro Phe Leu Phe Lys Val Leu Cys Ala Ala Gln Pro Leu Ser Ile 85 90 95		
15	Gln Val His Pro Asn Lys Arg Asn Ser Glu Ile Gly Phe Ala Lys Glu 100 105 110		
20	Asn Ala Ala Gly Ile Pro Met Asp Ala Ala Glu Arg Asn Tyr Lys Asp 115 120 125		
25	Pro Asn His Lys Pro Glu Leu Val Phe Ala Leu Thr Pro Phe Leu Ala 130 135 140		
30	Met Asn Ala Phe Arg Glu Phe Ser Glu Ile Val Ser Leu Leu Gln Pro 145 150 155 160		
35	Val Ala Gly Ala His Pro Ala Ile Ala His Phe Leu Gln Gln Pro Asn 165 170 175		
40	Ala Glu Arg Leu Ser Glu Leu Phe Ala Ser Leu Leu Asn Met Gln Gly 180 185 190		
45	Glu Glu Lys Ser Arg Ala Leu Ala Ile Leu Lys Ser Ala Leu Asp Ser 195 200 205		
50	Gln Gln Gly Glu Pro Trp Gln Thr Ile Arg Leu Ile Ser Glu Phe Tyr 210 215 220		
55	Pro Glu Asp Ser Gly Leu Phe Ser Pro Leu Leu Leu Asn Val Val Lys 225 230 235 240		
	Leu Asn Pro Gly Glu Ala Met Phe Leu Phe Ala Glu Thr Pro His Ala 245 250 255		
	Tyr Leu Gln Gly Val Ala Leu Glu Val Met Ala Asn Ser Asp Asn Val 260 265 270		
	Leu Arg Ala Gly Leu Thr Pro Lys Tyr Ile Asp Ile Pro Glu Leu Val 275 280 285		
	Ala Asn Val Lys Phe Glu Ala Lys Pro Ala Asn Gln Leu Leu Thr Gln 290 295 300		

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	Pro	Val	Lys	Gln	Gly	Ala	Glu	Leu	Asp	Phe	Pro	Ile	Pro	Val	Asp	Asp	
	305					310					315					320	
5	Phe	Ala	Phe	Ser	Leu	His	Asp	Leu	Ser	Asp	Lys	Glu	Thr	Thr	Ile	Ser	
					325					330					335		
10	Gln	Gln	Ser	Ala	Ala	Ile	Leu	Phe	Cys	Val	Glu	Gly	Asp	Ala	Thr	Leu	
				340					345					350			
15	Trp	Lys	Gly	Ser	Gln	Gln	Leu	Gln	Leu	Lys	Pro	Gly	Glu	Ser	Ala	Phe	
			355					360					365				
20	Ile	Ala	Ala	Asn	Glu	Ser	Pro	Val	Thr	Val	Lys	Gly	His	Gly	Arg	Leu	
		370					375					380					
25	Ala	Arg	Val	Tyr	Asn	Lys	Leu										
	385					390											
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	<212> PRT																
	<213> Shigella sonnei																
	<400> 19																
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Met Gln Ala Tyr Phe Asp Gln Leu Asp Arg Val Arg Tyr Glu Gly Ser
1 5 10 15

5 Lys Ser Ser Asn Pro Leu Ala Phe Arg His Tyr Asn Pro Asp Glu Leu
20 25 30

10 Val Leu Gly Lys Arg Met Glu Glu His Leu Arg Phe Ala Ala Cys Tyr
35 40 45

15 Trp His Thr Phe Cys Trp Asn Gly Ala Asp Met Phe Gly Val Gly Ala
50 55 60

Phe Asn Arg Pro Trp Gln Gln Pro Gly Glu Ala Leu Ala Leu Ala Lys
65 70 75 80

20 Arg Lys Ala Asp Val Ala Phe Glu Phe Phe His Lys Leu His Val Pro
85 90 95

25 Phe Tyr Cys Phe His Asp Val Asp Val Ser Pro Glu Gly Ala Ser Leu
100 105 110

30 Lys Glu Tyr Ile Asn Asn Phe Ala Gln Met Val Asp Val Leu Ala Ala
115 120 125

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	Lys	Gln	Glu	Glu	Ser	Gly	Val	Lys	Leu	Leu	Trp	Gly	Thr	Ala	Asn	Cys	
	130						135					140					
5	Phe	Thr	Asn	Pro	Arg	Tyr	Gly	Ala	Gly	Ala	Ala	Thr	Asn	Pro	Asp	Pro	
	145					150					155					160	
	Glu	Val	Phe	Ser	Trp	Ala	Ala	Thr	Gln	Val	Val	Thr	Ala	Met	Glu	Ala	
10					165					170					175		
	Thr	His	Lys	Leu	Gly	Gly	Glu	Asn	Tyr	Val	Leu	Trp	Gly	Gly	Arg	Glu	
				180					185					190			
15	Gly	Tyr	Glu	Thr	Leu	Leu	Asn	Thr	Asp	Leu	Arg	Gln	Glu	Arg	Glu	Gln	
			195					200					205				
	Leu	Gly	Arg	Phe	Met	Gln	Met	Val	Val	Glu	His	Lys	His	Lys	Ile	Gly	
20		210					215					220					
	Phe	Gln	Gly	Thr	Leu	Leu	Ile	Glu	Pro	Lys	Pro	Gln	Glu	Pro	Thr	Lys	
25		225					230				235					240	
	His	Gln	Tyr	Asp	Tyr	Asp	Ala	Ala	Thr	Val	Tyr	Gly	Phe	Leu	Lys	Gln	
				245						250					255		
30	Phe	Gly	Leu	Glu	Lys	Glu	Ile	Lys	Leu	Asn	Ile	Glu	Ala	Asn	His	Ala	
			260						265					270			
	Thr	Leu	Ala	Gly	His	Ser	Phe	His	His	Glu	Ile	Ala	Thr	Ala	Ile	Ala	
35			275					280					285				
	Leu	Gly	Leu	Phe	Gly	Ser	Val	Asp	Ala	Asn	Arg	Gly	Asp	Ala	Gln	Leu	
40		290					295					300					
	Gly	Trp	Asp	Thr	Asp	Gln	Phe	Pro	Asn	Ser	Val	Glu	Glu	Asn	Ala	Leu	
	305					310					315					320	
	Val	Met	Tyr	Glu	Ile	Leu	Lys	Ala	Gly	Gly	Phe	Thr	Thr	Gly	Gly	Leu	
45					325					330					335		
	Asn	Phe	Asp	Ala	Lys	Val	Arg	Arg	Gln	Ser	Thr	Asp	Lys	Tyr	Asp	Leu	
50				340					345					350			
	Phe	Tyr	Gly	His	Ile	Gly	Ala	Met	Asp	Thr	Met	Ala	Leu	Ala	Leu	Lys	
			355					360					365				
55	Ile	Ala	Ala	Arg	Met	Ile	Glu	Asp	Gly	Glu	Leu	Asp	Lys	Arg	Ile	Ala	
	370						375					380					

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	Gln	Arg	Tyr	Ser	Gly	Trp	Asn	Ser	Glu	Leu	Gly	Gln	Gln	Ile	Leu	Lys	385	390	395	400
5	Gly	Gln	Met	Ser	Leu	Ala	Asp	Leu	Ala	Lys	Tyr	Ala	Gln	Glu	His	Asn	405	410	415	
10	Leu	Ser	Pro	Val	His	Gln	Ser	Gly	Arg	Gln	Glu	Gln	Leu	Glu	Asn	Leu	420	425	430	
15	Val	Asn	His	Tyr	Leu	Phe	Asp	Lys									435	440		
	<210> 20																			
	<211> 391																			
	<212> PRT																			
	<213> Shigella dysenteriae																			
20	<400> 20																			
25	Met	Gln	Lys	Leu	Ile	Asn	Ser	Val	Gln	Asn	Tyr	Ala	Trp	Gly	Ser	Lys	1	5	10	15
30	Thr	Ala	Leu	Thr	Glu	Leu	Tyr	Gly	Met	Glu	Asn	Pro	Ser	Ser	Gln	Pro	20	25	30	
35	Met	Ala	Glu	Leu	Trp	Met	Gly	Ala	His	Pro	Lys	Ser	Ser	Ser	Arg	Val	35	40	45	
40	Gln	Asn	Ala	Ala	Gly	Asp	Ile	Val	Ser	Leu	Arg	Asp	Val	Ile	Glu	Ser	50	55	60	
45	Asp	Lys	Ser	Thr	Leu	Leu	Gly	Glu	Ala	Val	Ala	Lys	Arg	Phe	Gly	Glu	65	70	75	80
50	Leu	Pro	Phe	Leu	Phe	Lys	Val	Leu	Cys	Ala	Ala	Gln	Pro	Leu	Ser	Ile	85	90	95	
55	Gln	Val	His	Pro	Asn	Lys	His	Asn	Ser	Glu	Ile	Gly	Phe	Ala	Lys	Glu	100	105	110	
60	Asn	Ala	Ala	Gly	Ile	Pro	Met	Asp	Ala	Ala	Glu	Arg	Asn	Tyr	Lys	Asp	115	120	125	
65	Pro	Asn	His	Lys	Pro	Glu	Leu	Val	Phe	Ala	Leu	Thr	Pro	Phe	Leu	Ala	130	135	140	
70	Met	Asn	Ala	Phe	Arg	Glu	Phe	Ser	Glu	Ile	Val	Ser	Leu	Leu	Gln	Pro	145	150	155	160

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	Val	Ala	Gly	Ala	His	Pro	Ala	Ile	Ala	His	Phe	Leu	Gln	Gln	Pro	His	
					165					170					175		
5	Ala	Glu	His	Leu	Ser	Glu	Leu	Phe	Ala	Ser	Leu	Leu	Asn	Met	Gln	Gly	
				180					185					190			
10	Glu	Glu	Lys	Ser	Arg	Ala	Leu	Ala	Ile	Leu	Lys	Ser	Thr	Leu	Asp	Ser	
			195					200					205				
15	Gln	Gln	Gly	Glu	Pro	Trp	Gln	Thr	Ile	Arg	Leu	Ile	Ser	Glu	Phe	Tyr	
		210					215					220					
20	Pro	Glu	Asp	Ser	Gly	Leu	Phe	Ser	Pro	Leu	Leu	Leu	Asn	Val	Val	Lys	
	225					230					235					240	
25	Leu	Asn	Pro	Gly	Glu	Ala	Met	Phe	Leu	Phe	Ala	Glu	Thr	Pro	His	Ala	
					245					250					255		
30	Tyr	Leu	Gln	Gly	Val	Ala	Leu	Glu	Val	Met	Ala	Asn	Ser	Asp	Asn	Val	
			260						265					270			
35	Leu	Arg	Ala	Gly	Leu	Thr	Pro	Lys	Tyr	Ile	Asp	Ile	Pro	Glu	Leu	Val	
			275					280					285				
40	Ala	Asn	Val	Lys	Phe	Glu	Ala	Lys	Pro	Ala	Asn	Gln	Leu	Leu	Thr	Gln	
		290					295					300					
45	Pro	Val	Lys	Gln	Gly	Ala	Glu	Leu	Asp	Phe	Pro	Ile	Pro	Val	Asp	Asp	
	305					310					315				320		
50	Phe	Ala	Phe	Ser	Leu	His	Asp	Leu	Ser	Asp	Lys	Glu	Thr	Thr	Ile	Ser	
					325					330					335		
55	Gln	Gln	Ser	Ala	Ala	Ile	Leu	Phe	Cys	Val	Glu	Gly	Asp	Ala	Thr	Leu	
				340					345					350			
60	Trp	Lys	Gly	Ser	Gln	Gln	Leu	Gln	Leu	Lys	Pro	Gly	Glu	Ser	Ala	Phe	
			355					360					365				
65	Ile	Ala	Ala	Asn	Glu	Ser	Pro	Val	Thr	Val	Lys	Gly	His	Gly	Arg	Leu	
		370					375					380					
70	Ala	Arg	Val	Tyr	Asn	Lys	Leu										
	385					390											

<210> 21
<211> 300

<212> PRT

<213> Shigella dysenteriae

<400> 21

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	Met	Arg	Ile	Gly	Ile	Asp	Leu	Gly	Gly	Thr	Lys	Thr	Glu	Val	Ile	Ala	
	1				5					10					15		
5	Leu	Gly	Asp	Ala	Gly	Glu	Gln	Leu	Tyr	Arg	His	Arg	Leu	Pro	Thr	Pro	
				20					25					30			
10	Arg	Asp	Asp	Tyr	Arg	Gln	Thr	Ile	Glu	Thr	Ile	Ala	Thr	Leu	Val	Asp	
			35					40					45				
15	Met	Ala	Glu	Gln	Ala	Thr	Gly	Gln	Arg	Gly	Thr	Val	Gly	Met	Gly	Ile	
		50					55					60					
20	Pro	Gly	Ala	Ile	Ser	Pro	Tyr	Thr	Gly	Val	Val	Lys	Asn	Ala	Asn	Ser	
	65					70					75					80	
25	Thr	Trp	Leu	Asn	Gly	Gln	Pro	Phe	Asp	Lys	Asp	Leu	Ser	Ala	Arg	Leu	
				85						90					95		
30	Gln	Arg	Glu	Val	Arg	Leu	Ala	Asn	Asp	Ala	Asn	Cys	Leu	Ala	Val	Ser	
				100					105					110			
35	Glu	Ala	Val	Asp	Gly	Ala	Ala	Ala	Gly	Ala	Gln	Thr	Val	Phe	Ala	Val	
			115					120					125				
40	Ile	Ile	Gly	Thr	Gly	Cys	Gly	Ala	Gly	Val	Ala	Phe	Asn	Gly	Arg	Ala	
		130					135					140					
45	His	Ile	Gly	Gly	Asn	Gly	Thr	Ala	Gly	Glu	Trp	Gly	His	Asn	Pro	Leu	
	145					150					155					160	
50	Pro	Trp	Met	Asp	Glu	Asp	Glu	Leu	Arg	Tyr	Arg	Glu	Glu	Val	Pro	Cys	
				165						170					175		
55	Tyr	Cys	Gly	Lys	Gln	Gly	Cys	Ile	Glu	Thr	Phe	Ile	Ser	Gly	Thr	Gly	
				180					185					190			
60	Phe	Ala	Thr	Asp	Tyr	Arg	Arg	Leu	Ser	Gly	His	Ala	Leu	Lys	Gly	Ser	
			195					200					205				
65	Glu	Ile	Ile	Ser	Leu	Val	Glu	Glu	Ser	Asp	Pro	Val	Ala	Glu	Leu	Ala	
		210					215					220					
70	Leu	Arg	Arg	Tyr	Glu	Leu	Arg	Leu	Ala	Lys	Ser	Leu	Ala	His	Val	Val	
	225					230					235					240	

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Asn Ile Leu Asp Pro Asp Val Ile Val Leu Gly Gly Gly Met Ser Asn
245 250 255

5 Val Asp Arg Leu Tyr Gln Thr Val Pro Gln Leu Ile Lys Gln Phe Val
260 265 270

10 Phe Gly Gly Glu Cys Glu Thr Pro Val Arg Lys Ala Lys His Gly Asp
275 280 285

15 Ser Ser Gly Val Arg Gly Ala Ala Trp Leu Trp Pro
290 295 300

<210> 22
<211> 440
<212> PRT
<213> Shigella dysenteriae

20 <400> 22

25 Met Gln Ala Tyr Phe Asp Gln Leu Asp Arg Val Arg Tyr Glu Gly Ser
1 5 10 15

Lys Ser Ser Asn Pro Leu Ala Phe Arg His Tyr Asn Pro Asp Glu Leu
20 25 30

30 Val Leu Gly Lys Arg Met Glu Glu His Leu Arg Phe Ala Ala Cys Tyr
35 40 45

35 Trp His Thr Phe Cys Trp Asn Gly Ala Asp Met Phe Gly Val Gly Ala
50 55 60

40 Phe Asn Arg Pro Trp Gln Gln Pro Gly Glu Ala Leu Ala Leu Ala Lys
65 70 75 80

Arg Lys Ala Asp Val Ala Phe Glu Phe Phe His Lys Leu His Val Pro
85 90 95

45 Phe Tyr Cys Phe His Asp Val Asp Val Ser Pro Glu Gly Ala Ser Leu
100 105 110

50 Lys Glu Tyr Ile Asn Asn Phe Ala Gln Met Val Asp Val Leu Ala Gly
115 120 125

Lys Gln Glu Glu Ser Gly Val Lys Leu Leu Trp Gly Thr Ala Asn Cys
130 135 140

55 Phe Thr Asn Pro Arg Tyr Gly Ala Gly Ala Ala Thr Asn Pro Asp Pro
145 150 155 160

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	Glu	Val	Phe	Ser	Trp	Ala	Ala	Thr	Gln	Val	Val	Thr	Ala	Met	Glu	Ala	
					165					170					175		
5	Thr	His	Lys	Leu	Gly	Gly	Glu	Asn	Tyr	Val	Leu	Trp	Gly	Gly	Arg	Glu	
				180				185						190			
	Gly	Tyr	Glu	Thr	Leu	Leu	Asn	Thr	Asp	Leu	Arg	Gln	Glu	Arg	Glu	Gln	
10			195					200					205				
	Leu	Gly	Arg	Phe	Met	Gln	Met	Val	Val	Glu	His	Lys	His	Lys	Ile	Gly	
		210					215					220					
15																	
	Phe	Gln	Gly	Thr	Leu	Leu	Ile	Glu	Pro	Lys	Pro	Gln	Glu	Pro	Thr	Lys	
	225					230					235					240	
20	His	Gln	Tyr	Asp	Tyr	Asp	Ala	Ala	Thr	Val	Tyr	Gly	Phe	Leu	Lys	Gln	
				245						250					255		
	Phe	Gly	Leu	Glu	Lys	Glu	Ile	Lys	Leu	Asn	Ile	Glu	Ala	Asn	His	Ala	
25				260					265					270			
	Thr	Leu	Ala	Gly	His	Ser	Phe	His	His	Glu	Ile	Ala	Thr	Ala	Ile	Ala	
			275					280					285				
30																	
	Leu	Gly	Leu	Phe	Gly	Ser	Val	Asp	Ala	Asn	Arg	Gly	Asp	Ala	Gln	Leu	
		290					295					300					
35	Gly	Trp	Asp	Thr	Asp	Gln	Phe	Pro	Asn	Ser	Val	Glu	Glu	Asn	Ala	Leu	
	305					310					315					320	
	Val	Met	Tyr	Glu	Ile	Leu	Lys	Ala	Gly	Gly	Phe	Thr	Thr	Gly	Gly	Leu	
40					325					330					335		
	Asn	Phe	Asp	Ala	Lys	Val	Arg	Arg	Gln	Ser	Thr	Asp	Lys	Tyr	Asp	Leu	
				340					345					350			
45																	
	Phe	Tyr	Gly	His	Ile	Gly	Ala	Met	Asp	Thr	Met	Ala	Leu	Ala	Leu	Lys	
			355					360					365				
	Ile	Ala	Ala	Cys	Met	Ile	Glu	Asp	Gly	Glu	Leu	Asp	Lys	Arg	Ile	Ala	
50		370					375					380					
	Gln	Arg	Tyr	Ser	Gly	Trp	Asn	Ser	Glu	Leu	Gly	Gln	His	Ile	Leu	Lys	
	385					390					395					400	
55																	
	Gly	Gln	Met	Ser	Leu	Ala	Asp	Leu	Ala	Lys	Tyr	Ala	Gln	Glu	His	Asn	

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415

5 Leu Ser Pro Val His Gln Ser Gly Arg Gln Glu Gln Leu Glu Asn Leu
 420 425 430

10 Val Asn His Tyr Leu Phe Asp Lys
 435 440

<210> 23

<211> 350

<212> PRT

<213> Citrobacter koseri

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<400> 23

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EP 2 593 540 B1

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

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	180	185	190
5	Asp Glu Lys Ser Arg Ala Leu Ala Val Leu Lys Ala Ala Leu Asn Ser		
	195	200	205
10	Gln Gln Gly Glu Pro Trp Gln Thr Ile Arg Val Ile Ser Glu Phe Tyr		
	210	215	220
15	Pro Asp Asp Ser Gly Leu Phe Ser Pro Leu Leu Leu Asn Val Val Lys		
	225	230	235
20	Leu Asn Pro Gly Glu Ala Met Phe Leu Phe Ala Glu Thr Pro His Ala		
	245	250	255
25	Tyr Leu Gln Gly Val Ala Leu Glu Val Met Ala Asn Ser Asp Asn Val		
	260	265	270
30	Leu Arg Ala Gly Leu Thr Pro Lys Tyr Ile Asp Ile Pro Glu Leu Val		
	275	280	285
35	Ala Asn Val Lys Phe Val Ala Lys Pro Ala Asp Gln Leu Leu Thr Thr		
	290	295	300
40	Pro Val Lys His Gly Thr Glu Leu Asp Phe Pro Ile Pro Val Glu Asp		
	305	310	315
45	Phe Ala Phe Ser Leu His Asp Leu Ser Ala Gln Glu Thr Ala Ile Ser		
	325	330	335
50	Gln Gln Ser Ala Ala Ile Leu Phe Cys Val Glu Gly Glu Ala		
	340	345	350
55	<210> 24 <211> 444 <212> PRT <213> Citrobacter koseri <400> 24		

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Met Glu Phe Ile Met Gln Ala Tyr Phe Asp Gln Leu Asp Arg Val Arg
1 5 10 15

5 Tyr Glu Gly Pro Lys Ser Thr Asn Pro Leu Ala Phe Arg His Tyr Asn
20 25 30

10 Pro Asp Glu Ile Val Leu Gly Lys Arg Met Glu Asp His Leu Arg Phe
35 40 45

Ala Ala Cys Tyr Trp His Thr Phe Cys Trp Asn Gly Ala Asp Met Phe

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	50		55		60
5	Gly Val Gly Ala Phe Asn Arg Pro Trp Gln Gln Pro Gly Asp Ala Leu	65	70	75	80
10	Glu Leu Ala Lys Arg Lys Ala Asp Val Ala Phe Glu Phe Phe His Lys	85	90	95	
15	Leu Asn Val Pro Tyr Tyr Cys Phe His Asp Val Asp Val Ser Pro Glu	100	105	110	
20	Gly Ala Ser Leu Lys Glu Tyr Lys Asn Asn Phe Ala Gln Met Val Asp	115	120	125	
25	Val Leu Glu Ser Lys Gln Glu Gln Ser Gly Val Lys Leu Leu Trp Gly	130	135	140	
30	Thr Ala Asn Cys Phe Thr Asn Pro Arg Tyr Gly Ala Gly Ala Ala Thr	145	150	155	160
35	Asn Pro Asp Pro Glu Val Phe Ser Trp Ala Ala Thr Gln Val Val Asn	165	170	175	
40	Ala Met Asn Ala Thr His Lys Leu Gly Gly Glu Asn Tyr Val Leu Trp	180	185	190	
45	Gly Gly Arg Glu Gly Tyr Glu Thr Leu Leu Asn Thr Asp Leu Arg Gln	195	200	205	
50	Glu Arg Glu Gln Ile Gly Arg Phe Met Gln Met Val Val Glu His Lys	210	215	220	
55	His Lys Thr Gly Phe Gln Gly Thr Leu Leu Ile Glu Pro Lys Pro Gln	225	230	235	240
	Glu Pro Thr Lys His Gln Tyr Asp Tyr Asp Val Ala Thr Val Tyr Gly	245	250	255	
	Phe Leu Lys Gln Phe Gly Leu Glu Lys Glu Ile Lys Val Asn Ile Glu	260	265	270	
	Ala Asn His Ala Thr Leu Ala Gly His Ser Phe His His Glu Ile Ala	275	280	285	
	Ser Ala Ile Ala Leu Gly Ile Phe Gly Ser Val Asp Ala Asn Arg Gly	290	295	300	

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Asp Ala Gln Leu Gly Trp Asp Thr Asp Gln Phe Pro Val Ser Val Glu
305 310 315 320

5 Glu Asn Ala Leu Val Met Tyr Glu Ile Leu Lys Ala Gly Gly Phe Thr
325 330 335

10 Thr Gly Gly Leu Asn Phe Asp Ala Lys Val Arg Arg Gln Ser Thr Asp
340 345 350

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

15 Leu Ser Leu Lys Val Ala Ala Arg Met Ile Glu Asp Gly Glu Leu Asp
370 375 380

20 Lys Arg Val Ala Lys Arg Tyr Ser Gly Trp Asn Ser Glu Leu Gly Gln
385 390 395 400

25 Gln Ile Leu Lys Gly Gln Leu Ser Leu Ser Asp Val Ala His Tyr Ala
405 410 415

Glu Gln Arg Asn Leu Ala Pro Val His Gln Ser Gly His Gln Glu Leu
420 425 430

30 Leu Glu Asn Leu Val Asn His Tyr Leu Phe Asp Lys
435 440

<210> 25
35 <211> 391
<212> PRT
<213> Citrobacter rodentium

<400> 25

40 Met Gln Lys Leu Ile Asn Ser Val Gln Asn Tyr Ala Trp Gly Ser Lys
1 5 10 15

45 Thr Ala Leu Thr Glu Leu Tyr Gly Ile Ala Asn Pro Gln Gln Leu Pro
20 25 30

Met Ala Glu Leu Trp Met Gly Ala His Pro Lys Ser Ser Ser Gln Val
35 40 45

50 Val Asp Ala Ser Gly Gln Arg Val Ser Leu Arg Asp Val Ile Glu Ala
50 55 60

55 Asp Lys Ser Arg Leu Leu Gly Asp Ala Val Ala Arg Arg Phe Gly Glu
65 70 75 80

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	Leu	Pro	Phe	Leu	Phe	Lys	Val	Leu	Cys	Ala	Ala	Gln	Pro	Leu	Ser	Ile	
					85					90					95		
5	Gln	Val	His	Pro	Asn	Lys	Arg	Asn	Ser	Glu	Leu	Gly	Phe	Ala	Lys	Glu	
				100					105					110			
	Asn	Val	Ala	Gly	Ile	Pro	Met	Asp	Ala	Ala	Glu	Arg	Asn	Tyr	Lys	Asp	
10			115					120					125				
	Pro	Asn	His	Lys	Pro	Glu	Leu	Val	Phe	Ala	Leu	Thr	Pro	Phe	Leu	Ala	
		130					135					140					
15	Met	Asn	Ala	Phe	Arg	Glu	Phe	Ser	Glu	Ile	Val	Ser	Leu	Leu	Gln	Pro	
	145					150					155					160	
	Val	Ala	Gly	Ala	His	Thr	Ala	Ile	Ala	His	Phe	Leu	Glu	Ala	Pro	Asp	
20					165				170						175		
	Ala	Glu	Arg	Leu	Ser	Ala	Leu	Phe	Ala	Ala	Leu	Leu	Asn	Met	Gln	Gly	
25				180					185					190			
	Glu	Glu	Lys	Ser	Arg	Ala	Leu	Ala	Ile	Leu	Lys	Ala	Ala	Leu	Glu	Thr	
			195					200					205				
30	Gln	Ser	Gly	Glu	Pro	Trp	Gln	Thr	Ile	Arg	Leu	Ile	Gly	Glu	Phe	Tyr	
		210					215					220					
	Pro	Asp	Asp	Ser	Gly	Leu	Phe	Ser	Pro	Leu	Leu	Leu	Asn	Val	Val	Lys	
35	225					230					235					240	
	Leu	Asn	Pro	Gly	Glu	Ala	Met	Phe	Leu	Phe	Ala	Glu	Thr	Pro	His	Ala	
				245						250					255		
40	Tyr	Leu	Gln	Gly	Val	Ala	Leu	Glu	Val	Met	Ala	Asn	Ser	Asp	Asn	Val	
			260					265						270			
	Leu	Arg	Ala	Gly	Leu	Thr	Pro	Lys	Tyr	Ile	Asp	Ile	Pro	Glu	Leu	Val	
45			275					280					285				
	Ala	Asn	Val	Lys	Phe	Glu	Ala	Lys	Pro	Ala	Asp	Arg	Leu	Leu	Thr	Ala	
50		290					295					300					
	Pro	Leu	Thr	Ser	Gly	Ala	Glu	Gln	Asp	Phe	Pro	Ile	Pro	Val	Asp	Asp	
	305					310					315					320	
55	Phe	Ala	Phe	Ser	Leu	His	Asp	Leu	Ser	Ala	Gln	Gln	Ala	Glu	Ile	Ala	
					325					330					335		

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	Gln	Gln	Ser	Ala	Ala	Ile	Leu	Phe	Cys	Val	Glu	Gly	Glu	Ala	Thr	Leu
				340					345					350		
5	Arg	Lys	Gly	Glu	Gln	Ser	Leu	Thr	Leu	Lys	Pro	Gly	Glu	Ser	Ala	Phe
			355					360					365			
10	Ile	Ser	Ala	Ala	Glu	Ser	Pro	Val	Ser	Val	Ser	Gly	Ala	Gly	Arg	Leu
		370					375					380				
15	Ala	Arg	Val	Tyr	Asn	Lys	Leu									
	385					390										
	<210> 26															
	<211> 302															
	<212> PRT															
	<213> Citrobacter rodentium															
20	<400> 26															
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30																
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45																
50																
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EP 2 593 540 B1

Met Arg Ile Gly Ile Asp Leu Gly Gly Thr Lys Thr Glu Val Ile Ala
1 5 10 15

Leu Gly Glu Ser Gly Glu Gln Leu Phe Arg His Arg Leu Pro Thr Pro
20 25 30

Arg Asn Asp Tyr Arg Gln Thr Ile Glu Thr Ile Ala Thr Leu Val Glu
35 40 45

Met Ala Glu Lys Ala Thr Gly Gln Thr Gly Thr Val Gly Met Gly Ile
50 55 60

Pro Gly Ser Leu Ser Pro Tyr Thr Gly Val Val Lys Asn Ala Asn Ser
65 70 75 80

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

Gln Arg Glu Val Arg Leu Ala Asn Asp Ala Asn Cys Leu Ala Val Ser
100 105 110

Glu Ala Val Asp Gly Ala Ala Ala Gly Ala Gln Thr Val Phe Ala Val
115 120 125

Ile Ile Gly Thr Gly Cys Gly Ala Gly Val Ala Leu Asn Gly Arg Ala
130 135 140

His Ala Gly Gly Asn Gly Thr Ala Gly Glu Trp Gly His Asn Pro Leu
145 150 155 160

5 Pro Trp Met Asp Asp Asp Glu Leu Arg Tyr Arg Glu Glu Val Pro Cys
 165 170 175
 Tyr Cys Gly Lys Gln Gly Cys Ile Glu Thr Phe Ile Ser Gly Thr Gly
 180 185 190
 10 Phe Ala Thr Asp Tyr Gln Arg Leu Ser Gly Asn Ser Leu Thr Gly Asn
 195 200 205
 15 Glu Ile Met Arg Arg Val Glu Glu Gln Asp Pro Leu Ala Glu Leu Ala
 210 215 220
 20 Leu Gly Arg Tyr Glu Leu Arg Leu Ala Lys Ser Leu Ala His Val Val
 225 230 235 240
 Asn Ile Leu Asp Pro Asp Val Ile Val Leu Gly Gly Gly Met Ser Asn
 245 250 255
 25 Val Asp Arg Leu Tyr Asn Thr Leu Pro Ala Leu Ile Gly Gln Phe Val
 260 265 270
 30 Phe Gly Gly Glu Cys Glu Thr Pro Val Arg Lys Ala Leu His Gly Asp
 275 280 285
 Ser Ser Gly Val Arg Gly Ala Ala Trp Leu Trp Pro Gln Arg
 290 295 300
 35 <210> 27
 <211> 440
 <212> PRT
 <213> Citrobacter rodentium
 40 <400> 27
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EP 2 593 540 B1

Met Gln Ala Tyr Phe Asp Gln Leu Asp Arg Val Arg Phe Glu Gly Pro
1 5 10 15

5 Lys Ser Thr Asn Pro Leu Ala Phe Arg His Tyr Asn Pro Asp Glu Ile
20 25 30

10 Val Leu Gly Lys Arg Met Glu Asp His Leu Arg Phe Ala Ala Cys Tyr
35 40 45

Trp His Thr Phe Cys Trp Asn Gly Ala Asp Met Phe Gly Val Gly Ala
50 55 60

15 Phe Asp Arg Pro Trp Gln Gln Pro Gly Glu Ala Leu Ala Leu Ala Lys
65 70 75 80

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EP 2 593 540 B1

	Arg	Lys	Ala	Asp	Val	Ala	Phe	Glu	Phe	Phe	His	Lys	Leu	Asn	Val	Pro	
					85					90					95		
5	Phe	Tyr	Cys	Phe	His	Asp	Val	Asp	Val	Ser	Pro	Glu	Gly	Ala	Ser	Leu	
				100					105					110			
10	Lys	Glu	Tyr	Lys	Asn	Asn	Phe	Ala	Gln	Met	Val	Asp	Val	Leu	Ala	Ala	
			115					120					125				
15	Lys	Gln	Glu	Glu	Ser	Gly	Val	Lys	Leu	Leu	Trp	Gly	Thr	Ala	Asn	Cys	
		130					135					140					
20	Phe	Thr	Asn	Pro	Arg	Tyr	Gly	Ala	Gly	Ala	Ala	Thr	Asn	Pro	Asp	Pro	
	145					150					155					160	
25	Glu	Val	Phe	Ser	Trp	Ala	Ala	Thr	Gln	Val	Val	Thr	Ala	Met	Asp	Ala	
					165					170					175		
30	Thr	His	Lys	Leu	Gly	Gly	Glu	Asn	Tyr	Val	Leu	Trp	Gly	Gly	Arg	Glu	
				180					185					190			
35	Gly	Tyr	Glu	Thr	Leu	Leu	Asn	Thr	Asp	Leu	Arg	Gln	Glu	Arg	Glu	Gln	
			195					200					205				
40	Ile	Gly	Arg	Phe	Met	Gln	Met	Val	Val	Glu	His	Lys	His	Lys	Thr	Gly	
	210						215					220					
45	Phe	Gln	Gly	Thr	Leu	Leu	Ile	Glu	Pro	Lys	Pro	Gln	Glu	Pro	Thr	Lys	
	225					230					235					240	
50	His	Gln	Tyr	Asp	Tyr	Asp	Ala	Ala	Thr	Val	Tyr	Gly	Phe	Leu	Lys	Gln	
					245					250					255		
55	Phe	Gly	Leu	Glu	Lys	Glu	Ile	Lys	Leu	Asn	Ile	Glu	Ala	Asn	His	Ala	
				260					265					270			
60	Thr	Leu	Ala	Gly	His	Ser	Phe	His	His	Glu	Ile	Ala	Thr	Ala	Ile	Ala	
				275				280					285				
65	Leu	Gly	Leu	Phe	Gly	Ser	Val	Asp	Ala	Asn	Arg	Gly	Asp	Ala	Gln	Leu	
		290					295					300					
70	Gly	Trp	Asp	Thr	Asp	Gln	Phe	Pro	Asn	Ser	Val	Glu	Glu	Asn	Ala	Leu	
	305					310					315					320	
75	Val	Met	Tyr	Glu	Ile	Leu	Lys	Ala	Gly	Gly	Phe	Thr	Thr	Gly	Gly	Leu	
					325					330					335		

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Asn Phe Asp Ala Lys Val Arg Arg Gln Ser Thr Asp Lys Tyr Asp Leu
 340 345 350
 5
 Phe Tyr Gly His Ile Gly Ala Met Asp Thr Met Ala Leu Ser Leu Lys
 355 360 365
 10
 Ile Ala Ala Arg Met Ile Glu Asp Gly Glu Leu Asp Lys Arg Val Ala
 370 375 380
 15
 Lys Arg Tyr Ala Gly Trp Asn Ser Glu Leu Gly Gln Gln Ile Leu Lys
 385 390 395 400
 Gly Gln Met Ser Leu Ser Glu Leu Ala Gln Tyr Ala Glu Gln His Asn
 405 410 415
 20
 Leu Ala Pro Val His Gln Ser Gly His Gln Glu Leu Leu Glu Asn Leu
 420 425 430
 25
 Val Asn His Tyr Leu Phe Asn Lys
 435 440

<210> 28

<211> 416

30 <212> PRT

<213> Citrobacter youngae

<400> 28

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	Met	Cys	Arg	Leu	Leu	Pro	Leu	Gly	Phe	Leu	Gly	Leu	Ile	Ala	Ala	Glu	
	1				5					10					15		
5	Asn	Ser	Thr	Leu	Lys	Gln	Gly	Leu	Arg	Met	Gln	Lys	Leu	Ile	Asn	Ser	
				20					25					30			
	Val	Gln	Asn	Tyr	Ala	Trp	Gly	Ser	Lys	Thr	Ala	Leu	Thr	Glu	Leu	Tyr	
10			35					40					45				
	Gly	Met	Ala	Asn	Pro	Ala	Gln	Gln	Pro	Met	Ala	Glu	Leu	Trp	Met	Gly	
		50					55					60					
15																	
	Ala	His	Pro	Lys	Ser	Ser	Ser	Gln	Val	Leu	Gly	Ala	Asp	Gly	Gln	Thr	
	65					70					75					80	
20																	
	Ile	Ala	Leu	Arg	Asp	Val	Ile	Glu	Arg	Asp	Lys	Ser	Ala	Leu	Leu	Gly	
					85					90					95		
	Glu	Ala	Val	Ala	Lys	Arg	Phe	Gly	Glu	Leu	Pro	Phe	Leu	Phe	Lys	Val	
25				100					105					110			
30																	
35																	
40																	
45																	
50																	
55																	

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5 Leu Cys Ala Ala Gln Pro Leu Ser Ile Gln Val His Pro Asn Lys His
115 120 125

Asn Ser Glu Ile Gly Phe Ala Lys Glu Asn Ala Ala Gly Ile Pro Met
130 135 140

10 Asp Ala Ala Glu Arg Asn Tyr Lys Asp Pro Asn His Lys Pro Glu Leu
145 150 155 160

15 Val Phe Ala Leu Thr Pro Phe Leu Ala Met Asn Ala Phe Arg Glu Phe
165 170 175

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

20 Ile Ala His Phe Leu Gln Glu Pro Cys Ala Glu Arg Leu Ser Tyr Leu
195 200 205

25 Phe Ala Ser Leu Leu Asn Met Gln Asp Glu Glu Lys Ser His Ala Leu
210 215 220

30 Ala Ile Leu Lys Ser Ala Leu Asn Ser Gln Gln Gly Glu Pro Trp Gln
225 230 235 240

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

35 Ser Pro Leu Leu Leu Asn Val Val Lys Leu Asn Pro Gly Glu Ala Met
260 265 270

40 Phe Leu Phe Ala Glu Thr Pro His Ala Tyr Leu Gln Gly Val Ala Leu
275 280 285

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

45 Lys Tyr Ile Asp Ile Pro Glu Leu Val Ala Asn Val Lys Phe Val Ala
305 310 315 320

50 Lys Pro Ala Asn Gln Leu Leu Thr Thr Pro Ile Lys Thr Ala Gly Glu
325 330 335

Leu Asp Phe Pro Ile Pro Val Asp Asp Phe Ala Phe Ser Leu His Glu
340 345 350

55 Leu Thr Ala Asp Gly Ala Asp Ile Ser Gln Gln Ser Ala Ala Ile Leu

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		355				360				365						
5	Phe	Cys	Ile	Glu	Gly	Gln	Ala	Val	Leu	Ser	Lys	Gly	Glu	Gln	Arg	Leu
	370					375						380				
10	Val	Leu	Gln	Pro	Gly	Glu	Ser	Ala	Phe	Ile	Ser	Ala	Asn	Glu	Ser	Pro
	385					390					395					400
15	Val	Asn	Val	Ser	Gly	Thr	Gly	Arg	Leu	Ala	Arg	Val	Tyr	Asn	Lys	Leu
					405					410					415	
20	<210> 29															
	<211> 444															
	<212> PRT															
	<213> Citrobacter youngae															
25	<400> 29															
30																
35																
40																
45																
50																
55																

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Met Glu Leu Ile Met Gln Ala Tyr Phe Asp Gln Leu Asp Arg Val Arg
1 5 10 15

5 Phe Glu Gly Thr Lys Ser Thr Asn Pro Leu Ala Phe Arg His Tyr Asn
20 25 30

10 Pro Asp Glu Ile Val Leu Gly Lys Arg Met Glu Asp His Leu Arg Phe
35 40 45

15 Ala Ala Cys Tyr Trp His Thr Phe Cys Trp Asn Gly Ala Asp Met Phe
50 55 60

Gly Met Gly Ala Phe Asp Arg Pro Trp Gln Gln Pro Gly Glu Ala Leu
65 70 75 80

20 Ala Leu Ala Lys Arg Lys Ala Asp Val Ala Phe Glu Phe Phe His Lys
85 90 95

25 Leu Asn Val Pro Tyr Tyr Cys Phe His Asp Val Asp Val Ser Pro Glu
100 105 110

Gly Ala Ser Leu Lys Glu Tyr Lys Asn Asn Phe Ala Gln Met Val Asp
115 120 125

30 Val Leu Ala Ala Lys Gln Glu Gln Ser Gly Val Lys Leu Leu Trp Gly
130 135 140

35 Thr Ala Asn Cys Phe Thr Asn Pro Arg Tyr Gly Ala Gly Ala Ala Thr
145 150 155 160

40 Asn Pro Asp Pro Glu Val Phe Ser Trp Ala Ala Thr Gln Val Val Thr

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	165	170	175
5	Ala Met Asp Ala Thr His Lys Leu Gly Gly Glu Asn Tyr Val Leu Trp 180 185 190		
10	Gly Gly Arg Glu Gly Tyr Glu Thr Leu Leu Asn Thr Asp Leu Arg Gln 195 200 205		
15	Glu Arg Glu Gln Ile Gly Arg Phe Met Gln Leu Val Val Glu His Lys 210 215 220		
20	His Lys Ile Gly Phe Gln Gly Thr Leu Leu Ile Glu Pro Lys Pro Gln 225 230 235 240		
25	Glu Pro Thr Lys His Gln Tyr Asp Tyr Asp Ala Ala Thr Val Tyr Gly 245 250 255		
30	Phe Leu Lys Gln Phe Gly Leu Glu Lys Glu Ile Lys Leu Asn Ile Glu 260 265 270		
35	Ala Asn His Ala Thr Leu Ala Gly His Ser Phe His His Glu Ile Ala 275 280 285		
40	Thr Ala Ile Ala Leu Gly Leu Phe Gly Ser Val Asp Ala Asn Arg Gly 290 295 300		
45	Asp Ala Gln Leu Gly Trp Asp Thr Asp Gln Phe Pro Asn Ser Val Glu 305 310 315 320		
50	Glu Asn Ala Leu Val Met Tyr Glu Ile Leu Lys Ala Gly Gly Phe Thr 325 330 335		
55	Thr Gly Gly Leu Asn Phe Asp Ala Lys Val Arg Arg Gln Ser Thr Asp 340 345 350		
60	Lys Tyr Asp Leu Phe Tyr Gly His Ile Gly Ala Met Asp Thr Met Ala 355 360 365		
65	Leu Ser Leu Lys Ile Ala Ala Arg Met Ile Glu Asp Gly Gly Leu Asp 370 375 380		
70	Gln Arg Val Ala Lys Arg Tyr Ala Gly Trp Asn Gly Glu Leu Gly Gln 385 390 395 400		
75	Gln Ile Leu Lys Gly Gln Met Thr Leu Thr Glu Ile Ala Gln Tyr Ala 405 410 415		

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Glu Gln His Asn Leu Ala Pro Val His Gln Ser Gly His Gln Glu Gln
 420 425 430

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Leu Glu Asn Leu Val Asn His Tyr Leu Phe Asp Lys
 435 440

<210> 30

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<211> . 391

<212> PRT

<213> Salmonella enterica

<400> 30

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

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	Glu	Glu	Lys	Ser	Arg	Ala	Leu	Ala	Val	Leu	Lys	Ala	Ala	Leu	Asn	Ser	
			195					200					205				
5	Gln	Gln	Gly	Glu	Pro	Trp	Gln	Thr	Ile	Arg	Val	Ile	Ser	Glu	Tyr	Tyr	
		210					215					220					
10	Pro	Asp	Asp	Ser	Gly	Leu	Phe	Ser	Pro	Leu	Leu	Leu	Asn	Val	Val	Lys	
	225					230					235					240	
	Leu	Asn	Pro	Gly	Glu	Ala	Met	Phe	Leu	Phe	Ala	Glu	Thr	Pro	His	Ala	
					245					250					255		
15	Tyr	Leu	Gln	Gly	Val	Ala	Leu	Glu	Val	Met	Ala	Asn	Ser	Asp	Asn	Val	
				260					265					270			
20	Leu	Arg	Ala	Gly	Leu	Thr	Pro	Lys	Tyr	Ile	Asp	Ile	Pro	Glu	Leu	Val	
			275					280					285				
25	Ala	Asn	Val	Lys	Phe	Glu	Pro	Lys	Pro	Ala	Ser	Glu	Leu	Leu	Thr	Ala	
		290					295					300					
30	Pro	Val	Lys	Ser	Gly	Ala	Glu	Leu	Asp	Phe	Pro	Ile	Pro	Val	Asp	Asp	
	305					310					315					320	
	Phe	Ala	Phe	Ser	Leu	His	Asp	Leu	Ala	Leu	Gln	Glu	Thr	Ser	Ile	Gly	
					325					330					335		
35	Gln	His	Ser	Ala	Ala	Ile	Leu	Phe	Cys	Val	Glu	Gly	Glu	Ala	Val	Leu	
				340					345					350			
40	Arg	Lys	Asp	Glu	Gln	Arg	Leu	Val	Leu	Lys	Pro	Gly	Glu	Ser	Ala	Phe	
			355					360					365				
	Ile	Gly	Ala	Asp	Glu	Ser	Pro	Val	Asn	Ala	Ser	Gly	Thr	Gly	Arg	Leu	
		370					375					380					
45	Ala	Arg	Val	Tyr	Asn	Lys	Leu										
	385					390											
50	<210>	31															
	<211>	440															
	<212>	PRT															
	<213>	Salmonella enterica															
55	<400>	31															
	Met	Gln	Ala	Tyr	Phe	Asp	Gln	Leu	Asp	Arg	Val	Arg	Tyr	Glu	Gly	Pro	
	1				5					10					15		

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Gln Ser Thr Asn Pro Leu Ala Phe Arg His Tyr Asn Pro Asp Glu Leu
 20 25 30

5 Val Leu Gly Lys Arg Met Glu Asp His Leu Arg Phe Ala Ala Cys Tyr
 35 40 45

10 Trp His Thr Phe Cys Trp Asn Gly Ala Asp Met Phe Gly Val Gly Ala
 50 55 60

Phe Asn Arg Pro Trp Gln Gln Pro Gly Glu Ala Leu Glu Leu Ala Lys
 65 70 75 80

15 Arg Lys Ala Asp Val Ala Phe Glu Phe Phe His Lys Leu Asn Val Pro
 85 90 95

20 Phe Tyr Cys Phe His Asp Val Asp Val Ser Pro Glu Gly Ala Ser Leu
 100 105 110

25 Lys Glu Tyr Lys Asn Asn Phe Ala Gln Met Val Asp Val Leu Ala Ala
 115 120 125

Lys Gln Glu Gln Ser Gly Val Lys Leu Leu Trp Gly Thr Ala Asn Cys
 130 135 140

30 Phe Thr Asn Pro Arg Tyr Gly Ala Gly Ala Ala Thr Asn Pro Asp Pro
 145 150 155 160

35 Glu Val Phe Ser Trp Ala Ala Thr Gln Val Val Thr Ala Met Asn Ala
 165 170 175

Thr His Lys Leu Gly Gly Glu Asn Tyr Val Leu Trp Gly Gly Arg Glu
 180 185 190

40 Gly Tyr Glu Thr Leu Leu Asn Thr Asp Leu Arg Gln Glu Arg Glu Gln
 195 200 205

45 Ile Gly Arg Phe Met Gln Met Val Val Glu His Lys His Lys Met Gly
 210 215 220

50 Phe Gln Gly Thr Leu Leu Ile Glu Pro Lys Pro Gln Glu Pro Thr Lys
 225 230 235 240

His Gln Tyr Asp Tyr Asp Val Ala Thr Val Tyr Gly Phe Leu Lys Gln
 245 250 255

55 Phe Gly Leu Glu Lys Glu Ile Lys Val Asn Ile Glu Ala Asn His Ala
 260 265 270

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	Thr	Leu	Ala	Gly	His	Ser	Phe	His	His	Glu	Ile	Ala	Thr	Ala	Ile	Ala	
			275					280					285				
5	Leu	Gly	Ile	Phe	Gly	Ser	Val	Asp	Ala	Asn	Arg	Gly	Asp	Ala	Gln	Leu	
		290					295					300					
10	Gly	Trp	Asp	Thr	Asp	Gln	Phe	Pro	Ile	Ser	Val	Glu	Glu	Asn	Ala	Leu	
	305					310					315					320	
15	Val	Met	Tyr	Glu	Ile	Leu	Lys	Ala	Gly	Gly	Phe	Thr	Thr	Gly	Gly	Leu	
					325					330					335		
20	Asn	Phe	Asp	Ala	Lys	Val	Arg	Arg	Gln	Ser	Thr	Asp	Lys	Tyr	Asp	Leu	
				340					345					350			
25	Phe	Tyr	Gly	His	Ile	Gly	Ala	Met	Asp	Thr	Met	Ala	Leu	Ser	Leu	Lys	
			355					360					365				
30	Ile	Ala	Ala	Arg	Met	Val	Glu	Asp	Gly	Glu	Leu	Asp	Lys	Arg	Val	Ala	
		370					375					380					
35	Lys	Arg	Tyr	Ala	Gly	Trp	Asn	Gly	Glu	Leu	Gly	Gln	Gln	Ile	Leu	Lys	
	385					390					395					400	
40	Gly	Gln	Leu	Ser	Leu	Gly	Glu	Leu	Ala	Gln	Tyr	Ala	Glu	Gln	His	His	
					405					410					415		
45	Leu	Ala	Pro	Val	His	Gln	Ser	Gly	His	Gln	Glu	Leu	Leu	Glu	Asn	Leu	
				420					425					430			
50	Val	Asn	Arg	Tyr	Leu	Phe	Asp	Lys									
			435					440									
55	Met	Gln	Lys	Leu	Ile	Asn	Ser	Val	Gln	Asn	Tyr	Ala	Trp	Gly	Ser	Lys	
	1				5					10					15		
60	Thr	Ala	Leu	Thr	Glu	Leu	Tyr	Gly	Ile	Ala	Asn	Pro	Gln	Gln	Gln	Pro	
				20					25					30			
65	Met	Ala	Glu	Leu	Trp	Met	Gly	Ala	His	Pro	Lys	Ser	Ser	Ser	Arg	Ile	
			35					40					45				

<210> 32
 <211> 391
 <212> PRT
 <213> Salmonella typhimurium
 <400> 32

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	Thr	Thr	Ala	Asn	Gly	Glu	Thr	Val	Ser	Leu	Arg	Asp	Ala	Ile	Glu	Lys	
	50						55					60					
5	Asn	Lys	Thr	Ala	Met	Leu	Gly	Glu	Ala	Val	Ala	Asn	Arg	Phe	Gly	Glu	
	65					70					75					80	
	Leu	Pro	Phe	Leu	Phe	Lys	Val	Leu	Cys	Ala	Ala	Lys	Pro	Leu	Ser	Ile	
10					85					90					95		
	Gln	Val	His	Pro	Asn	Lys	Arg	Asn	Ser	Glu	Ile	Gly	Phe	Ala	Lys	Glu	
				100					105					110			
15	Asn	Ala	Ala	Gly	Ile	Pro	Met	Asp	Ala	Ala	Glu	Arg	Asn	Tyr	Lys	Asp	
			115					120					125				
	Pro	Asn	His	Lys	Pro	Glu	Leu	Val	Phe	Ala	Leu	Thr	Pro	Phe	Leu	Ala	
20							135						140				
	Met	Asn	Ala	Phe	Arg	Glu	Phe	Ser	Asp	Ile	Val	Ser	Leu	Leu	Gln	Pro	
25	145					150					155					160	
	Val	Ala	Gly	Ala	His	Ser	Ala	Ile	Ala	His	Phe	Leu	Gln	Val	Pro	Asn	
					165					170					175		
30	Ala	Glu	Arg	Leu	Ser	Gln	Leu	Phe	Ala	Ser	Leu	Leu	Asn	Met	Gln	Gly	
				180					185					190			
	Glu	Glu	Lys	Ser	Arg	Ala	Leu	Ala	Val	Leu	Lys	Ala	Ala	Leu	Asn	Ser	
35			195					200					205				
	Gln	Gln	Gly	Glu	Pro	Trp	Gln	Thr	Ile	Arg	Val	Ile	Ser	Glu	Tyr	Tyr	
		210					215					220					
40	Pro	Asp	Asp	Ser	Gly	Leu	Phe	Ser	Pro	Leu	Leu	Leu	Asn	Val	Val	Lys	
	225					230					235					240	
	Leu	Asn	Pro	Gly	Glu	Ala	Met	Phe	Leu	Phe	Ala	Glu	Thr	Pro	His	Ala	
45					245					250					255		
	Tyr	Leu	Gln	Gly	Val	Ala	Leu	Glu	Val	Met	Ala	Asn	Ser	Asp	Asn	Val	
50				260					265					270			
	Leu	Arg	Ala	Gly	Leu	Thr	Pro	Lys	Tyr	Ile	Asp	Ile	Pro	Glu	Leu	Val	
			275					280					285				
55	Ala	Asn	Val	Lys	Phe	Glu	Pro	Lys	Pro	Ala	Gly	Glu	Leu	Leu	Thr	Ala	
	290						295					300					

5 Pro Val Lys Ser Gly Ala Glu Leu Asp Phe Pro Ile Pro Val Asp Asp
305 310 315 320

Phe Ala Phe Ser Leu His Asp Leu Ala Leu Gln Glu Thr Ser Ile Gly
325 330 335

10 Gln His Ser Ala Ala Ile Leu Phe Cys Val Glu Gly Glu Ala Val Leu
340 345 350

15 Arg Lys Asp Glu Gln Arg Leu Val Leu Lys Pro Gly Glu Ser Ala Phe
355 360 365

Ile Gly Ala Asp Glu Ser Pro Val Asn Ala Ser Gly Thr Gly Arg Leu
20 370 375 380

Ala Arg Val Tyr Asn Lys Leu
385 390

25 <210> 33
<211> 391
<212> PRT
<213> Enterobacter cloacae

30 <400> 33

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Met Gln Lys Leu Ile Asn Ser Val Gln Asn Tyr Ala Trp Gly Ser Lys
1 5 10 15

5 Thr Ala Leu Thr Glu Leu Tyr Gly Ile Ala Asn Pro Asp Asn Leu Pro
20 25 30

10 Met Ala Glu Leu Trp Met Gly Ala His Pro Lys Ser Ser Ser Lys Val
35 40 45

15 Glu Asp Ala Ser Gly Gln Val Arg Ala Leu Arg Asp Val Ile Asp Ala
50 55 60

20 Asp Lys Ala Thr Leu Leu Gly Asn Ser Val Ala Ser Arg Phe Gly Glu
65 70 75 80

25 Leu Pro Phe Leu Phe Lys Val Leu Cys Ala Asp Gln Pro Leu Ser Ile
85 90 95

30 Gln Val His Pro Asn Lys Lys Ala Ser Glu Ile Gly Phe Ala Lys Glu
100 105 110

35 Asn Ala Ala Gly Ile Pro Leu Asp Ala Ala Glu Arg Asn Tyr Lys Asp
115 120 125

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5 Pro Asn His Lys Pro Glu Leu Val Phe Ala Leu Thr Pro Phe Leu Ala
 130 135 140
 Met Asn Ala Phe Arg Glu Phe Ser Glu Ile Ile Ser Leu Leu Gln Pro
 145 150 155 160
 10 Val Ala Gly Ala His Asn Ala Ile Ala His Phe Leu Ala Asn Pro Asn
 165 170 175
 Ala Glu Ser Leu Ser Gln Leu Phe Ala Ser Leu Leu Asn Met Gln Gly
 180 185 190
 15 Asp Glu Lys Ser His Ala Arg Ala Val Leu Lys Ala Ala Leu Asp Gly
 195 200 205
 20 Gln Gln Gly Glu Pro Trp Asp Thr Ile Arg Leu Ile Ser Ala Phe Tyr
 210 215 220
 25 Pro Asp Asp Ser Gly Leu Phe Ser Pro Leu Leu Leu Asn Val Val Lys
 225 230 235 240
 Leu Asn Pro Gly Glu Ala Met Phe Leu Phe Ala Glu Thr Pro His Ala
 245 250 255
 30 Tyr Leu Gln Gly Val Ala Leu Glu Val Met Ala Asn Ser Asp Asn Val
 260 265 270
 35 Leu Arg Ala Gly Leu Thr Pro Lys Tyr Ile Asp Ile Pro Glu Leu Val
 275 280 285
 Ala Asn Val Lys Phe Val Ala Lys Pro Ala Ala Glu Leu Leu Thr Gln
 290 295 300
 40 Pro Val Lys Asn Gly Ala Glu Leu Asp Phe Pro Ile Pro Val Asp Asp
 305 310 315 320
 45 Phe Ala Phe Ser Leu His Asp Leu Ser Gln Thr Glu Thr Thr Ile Ala
 325 330 335
 50 Gln Glu Ser Ala Ala Ile Leu Phe Cys Val Glu Gly Glu Ala Thr Leu
 340 345 350
 His Lys Gly Glu Gln Arg Leu Val Leu Lys Pro Gly Glu Ser Ala Phe
 355 360 365
 55 Val Ala Ala Asn Glu Ser Pro Val Ser Val Ser Gly Thr Gly Arg Leu

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370

375

380

5 Ala Arg Val Phe Asn Lys Leu
 385 390

<210> 34

<211> 461

<212> PRT

10 <213> Enterobacter cloacae

<400> 34

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EP 2 593 540 B1

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

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	195		200		205
5	Trp Gly Gly Arg Glu Gly Tyr Glu Thr Leu Leu Asn Thr Asp Leu Arg				
	210		215		220
	Gln Glu Arg Glu Gln Ile Gly Arg Phe Met Gln Met Val Val Glu His				
	225		230		235
10					
	Lys His Lys Ile Gly Phe Arg Gly Thr Leu Leu Ile Glu Pro Lys Pro				
		245		250	255
15					
	Gln Glu Pro Thr Lys His Gln Tyr Asp Tyr Asp Val Ala Thr Val Tyr				
		260		265	270
	Gly Phe Leu Lys Gln Phe Gly Leu Glu Lys Glu Ile Lys Val Asn Ile				
20		275		280	285
	Glu Ala Asn His Ala Thr Leu Ala Gly His Ser Phe His His Glu Ile				
	290		295		300
25					
	Ala Ser Ala Ile Ala Leu Gly Ile Phe Gly Ser Val Asp Ala Asn Arg				
	305		310		315
30					
	Gly Asp Ala Gln Leu Gly Trp Asp Thr Asp Gln Phe Pro Asn Ser Val				
		325		330	335
	Glu Glu Asn Ala Leu Val Met Tyr Glu Ile Ile Lys Ala Gly Gly Phe				
35		340		345	350
	Thr Thr Gly Gly Leu Asn Phe Asp Ala Lys Val Arg Arg Gln Ser Thr				
		355		360	365
40					
	Asp Lys Tyr Asp Leu Phe Tyr Gly His Ile Gly Ala Met Asp Thr Met				
	370		375		380
	Ala Leu Ala Leu Lys Val Ala Ala Arg Met Ile Glu Asp Gly Glu Leu				
45		385		390	395
	Asp Lys Arg Val Ala Lys Arg Tyr Ser Gly Trp Asn Ser Glu Leu Gly				
		405		410	415
50					
	Gln Gln Ile Leu Lys Gly Gln Leu Ser Leu Ala Glu Ile Ala Lys Tyr				
		420		425	430
55					
	Ala Glu Gln His Gln Leu Ala Pro Gln His Gln Ser Gly His Gln Glu				
	435		440		445

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Leu Leu Glu Asn Leu Val Asn His Tyr Leu Phe Asp Lys
450 455 460

5 <210> 35
<211> 391
<212> PRT
<213> Enterobacter cancerogenus

10 <400> 35

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	Met	Gln	Lys	Leu	Ile	Asn	Ser	Val	Gln	Asn	Tyr	Ala	Trp	Gly	Ser	Lys	
	1				5					10					15		
5	Thr	Ala	Leu	Thr	Asp	Leu	Tyr	Gly	Ile	Ala	Asn	Pro	Asp	Gln	Leu	Pro	
				20					25					30			
10	Met	Ala	Glu	Leu	Trp	Met	Gly	Ala	His	Pro	Lys	Ser	Ser	Ser	Lys	Ile	
			35					40					45				
	Glu	Asp	Ala	Ser	Gly	Gln	Val	Arg	Ser	Leu	Arg	Asp	Ala	Ile	Asp	Ala	
		50					55					60					
15	Asp	Lys	Thr	Ala	Leu	Leu	Gly	Asp	Lys	Val	Ala	Ser	Arg	Phe	Gly	Glu	
	65					70					75					80	
20	Leu	Pro	Phe	Leu	Phe	Lys	Val	Leu	Cys	Ala	Asp	Gln	Pro	Leu	Ser	Ile	
					85					90					95		
25	Gln	Val	His	Pro	Asn	Lys	Lys	Ala	Ser	Glu	Ile	Gly	Phe	Ala	Lys	Glu	
				100					105					110			
30	Asn	Ala	Ala	Gly	Ile	Pro	Leu	Asp	Ala	Ala	Glu	Arg	Asn	Tyr	Lys	Asp	
			115					120					125				
35	Pro	Asn	His	Lys	Pro	Glu	Leu	Val	Phe	Ala	Leu	Thr	Pro	Phe	Leu	Ala	
		130					135					140					
40	Met	Asn	Ala	Phe	Arg	Glu	Phe	Ser	Glu	Ile	Ile	Ser	Leu	Leu	Gln	Pro	
	145					150					155					160	
	Val	Ala	Gly	Ala	Asn	Asn	Ala	Ile	Ala	His	Phe	Leu	Glu	Asn	Pro	Asn	
					165					170					175		
45	Ala	Glu	Gly	Leu	Ser	Gln	Leu	Phe	Ala	Ser	Leu	Leu	Asn	Met	Gln	Gly	
				180					185					190			
50	Glu	Glu	Lys	Ser	His	Ala	Leu	Ala	Ile	Leu	Lys	Ala	Ala	Leu	Asn	Asp	
			195					200					205				

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	Gln	Gln	Gly	Glu	Pro	Trp	Asp	Thr	Ile	Arg	Val	Ile	Ser	Glu	Phe	Tyr	
	210						215					220					
5	Pro	Asp	Asp	Ser	Gly	Leu	Phe	Ser	Pro	Leu	Leu	Leu	Asn	Val	Val	Lys	
	225					230					235					240	
	Leu	Asn	Pro	Gly	Glu	Ala	Met	Phe	Leu	Phe	Ala	Glu	Thr	Pro	His	Ala	
10					245					250					255		
	Tyr	Leu	Gln	Gly	Val	Ala	Leu	Glu	Val	Met	Ala	Asn	Ser	Asp	Asn	Val	
				260					265					270			
15	Leu	Arg	Ala	Gly	Leu	Thr	Pro	Lys	Tyr	Ile	Asp	Ile	Pro	Glu	Leu	Val	
			275					280					285				
20	Ala	Asn	Val	Lys	Phe	Val	Ala	Lys	Pro	Ala	Ala	Glu	Leu	Leu	Thr	Gln	
		290					295					300					
	Pro	Val	Lys	Asn	Gly	Ala	Glu	Leu	Asp	Phe	Pro	Ile	Pro	Val	Asp	Asp	
25	305					310					315					320	
	Phe	Ala	Phe	Ser	Leu	His	Asp	Leu	Ser	Ala	Asp	Glu	Thr	Ala	Ile	Ala	
					325					330					335		
30	Gln	Glu	Ser	Ala	Ala	Ile	Leu	Phe	Cys	Val	Glu	Gly	Glu	Ala	Thr	Leu	
				340					345					350			
35	His	Lys	Gly	Glu	Gln	Arg	Leu	Val	Leu	Lys	Pro	Gly	Glu	Ser	Ala	Phe	
			355					360					365				
	Val	Ala	Ala	Asn	Glu	Ser	Pro	Val	Ser	Ile	Ser	Gly	Lys	Gly	Arg	Leu	
40		370					375					380					
	Ala	Arg	Val	Phe	Asn	Lys	Leu										
	385					390											
45	<210> 36																
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	<212> PRT																
	<213> Cronobacter turicensis																
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Met	Gln	Lys	Leu	Ile	Asn	Ser	Val	Gln	Asn	Tyr	Ala	Trp	Gly	Ser	Lys
1				5					10					15	

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Thr	Ala	Leu	Thr	Glu	Leu	Tyr	Gly	Val	Ala	Asn	Pro	Glu	Gly	Leu	Pro
			20					25					30		

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	Met	Ala	Glu	Leu	Trp	Met	Gly	Ala	His	Pro	Lys	Ser	Ser	Ser	Lys	Ile
			35					40					45			
5	Gln	Asp	Asp	Gln	Gly	Gln	Thr	Arg	Ala	Leu	Arg	Glu	Val	Ile	Asp	Ala
		50					55					60				
10	Asp	Lys	Thr	Ala	Leu	Leu	Gly	Ala	Pro	Val	Ala	Glu	Arg	Phe	Gly	Glu
	65					70					75					80
	Leu	Pro	Phe	Leu	Phe	Lys	Val	Leu	Cys	Ala	Asp	Gln	Pro	Leu	Ser	Ile
				85						90					95	
15	Gln	Val	His	Pro	Asn	Lys	Gln	Ala	Ser	Glu	Glu	Gly	Phe	Ala	Arg	Glu
				100					105					110		
20	Asn	Ala	Ala	Gly	Ile	Pro	Leu	Asp	Ala	Ala	Glu	Arg	Asn	Tyr	Lys	Asp
			115					120					125			
25	Pro	Asn	His	Lys	Pro	Glu	Leu	Val	Phe	Ala	Leu	Thr	Pro	Phe	Leu	Ala
		130					135					140				
30	Met	Asn	Ala	Phe	Arg	Glu	Phe	Ser	Glu	Ile	Val	Ser	Leu	Leu	Gln	Pro
	145					150					155					160
	Val	Ala	Gly	Ala	His	Thr	Ala	Ile	Ala	His	Phe	Leu	Ala	Glu	Pro	Asn
				165						170					175	
35	Ala	Asp	Arg	Leu	Arg	Asp	Leu	Phe	Ala	Gly	Leu	Leu	Asn	Met	Gln	Gly
				180					185					190		
40	Glu	Glu	Lys	Ser	Arg	Ala	Leu	Ala	Val	Leu	Lys	Ala	Thr	Val	Ala	Ser
			195					200					205			
45	Gln	Gln	Gly	Glu	Pro	Trp	Asp	Thr	Ile	Arg	Phe	Ile	Ala	Gln	Phe	Tyr
		210					215					220				
	Pro	Asp	Asp	Ser	Gly	Leu	Phe	Ser	Pro	Leu	Leu	Leu	Asn	Val	Val	Lys
	225					230					235					240
50	Leu	Asn	Pro	Gly	Glu	Ala	Met	Phe	Leu	Phe	Ala	Glu	Thr	Pro	His	Ala
				245						250					255	
55	Tyr	Leu	Gln	Gly	Val	Ala	Leu	Glu	Val	Met	Ala	Asn	Ser	Asp	Asn	Val
			260					265						270		
	Leu	Arg	Ala	Gly	Leu	Thr	Pro	Lys	Tyr	Ile	Asp	Ile	Pro	Glu	Leu	Val
		275						280					285			

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Ala Asn Val Lys Phe Val Ala Lys Pro Ala Ala Gln Leu Leu Thr Gln
290 295 300

5 Pro Glu Lys Asp Gly Ala Ala Leu Glu Phe Pro Ile Pro Val Glu Asp
305 310 315 320

10 Phe Ala Phe Ser Leu His Asp Leu Gln Ser Gln Pro Gln Arg Leu Ala
325 330 335

Gln Glu Ser Ala Ala Ile Leu Phe Cys Val Glu Gly Glu Ala Val Leu
340 345 350

15 Ser Lys Gly Asp Glu Arg Leu Thr Leu Lys Pro Gly Glu Ser Ala Phe
355 360 365

20 Val Ala Ala Asn Glu Ser Pro Val Asn Val Ser Gly Val Gly Arg Leu
370 375 380

25 Ala Arg Val Tyr Asn Lys Leu Asn
385 390

<210> 37
<211> 392
<212> PRT
30 <213> Cronobacter sakazakii

<400> 37

35 Met Gln Lys Leu Ile Asn Ser Val Gln Asn Tyr Ala Trp Gly Ser Lys
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Thr Ala Leu Thr Glu Leu Tyr Gly Val Ala Asn Pro Glu Gly Leu Pro
20 25 30

40 Met Ala Glu Leu Trp Met Gly Ala His Pro Lys Ser Ser Ser Lys Ile
35 40 45

45 Gln Asp Ala Gln Gly Gln Thr Arg Ala Leu Arg Glu Val Ile Asp Ala
50 55 60

50 Asp Lys Thr Ala Leu Leu Gly Ala Pro Val Ala Glu Arg Phe Gly Glu
65 70 75 80

Leu Pro Phe Leu Phe Lys Val Leu Cys Ala Asp Gln Pro Leu Ser Ile
85 90 95

55 Gln Val His Pro Asn Lys Gln Ala Ser Glu Glu Gly Phe Ala Arg Glu
100 105 110

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	Asn	Ala	Ala	Gly	Ile	Pro	Leu	Ser	Ala	Ala	Glu	Arg	Asn	Tyr	Lys	Asp
			115					120					125			
5	Pro	Asn	His	Lys	Pro	Glu	Leu	Val	Phe	Ala	Leu	Thr	Pro	Phe	Leu	Ala
		130					135					140				
10	Met	Asn	Ala	Phe	Arg	Glu	Phe	Ser	Glu	Ile	Val	Ser	Leu	Leu	Gln	Pro
	145					150					155					160
	Val	Ala	Gly	Ala	His	Thr	Ala	Ile	Ala	His	Phe	Leu	Ala	Glu	Pro	Asn
				165						170					175	
15	Ala	Asp	Arg	Leu	Arg	Glu	Leu	Phe	Ala	Gly	Leu	Leu	Asn	Met	Gln	Gly
				180					185					190		
20	Glu	Glu	Lys	Ser	Arg	Ala	Leu	Ala	Val	Leu	Lys	Ala	Thr	Val	Ala	Ser
			195					200					205			
25	Gln	Gln	Gly	Glu	Pro	Trp	Glu	Thr	Ile	Arg	Phe	Ile	Ala	Gln	Phe	Tyr
	210						215					220				
	Pro	Asp	Asp	Ser	Gly	Leu	Phe	Ser	Pro	Leu	Leu	Leu	Asn	Val	Val	Lys
	225					230					235					240
30	Leu	Asn	Pro	Gly	Glu	Ala	Met	Phe	Leu	Phe	Ala	Glu	Thr	Pro	His	Ala
				245						250					255	
35	Tyr	Leu	Gln	Gly	Val	Ala	Leu	Glu	Val	Met	Ala	Asn	Ser	Asp	Asn	Val
			260						265					270		
40	Leu	Arg	Ala	Gly	Leu	Thr	Pro	Lys	Tyr	Ile	Asp	Ile	Pro	Glu	Leu	Val
		275						280					285			
	Ala	Asn	Val	Lys	Phe	Val	Ala	Lys	Pro	Ala	Gly	Glu	Leu	Leu	Thr	Gln
	290						295					300				
45	Pro	Val	Lys	Gln	Glu	Ser	Ala	Leu	Glu	Phe	Pro	Ile	Pro	Val	Glu	Asp
	305					310					315					320
50	Phe	Ala	Phe	Ser	Leu	His	Asp	Leu	Asn	Ser	Gln	Pro	Gln	Ser	Leu	Ala
				325						330					335	
	Gln	Glu	Ser	Ala	Ala	Ile	Leu	Phe	Cys	Val	Glu	Gly	Glu	Ala	Val	Leu
			340						345					350		
55	Ser	Lys	Gly	Asp	Glu	Arg	Leu	Thr	Leu	Lys	Pro	Gly	Glu	Ser	Ala	Phe
			355					360					365			

5 Val Ala Ala Ser Glu Ser Pro Val Arg Ala Ser Gly Val Gly Arg Leu
370 375 380

Ala Arg Val Tyr Asn Lys Leu Asn
385 390

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<210> 38

<211> 392

<212> PRT

<213> Klebsiella pneumoniae

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<400> 38

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

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	Glu	Glu	Lys	Ala	Lys	Ala	Leu	Gln	Val	Leu	Arg	Asp	Val	Leu	Ala	Arg	
			195					200					205				
5	Glu	Gln	Gly	Glu	Pro	Trp	Gln	Thr	Ile	Arg	Leu	Ile	Ala	Glu	Phe	Tyr	
		210					215					220					
10	Pro	Asp	Asp	Ser	Gly	Leu	Phe	Ser	Pro	Leu	Leu	Leu	Asn	Val	Val	Lys	
	225					230					235					240	
	Leu	Asn	Pro	Gly	Glu	Ala	Met	Phe	Leu	Phe	Ala	Glu	Thr	Pro	His	Ala	
					245					250					255		
15	Tyr	Leu	Gln	Gly	Val	Ala	Leu	Glu	Val	Met	Ala	Asn	Ser	Asp	Asn	Val	
				260					265					270			
20	Leu	Arg	Ala	Gly	Leu	Thr	Pro	Lys	Tyr	Ile	Asp	Ile	Pro	Glu	Leu	Val	
			275					280					285				
25	Ala	Asn	Val	Lys	Phe	Glu	Ala	Lys	Pro	Ala	Gly	Glu	Leu	Leu	Thr	Gln	
		290					295					300					
30	Pro	Gln	Gln	His	Gly	Ala	Glu	Leu	Asp	Phe	Pro	Ile	Pro	Val	Glu	Asp	
	305					310					315					320	
	Phe	Ala	Phe	Ser	Leu	His	Asp	Leu	Ser	Ala	Glu	Ala	Ser	Asp	Leu	Ala	
					325					330					335		
35	Gln	Ala	Ser	Ala	Ala	Ile	Ile	Phe	Cys	Val	Asp	Gly	Glu	Ala	Val	Leu	
				340					345					350			
40	His	Lys	Gly	Asp	Gln	Ser	Leu	Thr	Leu	Lys	Pro	Gly	Glu	Ser	Ala	Phe	
			355					360					365				
	Val	Ala	Ala	Asn	Glu	Ser	Pro	Val	Gln	Val	Ser	Gly	Arg	Gly	Arg	Val	
		370					375					380					
45	Ala	Arg	Val	Phe	Asn	Lys	Leu	Gln									
	385					390											
50	<210>	39															
	<211>	304															
	<212>	PRT															
	<213>	Klebsiella pneumoniae															
55	<400>	39															
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	1				5					10					15		

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	Leu	Ser	Asp	Gln	Gly	Glu	Gln	Leu	Phe	Arg	His	Arg	Leu	Pro	Thr	Pro	
				20					25					30			
5	Arg	Glu	Asp	Tyr	Arg	Gln	Thr	Ile	Glu	Thr	Ile	Ala	Thr	Leu	Val	Ala	
			35					40					45				
10	Met	Ala	Glu	Gln	Ala	Thr	Gly	Gln	Gln	Gly	Thr	Val	Gly	Met	Gly	Ile	
		50					55					60					
	Pro	Gly	Ala	Ile	Ser	Pro	Tyr	Thr	Gly	Val	Val	Lys	Asn	Ala	Asn	Ser	
	65					70					75					80	
15	Thr	Trp	Leu	Asn	Gly	Gln	Pro	Phe	Asp	Lys	Asp	Leu	Ser	Leu	Arg	Leu	
				85						90					95		
20	Glu	Arg	Glu	Val	Arg	Leu	Ala	Asn	Asp	Ala	Asn	Cys	Leu	Ala	Val	Ser	
				100					105					110			
25	Glu	Ala	Val	Asp	Gly	Ala	Ala	Ala	Gly	Ala	Gln	Thr	Val	Phe	Ala	Val	
			115					120					125				
30	Ile	Ile	Gly	Thr	Gly	Cys	Gly	Ala	Gly	Val	Ala	Leu	Asn	Gly	Arg	Ala	
		130					135						140				
35	His	Ile	Gly	Gly	Asn	Gly	Asn	Ala	Gly	Glu	Trp	Gly	His	Asn	Pro	Leu	
	145					150					155					160	
40	Pro	Trp	Met	Asn	Asp	Asp	Glu	Leu	Arg	Tyr	Arg	Ala	Glu	Val	Pro	Cys	
				165						170					175		
45	Tyr	Cys	Gly	Lys	Gln	Gly	Cys	Ile	Glu	Thr	Phe	Ile	Ser	Gly	Thr	Gly	
				180					185					190			
50	Phe	Ala	Thr	Asp	Tyr	Gln	Arg	Leu	Ser	Gly	Lys	Gly	Leu	Thr	Gly	Ser	
			195					200					205				
55	Glu	Ile	Met	Arg	Leu	Val	Gly	Glu	Gly	Asp	Glu	Lys	Ala	Glu	Leu	Ala	
		210					215					220					
60	Leu	Ser	Arg	Tyr	Glu	Gln	Arg	Leu	Ala	Lys	Ser	Leu	Ala	His	Val	Val	
	225					230					235					240	
65	Asn	Ile	Leu	Asp	Pro	Asp	Val	Ile	Val	Leu	Gly	Gly	Gly	Met	Ser	Asn	
				245						250				255			
70	Val	Glu	Arg	Leu	Tyr	Gln	Thr	Val	Pro	Asp	Leu	Val	Lys	Gln	Trp	Val	

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260 265 270

5 Phe Gly Gly Glu Cys Glu Thr Pro Ile Arg Lys Ala Leu His Gly Asp
275 280 285

10 Ser Ser Gly Val Arg Gly Ala Ala Trp Leu Trp Pro Leu Gln Gly Thr
290 295 300

<210> 40
<211> 392
<212> PRT
15 <213> Serratia odorifera

<400> 40

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Met Gln Lys Met Thr Asn Ala Val Gln Asn Tyr Ala Trp Gly Ser His
1 5 10 15

5
Glu Ala Leu Thr Lys Leu Tyr Gly Ile Ala Asn Pro Gln Gly Lys Pro
20 25 30

10
Met Ala Glu Leu Trp Met Gly Ala His Pro Lys Ser Ser Ser Gln Val
35 40 45

15
Pro Asp Ala Asn Gly Asp Leu Arg Ser Leu Arg Asp Val Ile Asn Gln
50 55 60

20
Asp Gln Pro Lys Gln Leu Gly Gln Asp Val Ala Thr Arg Phe Gly Glu
65 70 75 80

25
Leu Pro Phe Leu Phe Lys Val Leu Cys Ala Asp Gln Pro Leu Ser Ile
85 90 95

30
Gln Val His Pro Ser Lys Ser Ala Ala Glu Val Gly Tyr Ala Arg Glu
100 105 110

35
Asn Ala Ala Gly Ile Pro Leu Asp Ala Ala Glu Arg Asn Tyr Lys Asp
115 120 125

40
Pro Asn His Lys Pro Glu Leu Val Phe Ala Leu Thr Pro Phe Leu Ala
130 135 140

45
Met Asn Gly Phe Arg Glu Leu Ala Asp Ile Val Ala Leu Leu Gln Pro
145 150 155 160

50
Ile Ala Gly Ala His His Asp Ile Ala Ala Phe Leu Gln Gln Pro Asp
165 170 175

55
Thr Glu Arg Leu Ala Lys Leu Phe Ala Ser Leu Leu Gly Met Ser Gly

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	180	185	190
5	Glu Gln Lys Ser Leu Ala Leu Gly Val Leu Lys Ala Ala Leu Asn Asn 195 200 205		
10	Gln Gln Gly Glu Pro Trp Asp Thr Val Arg Phe Ile Ala Gly Phe Tyr 210 215 220		
15	Pro Glu Asp Ser Gly Leu Phe Ser Pro Leu Leu Leu Asn Val Val Lys 225 230 235 240		
20	Leu Ala Pro Gly Glu Ala Met Phe Leu Tyr Ala Glu Thr Pro His Ala 245 250 255		
25	Tyr Leu Gln Gly Val Ala Leu Glu Val Met Ala Asn Ser Asp Asn Val 260 265 270		
30	Leu Arg Ala Gly Leu Thr Pro Lys Phe Ile Asp Ile Pro Glu Leu Leu 275 280 285		
35	Ala Asn Leu Gln Phe Arg Pro Gln Pro Ala Ser Gly Leu Leu Thr Gln 290 295 300		
40	Pro Glu Lys Arg Gly Asp Glu Leu Phe Phe Pro Ile Pro Val Glu Asp 305 310 315 320		
45	Phe Ala Phe Ser Leu His Asp Leu Ser Ser Ser Ala Gln Ser Leu Ala 325 330 335		
50	Gln Arg Ser Ala Ala Ile Val Phe Cys Val Glu Gly Glu Ala Arg Leu 340 345 350		
55	Glu Lys Gln Gly Gln Gln Ile Thr Leu His Pro Gly Glu Ser Cys Phe 355 360 365		
60	Ile Gly Ala Phe Glu Ser Pro Val Ser Val Ser Gly Ser Gly Arg Ile 370 375 380		
65	Ala Arg Val Tyr Asn Gln Leu Ser 385 390		

<210> 41

<211> 491

<212> PRT

<213> Escherichia fergusonii

<400> 41

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Met Ser Thr Gln Tyr Asn Ser Ser Tyr Ile Phe Ser Ile Thr Leu Val

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	1			5				10				15				
5	Ala	Thr	Leu	Gly	Gly	Leu	Leu	Phe	Gly	Tyr	Asp	Thr	Ala	Val	Ile	Ser
				20					25					30		
10	Gly	Thr	Val	Glu	Ser	Leu	Asn	Thr	Val	Phe	Val	Ala	Pro	Gln	Asn	Leu
			35					40					45			
15	Ser	Glu	Ser	Ala	Ala	Asn	Ser	Leu	Leu	Gly	Phe	Cys	Val	Ala	Ser	Ala
		50					55					60				
20	Leu	Ile	Gly	Cys	Ile	Ile	Gly	Gly	Ala	Leu	Gly	Gly	Tyr	Cys	Ser	Asn
	65					70					75					80
25	Arg	Phe	Gly	Arg	Arg	Asp	Ser	Leu	Lys	Ile	Ala	Ala	Val	Leu	Phe	Phe
					85					90					95	
30	Ile	Ser	Gly	Val	Gly	Ser	Ala	Trp	Pro	Glu	Leu	Gly	Phe	Thr	Ser	Ile
				100					105					110		
35	Asn	Pro	Asp	Asn	Thr	Val	Pro	Val	Tyr	Leu	Ala	Gly	Tyr	Val	Pro	Glu
			115					120					125			
40	Phe	Val	Ile	Tyr	Arg	Ile	Ile	Gly	Gly	Ile	Gly	Val	Gly	Leu	Ala	Ser
		130					135					140				
45	Met	Leu	Ser	Pro	Met	Tyr	Ile	Ala	Glu	Leu	Ala	Pro	Ala	His	Ile	Arg
	145					150					155					160
50	Gly	Lys	Leu	Val	Ser	Phe	Asn	Gln	Phe	Ala	Ile	Ile	Phe	Gly	Gln	Leu
					165					170					175	
55	Leu	Val	Tyr	Cys	Val	Asn	Tyr	Phe	Ile	Ala	Arg	Ser	Gly	Asp	Ala	Thr
				180					185					190		
60	Trp	Leu	Asn	Thr	Asp	Gly	Trp	Arg	Tyr	Met	Phe	Ala	Ser	Glu	Cys	Ile
			195					200					205			
65	Pro	Ala	Leu	Leu	Phe	Leu	Met	Leu	Leu	Tyr	Thr	Val	Pro	Glu	Ser	Pro
		210					215					220				
70	Arg	Trp	Leu	Met	Ser	Arg	Gly	Lys	Gln	Glu	Gln	Ala	Glu	Gly	Ile	Leu
	225					230					235					240
75	Arg	Lys	Ile	Met	Gly	Asn	Thr	Leu	Ala	Thr	Gln	Ala	Val	Gln	Glu	Ile
					245					250					255	

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Lys His Ser Leu Asp His Gly Arg Lys Thr Gly Gly Arg Leu Leu Met
 260 265 270
 5
 Phe Gly Val Gly Val Ile Val Ile Gly Val Met Leu Ser Ile Phe Gln
 275 280 285
 10
 Gln Phe Val Gly Ile Asn Val Val Leu Tyr Tyr Ala Pro Glu Val Phe
 290 295 300
 15
 Lys Thr Leu Gly Ala Ser Thr Asp Ile Ala Leu Leu Gln Thr Ile Ile
 305 310 315 320
 Val Gly Val Ile Asn Leu Thr Phe Thr Val Leu Ala Ile Met Thr Val
 325 330 335
 20
 Asp Lys Phe Gly Arg Lys Pro Leu Gln Ile Ile Gly Ala Leu Gly Met
 340 345 350
 25
 Ala Ile Gly Met Phe Ser Leu Gly Thr Ala Phe Tyr Thr Gln Ala Pro
 355 360 365
 Gly Ile Val Ala Leu Leu Ser Met Leu Phe Tyr Val Ala Ala Phe Ala
 370 375 380
 30
 Met Ser Trp Gly Pro Val Cys Trp Val Leu Leu Ser Glu Ile Phe Pro
 385 390 395 400
 35
 Asn Ala Ile Arg Gly Lys Ala Leu Ala Ile Ala Val Ala Ala Gln Trp
 405 410 415
 40
 Leu Ala Asn Tyr Phe Val Ser Trp Thr Phe Pro Met Met Asp Lys Asn
 420 425 430
 Ser Trp Leu Val Ala His Phe His Asn Gly Phe Ser Tyr Trp Ile Tyr
 435 440 445
 45
 Gly Cys Met Gly Val Leu Ala Ala Leu Phe Met Trp Lys Phe Val Pro
 450 455 460
 50
 Glu Thr Lys Gly Lys Thr Leu Glu Glu Leu Glu Ala Leu Trp Glu Pro
 465 470 475 480
 55
 Glu Thr Lys Lys Thr Gln Gln Thr Ala Thr Leu
 485 490

<210> 42

<211> 490

<212> PRT

<213> *Shigella flexneri*

<400> 42

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Met Asn Thr Gln Tyr Asn Ser Ser Tyr Ile Phe Ser Ile Thr Leu Val
 1 5 10 15
 5 Ala Thr Leu Gly Gly Leu Leu Phe Gly Tyr Asp Thr Ala Val Ile Ser
 20 25 30
 10 Gly Thr Val Glu Ser Leu Asn Thr Val Phe Val Ala Pro Gln Asn Leu
 35 40 45
 Ser Glu Ser Ala Ala Asn Ser Leu Leu Gly Phe Cys Val Ala Ser Ala
 50 55 60
 15 Leu Ile Gly Cys Ile Ile Gly Gly Ala Leu Gly Gly Tyr Cys Ser Asn
 65 70 75 80
 20 Arg Phe Gly Arg Arg Asp Ser Leu Lys Ile Ser Ala Val Leu Phe Phe
 85 90 95
 25 Ile Ser Gly Val Gly Ser Ala Trp Pro Glu Leu Gly Phe Thr Ser Ile
 100 105 110
 Asn Pro Asp Asn Thr Val Pro Ile Tyr Leu Ala Gly Tyr Val Pro Glu
 115 120 125
 30 Phe Val Ile Tyr Arg Ile Ile Gly Gly Ile Gly Val Gly Leu Ala Ser
 130 135 140
 35 Met Leu Ser Pro Met Tyr Ile Ala Glu Leu Ala Pro Ala His Ile Arg
 145 150 155 160
 40 Gly Lys Leu Val Ser Phe Asn Gln Phe Ala Ile Ile Phe Gly Gln Leu
 165 170 175
 Leu Val Tyr Cys Val Asn Tyr Phe Ile Ala Arg Ser Gly Asp Ala Ser
 180 185 190
 45 Trp Leu Asn Thr Asp Gly Arg Arg Tyr Met Phe Ala Ser Glu Cys Ile
 195 200 205
 50 Pro Ala Leu Leu Phe Leu Met Leu Leu Tyr Thr Val Pro Glu Ser Pro
 210 215 220
 55 Arg Trp Leu Met Ser Arg Gly Lys Gln Glu Gln Thr Glu Ser Ile Leu
 225 230 235 240

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Arg Lys Ile Met Gly Asn Thr Leu Ala Thr Gln Ala Val Gln Glu Ile
 245 250 255
 5
 Lys His Ser Leu Asp His Gly Arg Lys Thr Gly Gly Arg Leu Leu Met
 260 265 270
 10
 Phe Gly Val Gly Val Ile Val Ile Gly Val Met Leu Ser Ile Phe Gln
 275 280 285
 Gln Phe Val Gly Ile Asn Val Val Leu Tyr Tyr Ala Pro Glu Val Phe
 290 295 300
 15
 Lys Thr Leu Gly Ala Ser Thr Asp Ile Ala Leu Leu Gln Thr Ile Ile
 305 310 315 320
 20
 Val Gly Val Ile Asn Leu Thr Phe Thr Val Leu Ala Ile Met Thr Val
 325 330 335
 Asp Lys Phe Gly Arg Lys Pro Leu Gln Ile Ile Gly Ala Leu Gly Met
 340 345 350
 25
 Ala Ile Gly Met Phe Ser Leu Gly Thr Ala Phe Tyr Thr Gln Ala Ser
 355 360 365
 30
 Gly Ile Val Ala Leu Leu Ser Met Leu Phe Tyr Val Ala Ala Phe Ala
 370 375 380
 Met Ser Trp Gly Pro Val Cys Trp Val Leu Leu Ser Glu Ile Phe Pro
 385 390 395 400
 35
 Asn Ala Ile Arg Gly Lys Ala Leu Ala Ile Ala Val Ala Ala Gln Trp
 405 410 415
 40
 Leu Ala Asn Tyr Phe Val Ser Trp Thr Phe Pro Met Met Asp Lys Asn
 420 425 430
 Ser Trp Leu Val Ala His Phe His Asn Gly Phe Ser Tyr Trp Ile Tyr
 435 440 445
 45
 Gly Cys Met Gly Val Leu Ala Ala Leu Phe Met Trp Lys Phe Val Pro
 450 455 460
 50
 Glu Thr Lys Gly Lys Thr Leu Glu Glu Leu Glu Ala Leu Trp Glu Pro
 465 470 475 480
 55
 Glu Thr Lys Lys Thr Gln Gln Thr Ala Thr
 485 490

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<210> 43
 <211> 491
 <212> PRT
 <213> Salmonella enterica

5
 <400> 43

10	Met	Asn	Thr	His	Tyr	Asn	Ser	Arg	Tyr	Ile	Phe	Ser	Ile	Thr	Leu	Val	1	5	10	15
	Ala	Thr	Leu	Gly	Gly	Leu	Leu	Phe	Gly	Tyr	Asp	Thr	Ala	Val	Ile	Ser	20	25	30	
15	Gly	Thr	Val	Glu	Ser	Leu	Asn	Thr	Val	Phe	Val	Ala	Pro	Gln	His	Leu	35	40	45	
20	Ser	Glu	Ser	Ala	Ala	Asn	Ser	Leu	Leu	Gly	Phe	Cys	Val	Ala	Ser	Ala	50	55	60	
25	Leu	Ile	Gly	Cys	Ile	Ile	Gly	Gly	Ala	Leu	Gly	Gly	Tyr	Cys	Ser	Asn	65	70	75	
	Arg	Phe	Gly	Arg	Arg	Asp	Ser	Leu	Lys	Ile	Ala	Ala	Leu	Leu	Phe	Phe	85	90	95	
30	Ile	Ser	Gly	Ile	Gly	Ser	Ala	Trp	Pro	Glu	Leu	Gly	Phe	Thr	Thr	Ile	100	105	110	
35	Asn	Pro	Asp	Asn	Ala	Val	Pro	Val	Tyr	Leu	Ala	Gly	Tyr	Val	Pro	Glu	115	120	125	
40	Phe	Val	Ile	Tyr	Arg	Ile	Ile	Gly	Gly	Ile	Gly	Val	Gly	Leu	Ala	Ser	130	135	140	
45	Met	Leu	Ser	Pro	Met	Tyr	Ile	Ala	Glu	Leu	Ala	Pro	Ala	His	Ile	Arg	145	150	155	
	Gly	Lys	Leu	Val	Ser	Phe	Asn	Gln	Phe	Ala	Ile	Ile	Phe	Gly	Gln	Leu	165	170	175	
50	Leu	Val	Tyr	Cys	Val	Asn	Tyr	Phe	Ile	Ala	Arg	Ser	Gly	Asp	Ala	Asn	180	185	190	
55	Trp	Leu	Asn	Thr	Asp	Gly	Trp	Arg	Tyr	Met	Phe	Ala	Ser	Glu	Ser	Ile	195	200	205	
	Pro	Ala	Leu	Leu	Phe	Leu	Leu	Leu	Leu	Tyr	Thr	Val	Pro	Glu	Ser	Pro	210	215	220	

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	Arg	Trp	Leu	Met	Ala	Arg	Gly	Lys	His	Glu	Gln	Ala	Glu	Gly	Ile	Leu	
	225					230					235					240	
5	Arg	Lys	Ile	Met	Gly	Ser	Ser	Leu	Thr	Thr	Gln	Ala	Met	Gln	Glu	Ile	
					245					250					255		
10	Asn	Gln	Ser	Leu	Glu	His	Gly	Arg	Lys	Thr	Gly	Gly	Arg	Leu	Leu	Met	
				260					265					270			
	Phe	Gly	Val	Gly	Val	Ile	Val	Ile	Gly	Val	Met	Leu	Ser	Val	Phe	Gln	
			275					280					285				
15	Gln	Phe	Val	Gly	Ile	Asn	Val	Val	Leu	Tyr	Tyr	Ala	Pro	Glu	Val	Phe	
	290						295					300					
20	Lys	Thr	Leu	Gly	Ala	Ser	Thr	Asp	Val	Ala	Leu	Leu	Gln	Thr	Ile	Ile	
	305					310					315					320	
	Val	Gly	Val	Ile	Asn	Leu	Ser	Phe	Thr	Val	Leu	Ala	Ile	Met	Thr	Val	
25					325					330					335		
	Asp	Lys	Phe	Gly	Arg	Lys	Pro	Leu	Gln	Ile	Ile	Gly	Ala	Leu	Gly	Met	
				340					345					350			
30	Ala	Leu	Gly	Met	Phe	Ser	Leu	Gly	Thr	Ala	Phe	Tyr	Thr	Gln	Ala	Ser	
			355					360					365				
35	Gly	Leu	Ile	Ala	Leu	Leu	Ser	Met	Leu	Phe	Tyr	Val	Ala	Ala	Phe	Ala	
	370						375					380					
	Met	Ser	Trp	Gly	Pro	Val	Cys	Trp	Val	Leu	Leu	Ala	Glu	Ile	Phe	Pro	
40	385					390					395					400	
	Asn	Ala	Ile	Arg	Gly	Lys	Ala	Leu	Ala	Ile	Ala	Val	Ala	Ala	Gln	Trp	
					405					410					415		
45	Leu	Ala	Asn	Tyr	Phe	Val	Ser	Trp	Thr	Phe	Pro	Met	Met	Asp	Lys	Asn	
				420					425					430			
	Ser	Trp	Leu	Val	Ser	His	Phe	His	Asn	Gly	Phe	Ser	Tyr	Trp	Ile	Tyr	
50			435					440					445				
	Gly	Cys	Met	Gly	Ile	Leu	Ala	Ala	Leu	Phe	Met	Trp	Lys	Phe	Val	Pro	
	450						455					460					
55	Glu	Thr	Lys	Gly	Lys	Thr	Leu	Glu	Glu	Leu	Glu	Lys	Leu	Trp	Thr	Pro	

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465

470

475

480

5 Ala Glu Glu Lys Thr Pro Lys Ala Ala Ile Gln
485 490

<210> 44

<211> 491

10 <212> PRT

<213> Citrobacter youngae

<400> 44

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Met Asn Thr Gln Tyr Asn Ser Ser Tyr Ile Phe Ser Ile Thr Leu Val
1 5 10 15

Ala Thr Leu Gly Gly Leu Leu Phe Gly Tyr Asp Thr Ala Val Ile Ser
20 25 30

Gly Thr Val Glu Ser Leu Asn Thr Val Phe Val Ala Pro Gln His Leu
35 40 45

Ser Glu Ser Ala Ala Asn Ser Leu Leu Gly Phe Cys Val Ala Ser Ala
50 55 60

Leu Ile Gly Cys Ile Ile Gly Gly Ala Leu Gly Gly Tyr Cys Ser Asn
65 70 75 80

Arg Phe Gly Arg Arg Asp Ser Leu Lys Ile Ala Ala Leu Leu Phe Phe
85 90 95

Ile Ser Gly Ile Gly Ser Ala Trp Pro Glu Leu Gly Phe Thr Thr Ile
100 105 110

Asn Pro Asp Asn Ala Val Pro Val Tyr Leu Ala Gly Tyr Val Pro Glu
115 120 125

Phe Val Ile Tyr Arg Ile Ile Gly Gly Ile Gly Val Gly Leu Ala Ser
130 135 140

Met Leu Ser Pro Met Tyr Ile Ala Glu Leu Ala Pro Ala His Ile Arg
145 150 155 160

Gly Lys Leu Val Ser Phe Asn Gln Phe Ala Ile Ile Phe Gly Gln Leu
165 170 175

Leu Val Tyr Cys Val Asn Tyr Phe Ile Ala Arg Ser Gly Asp Ala Asn
180 185 190

Trp Leu Asn Thr Asp Gly Trp Arg Tyr Met Phe Ala Ser Glu Ser Ile

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	195						200						205				
5	Pro	Ala	Leu	Leu	Phe	Leu	Leu	Leu	Leu	Tyr	Thr	Val	Pro	Glu	Ser	Pro	
	210						215					220					
	Arg	Trp	Leu	Met	Ala	Arg	Gly	Lys	His	Glu	Gln	Ala	Glu	Gly	Ile	Leu	
	225					230					235					240	
10	Arg	Lys	Ile	Met	Gly	Asn	Ser	Leu	Ala	Thr	Gln	Ala	Met	Gln	Glu	Ile	
					245					250					255		
15	Asn	Gln	Ser	Leu	Glu	His	Gly	Arg	Lys	Thr	Gly	Gly	Arg	Leu	Leu	Met	
				260					265					270			
	Phe	Gly	Val	Gly	Val	Ile	Val	Ile	Gly	Val	Met	Leu	Ser	Ile	Phe	Gln	
20			275					280					285				
	Gln	Phe	Val	Gly	Ile	Asn	Val	Val	Leu	Tyr	Tyr	Ala	Pro	Glu	Val	Phe	
		290					295					300					
25	Lys	Thr	Leu	Gly	Ala	Ser	Thr	Asp	Val	Ala	Leu	Leu	Gln	Thr	Ile	Ile	
	305					310					315					320	
30	Val	Gly	Val	Ile	Asn	Leu	Ser	Phe	Thr	Val	Leu	Ala	Ile	Met	Thr	Val	
					325					330					335		
	Asp	Lys	Phe	Gly	Arg	Lys	Pro	Leu	Gln	Ile	Ile	Gly	Ala	Leu	Gly	Met	
35				340					345					350			
	Ala	Leu	Gly	Met	Phe	Ser	Leu	Gly	Thr	Ala	Phe	Tyr	Thr	Gln	Ala	Ser	
			355					360					365				
40	Gly	Leu	Ile	Ala	Leu	Leu	Ser	Met	Leu	Phe	Tyr	Val	Ala	Ala	Phe	Ala	
		370					375					380					
	Met	Ser	Trp	Gly	Pro	Val	Cys	Trp	Val	Leu	Leu	Ala	Glu	Ile	Phe	Pro	
45	385					390					395					400	
	Asn	Ala	Ile	Arg	Gly	Lys	Ala	Leu	Ala	Ile	Ala	Val	Ala	Ala	Gln	Trp	
					405					410					415		
50	Leu	Ala	Asn	Tyr	Phe	Val	Ser	Trp	Thr	Phe	Pro	Met	Met	Asp	Lys	Asn	
				420					425					430			
55	Ser	Trp	Leu	Val	Ser	His	Phe	His	Asn	Gly	Phe	Ser	Tyr	Trp	Ile	Tyr	
			435					440					445				

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Gly Cys Met Gly Ile Leu Ala Ala Leu Phe Met Trp Lys Phe Val Pro
450 455 460

5 Glu Thr Lys Gly Lys Thr Leu Glu Glu Leu Glu Glu Leu Trp Glu Pro
465 470 475 480

10 Ala Ala Glu Lys Ala Lys Pro Ala Ser Ala Gln
485 490

<210> 45
<211> 484
<212> PRT
15 <213> Acidobacterium capsulatum

<400> 45

20 Met Ala Arg Leu Asn Lys Tyr Val Val Phe Leu Ala Leu Ile Ala Thr
1 5 10 15

25 Phe Gly Gly Leu Leu Phe Gly Tyr Asp Thr Ala Val Ile Asn Gly Ala
20 25 30

30 Val Asp Ser Leu Lys Ala Tyr Phe Ile Asn Pro Arg Phe Ser Asp Leu
35 40 45

30 Ala Asn Pro Ala Gln Ala Asp Ala Ala Ser Ser Leu Leu Gly Phe Val
50 55 60

35 Val Ser Ser Ala Leu Ile Gly Cys Ile Ile Gly Gly Leu Met Gly Gly
65 70 75 80

40 Trp Val Ser Thr Val Ile Gly Arg Lys Arg Gly Leu Val Ile Ala Ala
85 90 95

45 Val Leu Phe Leu Ile Ser Ala Leu Gly Ala Ser Ala Pro Glu Phe Pro
100 105 110

45 Phe Ala Pro Ile Gly His Gly Gly Pro Ala Tyr Met Trp Asn Phe Val
115 120 125

50 Ile Tyr Arg Ile Leu Gly Gly Ile Gly Val Gly Leu Ala Ser Met Leu
130 135 140

55 Ser Pro Met Tyr Ile Ala Glu Ile Ala Pro Pro Lys Val Arg Gly Asn
145 150 155 160

Leu Val Ala Trp Asn Gln Phe Ala Ile Ile Phe Gly Met Leu Val Ile
165 170 175

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Tyr Phe Val Asn Tyr Gly Ile Ser Lys Gly Gly Asn Gly Asp Ala Trp
 180 185 190
 5 Leu Asn Ser Ile Gly Trp Arg Tyr Met Phe Leu Ser Gly Ala Ile Pro
 195 200 205
 10 Ala Ser Ile Phe Leu Leu Leu Leu Leu Phe Val Pro Glu Thr Pro Arg
 210 215 220
 Tyr Leu Met Met Lys Gly Gln Glu Ala Lys Ala Arg Thr Val Leu Asp
 225 230 235 240
 15 Lys Leu Val Thr Lys Glu Glu Ala Asp Arg Glu Leu Arg Glu Ile Arg
 245 250 255
 20 Ala Ser Leu Ser Gln Asn His Ser Gly Lys Leu Phe Ser Phe Gly Ala
 260 265 270
 Phe Leu Ile Phe Ser Gly Met Leu Leu Ser Ile Phe Gln Gln Phe Val
 275 280 285
 25 Gly Ile Asn Val Val Leu Tyr Tyr Ala Thr Asp Ile Phe Lys Gly Met
 290 295 300
 30 Gly Met Ser Thr Asn Ala Ala Leu Met Gln Thr Ile Ile Val Gly Ala
 305 310 315 320
 Val Asn Leu Thr Phe Thr Val Ile Ala Ile Leu Thr Val Asp Arg Phe
 325 330 335
 35 Gly Arg Arg Pro Leu Gln Val Val Gly Gly Leu Ile Met Ala Ala Ser
 340 345 350
 40 Met Thr Trp Leu Gly Ile Glu Leu Trp Thr Gly Gly Lys Gly Leu Gly
 355 360 365
 45 Ala Leu Ile Ala Met Leu Val Tyr Thr Ala Gly Phe Ala Val Ser Trp
 370 375 380
 Gly Pro Val Thr Trp Val Leu Leu Ser Glu Ile Phe Pro Asn Gln Ile
 385 390 395 400
 50 Arg Gly Lys Ala Met Ala Ile Ala Val Ala Val Gln Trp Val Ala Asn
 405 410 415
 55 Tyr Leu Val Ser Trp Thr Phe Pro Ile Leu Asn Asn Asn Pro Phe Leu
 420 425 430

Val Lys His Phe His His Gly Phe Ala Tyr Trp Ile Tyr Gly Val Met
 435 440 445
 5
 Ser Ile Leu Ala Ala Leu Phe Val Trp Arg Lys Val Pro Glu Thr Lys
 450 455 460
 10
 Gly Arg Thr Leu Glu Gln Met Glu Ser Leu Trp Gly Ser Leu Lys Lys
 465 470 475 480
 Ala Ala Ile Gly
 15
 <210> 46
 <211> 484
 <212> PRT
 <213> Shigella flexneri
 20
 <400> 46
 Met Tyr Ile Gly Ile Asp Leu Gly Thr Ser Gly Val Lys Val Ile Leu
 1 5 10 15
 25
 Leu Asn Glu Gln Gly Glu Val Val Ala Ser Gln Thr Glu Lys Leu Thr
 20 25 30
 30
 Val Ser Arg Pro His Pro Leu Trp Ser Glu Gln Asp Pro Glu Gln Trp
 35 40 45
 35
 Trp Gln Ala Thr Asp Arg Ala Met Lys Ala Leu Gly Asp Gln His Ser
 50 55 60
 40
 Leu Gln Asp Val Lys Ala Leu Gly Ile Ala Gly Gln Met His Gly Ala
 65 70 75 80
 45
 Thr Leu Leu Asp Ala Gln Gln Arg Val Leu Arg Pro Ala Ile Leu Trp
 85 90 95
 50
 Asn Asp Gly Arg Cys Ala Gln Glu Cys Thr Leu Leu Glu Ala Arg Val
 100 105 110
 55
 Pro Gln Ser Arg Val Ile Thr Gly Asn Leu Met Met Pro Gly Phe Thr
 115 120 125
 Ala Pro Lys Leu Leu Trp Val Gln Arg His Glu Pro Glu Ile Phe Arg
 130 135 140
 55
 Gln Ile Asp Lys Val Leu Leu Pro Lys Asp Tyr Leu Arg Leu Arg Met
 145 150 155 160

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5 Thr Gly Glu Phe Ala Ser Asp Met Ser Asp Ala Ala Gly Thr Met Trp
165 170 175

10 Leu Asp Val Ala Lys Arg Asp Trp Ser Asp Val Met Leu Gln Ala Cys
180 185 190

15 Asp Leu Ser Arg Asp Gln Met Pro Ala Leu Tyr Glu Gly Ser Glu Ile
195 200 205

20 Thr Gly Ala Leu Leu Pro Glu Val Ala Lys Ala Trp Gly Met Ala Thr
210 215 220

25 Val Pro Val Val Ala Gly Gly Gly Asp Asn Ala Ala Gly Ala Val Gly
225 230 235 240

30 Val Gly Met Val Asp Ala Asn Gln Ala Met Leu Ser Leu Gly Thr Ser
245 250 255

35 Gly Val Tyr Phe Ala Val Ser Glu Gly Phe Leu Ser Lys Pro Glu Ser
260 265 270

40 Ala Val His Ser Phe Cys His Ala Leu Pro Gln Arg Trp His Leu Met
275 280 285

45 Ser Val Met Leu Ser Ala Ala Ser Cys Leu Asp Trp Ala Ala Lys Leu
290 295 300

50 Thr Gly Leu Cys Asn Val Pro Ala Leu Ile Ala Ala Ala Gln Gln Ala
305 310 315 320

55 Asp Glu Ser Ala Glu Pro Val Trp Phe Leu Pro Tyr Leu Ser Gly Glu
325 330 335

60 Arg Thr Pro His Asn Asn Pro Gln Ala Lys Gly Val Phe Phe Gly Leu
340 345 350

65 Thr His Gln His Gly Pro Asn Glu Leu Ala Arg Ala Val Leu Glu Gly
355 360 365

70 Val Gly Tyr Ala Leu Ala Asp Gly Met Asp Val Val His Ala Cys Gly
370 375 380

75 Ile Lys Pro Gln Ser Val Thr Leu Ile Gly Gly Gly Ala Arg Ser Glu
385 390 395 400

80 Tyr Trp Arg Gln Met Leu Ala Asp Ile Ser Gly Gln Gln Leu Asp Tyr
405 410 415

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Arg Thr Gly Gly Asp Val Gly Pro Ala Leu Gly Ala Ala Arg Leu Ala
420 425 430

5 Gln Ile Ala Ala Asn Pro Glu Lys Ser Leu Ile Glu Leu Leu Pro Gln
435 440 445

10 Leu Pro Leu Glu Gln Ser His Leu Pro Asp Ala Gln Arg Tyr Ala Ala
450 455 460

Tyr Gln Pro Arg Arg Glu Thr Phe Arg Arg Leu Tyr Gln Gln Leu Leu
465 470 475 480

15 Pro Leu Met Ala

<210> 47
<211> 484
20 <212> PRT
<213> Citrobacter rodentium

<400> 47

25 Met Tyr Ile Gly Ile Asp Leu Gly Thr Ser Gly Val Lys Ala Ile Leu
1 5 10 15

30 Leu Asn Glu Gln Gly Glu Val Val Ala Ala Gln Thr Glu Lys Leu Thr
20 25 30

35 Val Ser Arg Pro His Pro Leu Trp Ser Glu Gln Asp Pro Glu Gln Trp
35 40 45

Trp Leu Ala Thr Asp Arg Ala Val Lys Ala Leu Gly Lys Gln His Ser
50 55 60

40 Leu Ser Glu Val Lys Ala Leu Gly Ile Ala Gly Gln Met His Gly Ala
65 70 75 80

45 Thr Leu Leu Asp Asp Gln Gln Arg Val Leu Arg Pro Ala Ile Leu Trp
85 90 95

Asn Asp Gly Arg Cys Gly Glu Glu Cys Ala Leu Leu Glu Ala Gln Ala
100 105 110

50 Pro Gln Ser Arg Ala Ile Thr Gly Asn Leu Met Met Pro Gly Phe Thr
115 120 125

55 Ala Pro Lys Leu Leu Trp Val Lys Cys His Glu Pro Glu Ile Phe Arg
130 135 140

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Gln Val Ala Lys Val Leu Leu Pro Lys Asp Tyr Leu Arg Leu Arg Met
 145 150 155 160

5 Thr Gly Glu Phe Ala Ser Asp Met Ser Asp Ala Ala Gly Thr Met Trp
 165 170 175

10 Leu Asp Val Glu Lys Arg Asp Trp Ser Asp Val Met Leu Glu Ala Cys
 180 185 190

15 Gly Leu Thr Arg Asp His Met Pro Ala Leu Phe Glu Gly Ser Asp Ile
 195 200 205

Ser Gly Thr Leu Leu Pro Glu Ile Ala Thr Ala Trp Gly Met Pro Ala
 210 215 220

20 Ala Pro Val Val Ala Gly Gly Gly Asp Asn Ala Ala Gly Ala Val Gly
 225 230 235 240

25 Val Gly Met Ile Asp Ala Asn Gln Ala Met Leu Ser Leu Gly Thr Ser
 245 250 255

Gly Val Tyr Phe Ala Val Ser Asp Gly Phe Leu Ser Lys Pro Glu Ser
 260 265 270

30 Ala Val His Ser Phe Cys His Ala Leu Pro Gly Arg Trp His Leu Met
 275 280 285

35 Ser Val Met Leu Ser Ala Ala Ser Cys Leu Asp Trp Ala Ala Lys Leu
 290 295 300

40 Thr Gly Leu Glu Thr Val Pro Ala Leu Leu Asp Ala Ala Gln Thr Ala
 305 310 315 320

Asp Thr Ser Ala Asp Pro Val Trp Phe Leu Pro Tyr Leu Ser Gly Glu
 325 330 335

45 Arg Thr Pro His Asn Asn Pro Gln Ala Lys Gly Val Phe Phe Gly Leu
 340 345 350

50 Thr His Gln His Gly Pro Ala Glu Leu Ala Arg Ala Val Leu Glu Gly
 355 360 365

Val Gly Tyr Ala Leu Ala Asp Gly Met Asp Val Val His Ala Cys Gly
 370 375 380

55 Val Lys Pro Gln Ser Ile Thr Leu Ile Gly Gly Gly Ala Arg Ser Ala

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	385		390		395		400									
5	Tyr	Trp	Arg	Gln	Met	Leu	Ala	Asp	Ile	Ser	Gly	Leu	Gln	Leu	Asp	Tyr
				405						410					415	
10	Arg	Thr	Gly	Gly	Asp	Val	Gly	Pro	Ala	Leu	Gly	Ala	Ala	Arg	Leu	Ala
				420					425					430		
15	Gln	Ile	Ala	Met	Asn	Pro	Glu	Lys	Pro	Leu	Ser	Asp	Leu	Leu	Pro	Gln
			435					440					445			
20	Leu	Pro	Leu	Glu	Gln	Thr	His	Arg	Pro	Asp	Ala	Gln	Arg	His	Ala	Asp
		450					455					460				
25	Tyr	Gln	Gln	Arg	Arg	Glu	Thr	Phe	Arg	Arg	Leu	Tyr	Gln	Gln	Leu	Leu
	465					470					475					480
30	Pro	Leu	Met	Ser												
35	<210>	48														
40	<211>	356														
45	<212>	PRT														
50	<213>	Shigella flexneri														
55	<400>	48														

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	Met	Leu	Lys	Ile	Gly	Tyr	Val	Tyr	Arg	Gly	Asp	Cys	Tyr	Leu	Leu	Lys
	1				5					10					15	
5	Leu	Ser	Ser	Asn	Tyr	Arg	Arg	Pro	Tyr	Thr	Met	Lys	Ile	Lys	Asn	Ile
				20					25					30		
	Leu	Leu	Thr	Leu	Cys	Thr	Ser	Leu	Leu	Leu	Thr	Asn	Val	Ala	Ala	His
10			35					40					45			
	Ala	Lys	Glu	Val	Lys	Ile	Gly	Met	Ala	Ile	Asp	Asp	Leu	Arg	Leu	Glu
	50						55					60				
15	Arg	Trp	Gln	Lys	Asp	Arg	Asp	Ile	Phe	Val	Lys	Lys	Ala	Glu	Ser	Leu
	65					70					75					80
	Gly	Ala	Lys	Val	Phe	Val	Gln	Ser	Ala	Asn	Gly	Asn	Glu	Glu	Thr	Gln
20					85					90					95	
	Met	Ser	Gln	Ile	Glu	Asn	Met	Ile	Asn	Arg	Gly	Val	Asp	Val	Leu	Val
25				100					105					110		
	Ile	Ile	Pro	Tyr	Asn	Gly	Gln	Val	Leu	Ser	Asn	Val	Val	Lys	Glu	Ala

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	115	120	125
5	Lys Gln Glu Gly Ile Lys Val Leu Ala Tyr Asp Arg Met Ile Asn Asp 130 135 140		
10	Ala Asp Ile Asp Phe Tyr Ile Ser Phe Asp Asn Glu Lys Val Gly Glu 145 150 155 160		
15	Leu Gln Ala Lys Ala Leu Val Asp Ile Val Pro Gln Gly Asn Tyr Phe 165 170 175		
20	Leu Met Gly Gly Ser Pro Val Asp Asn Asn Ala Lys Leu Phe Arg Ala 180 185 190		
25	Gly Gln Met Lys Val Leu Lys Pro Tyr Val Asp Ser Gly Lys Ile Lys 195 200 205		
30	Val Val Gly Asp Gln Trp Val Asp Gly Trp Leu Pro Glu Asn Ala Leu 210 215 220		
35	Lys Ile Met Glu Asn Ala Leu Thr Ala Asn Asn Asn Lys Ile Asp Ala 225 230 235 240		
40	Val Val Ala Ser Asn Asp Ala Thr Ala Gly Gly Ala Ile Gln Ala Leu 245 250 255		
45	Ser Ala Gln Gly Leu Ser Gly Lys Val Ala Ile Ser Gly Gln Asp Ala 260 265 270		
50	Asp Leu Ala Gly Ile Lys Arg Ile Ala Ala Gly Thr Gln Thr Met Thr 275 280 285		
55	Val Tyr Lys Pro Ile Thr Leu Leu Ala Asn Thr Ala Ala Glu Ile Ala 290 295 300		
	Val Glu Leu Gly Asn Gly Gln Glu Pro Lys Ala Asp Thr Ser Leu Asn 305 310 315 320		
	Asn Gly Leu Lys Asp Val Pro Ser Arg Leu Leu Thr Pro Ile Asp Val 325 330 335		
	Asn Lys Asn Asn Ile Lys Asp Thr Val Ile Lys Asp Gly Phe His Lys 340 345 350		
	Glu Ser Glu Leu 355		

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<210> 49
<211> 330
<212> PRT
<213> Citrobacter koseri

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<400> 49

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Met Lys Ile Lys Asn Ile Leu Leu Thr Val Cys Thr Ser Leu Leu Leu
 1 5 10 15
 5
 Thr Ser Ile Thr Gly His Ala Lys Glu Val Lys Ile Gly Met Ala Ile
 20 25 30
 10
 Asp Asp Leu Arg Leu Glu Arg Trp Gln Lys Asp Arg Asp Ile Phe Val
 35 40 45
 15
 Ser Lys Ala Glu Ser Leu Gly Ala Lys Val Phe Val Gln Ser Ala Asn
 50 55 60
 20
 Gly Asn Glu Glu Thr Gln Met Ser Gln Ile Glu Asn Met Ile Asn Arg
 65 70 75 80
 25
 Gly Val Asp Val Leu Val Ile Ile Pro Tyr Asn Gly Gln Val Leu Ser
 85 90 95
 30
 Asn Val Val Lys Glu Ala Lys Gln Glu Gly Ile Lys Val Leu Ala Tyr
 100 105 110
 35
 Asp Arg Met Ile Asn Asp Ala Asp Ile Asp Phe Tyr Ile Ser Phe Asp
 115 120 125
 40
 Asn Glu Lys Val Gly Glu Leu Gln Ala Gln Ala Leu Val Asp Lys Val
 130 135 140
 45
 Pro Glu Gly Asn Tyr Phe Leu Met Gly Gly Ser Pro Val Asp Asn Asn
 145 150 155 160
 50
 Ala Lys Leu Phe Arg Ala Gly Gln Met Lys Val Leu Lys Pro Tyr Ile
 165 170 175
 55
 Asp Ser Gly Lys Ile Asn Val Val Gly Asp Gln Trp Val Asp Gly Trp
 180 185 190
 60
 Leu Pro Glu Asn Ala Leu Lys Ile Met Glu Asn Ala Leu Thr Ala Asn
 195 200 205
 65
 Asn Asn Lys Ile Asp Ala Val Val Ala Ser Asn Asp Ala Thr Ala Gly
 210 215 220

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	Gly	Ala	Ile	Gln	Ala	Leu	Ser	Ala	Gln	Gly	Leu	Ser	Gly	Lys	Val	Ala	225	230	235	240
5	Ile	Ser	Gly	Gln	Asp	Ala	Asp	Leu	Ala	Gly	Ile	Lys	Arg	Ile	Val	Ala	245	250	255	
10	Gly	Thr	Gln	Thr	Met	Thr	Val	Tyr	Lys	Pro	Ile	Thr	Leu	Leu	Ala	Asn	260	265	270	
15	Thr	Ala	Ala	Glu	Ile	Ala	Val	Glu	Leu	Gly	Asn	Gly	Gln	Gln	Pro	Lys	275	280	285	
20	Ala	Asp	Ala	Thr	Leu	Asn	Asn	Gly	Leu	Lys	Asp	Val	Pro	Ser	Arg	Leu	290	295	300	
25	Leu	Thr	Pro	Ile	Asp	Val	Asn	Lys	Thr	Asn	Ile	Asp	Glu	Thr	Val	Ile	305	310	315	320
30	Lys	Asp	Gly	Phe	His	Lys	Lys	Ser	Asp	Leu	325	330								
	<210> 50																			
	<211> 513																			
	<212> PRT																			
	<213> Shigella boydii																			
	<400> 50																			
35	Met	Pro	Tyr	Leu	Leu	Glu	Met	Lys	Asn	Ile	Thr	Lys	Thr	Phe	Gly	Ser	1	5	10	15
40	Val	Lys	Ala	Ile	Asp	Asn	Val	Ser	Leu	Arg	Leu	Asn	Ala	Gly	Glu	Ile	20	25	30	
45	Val	Ser	Leu	Cys	Gly	Glu	Asn	Gly	Ser	Gly	Lys	Ser	Thr	Leu	Met	Lys	35	40	45	
50	Val	Leu	Cys	Gly	Ile	Tyr	Pro	His	Gly	Ser	Tyr	Glu	Gly	Glu	Ile	Ile	50	55	60	
55	Phe	Ala	Gly	Glu	Glu	Ile	Gln	Ala	Ser	His	Ile	Arg	Asp	Thr	Glu	Arg	65	70	75	80
	Lys	Gly	Ile	Ala	Ile	Ile	His	Gln	Glu	Leu	Ala	Leu	Val	Lys	Glu	Leu	85	90	95	
	Thr	Val	Leu	Glu	Asn	Ile	Phe	Leu	Gly	Asn	Glu	Ile	Thr	His	Asn	Gly	100	105	110	

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	Ile	Met	Asp	Tyr	Asp	Leu	Met	Thr	Leu	Arg	Cys	Gln	Lys	Leu	Leu	Ala	
			115					120					125				
5	Gln	Val	Ser	Leu	Ser	Ile	Ser	Pro	Asp	Thr	Arg	Val	Gly	Asp	Leu	Gly	
		130					135					140					
10	Leu	Gly	Gln	Gln	Gln	Leu	Val	Glu	Ile	Ala	Lys	Ala	Leu	Asn	Lys	Gln	
	145					150					155					160	
	Val	Arg	Leu	Leu	Ile	Leu	Asp	Glu	Pro	Thr	Ala	Ser	Leu	Thr	Glu	Gln	
					165					170					175		
15	Glu	Thr	Ser	Val	Leu	Leu	Asp	Ile	Ile	Arg	Asp	Leu	Gln	Gln	His	Gly	
				180					185					190			
20	Ile	Ala	Cys	Ile	Tyr	Ile	Ser	His	Lys	Leu	Asn	Glu	Val	Lys	Ala	Ile	
			195					200					205				
	Ser	Asp	Thr	Ile	Cys	Val	Ile	Arg	Asp	Gly	Gln	His	Ile	Gly	Thr	Arg	
		210					215					220					
25	Asp	Ala	Ala	Gly	Met	Ser	Glu	Asp	Asp	Ile	Ile	Thr	Met	Met	Val	Gly	
	225					230					235					240	
30	Arg	Glu	Leu	Thr	Ala	Leu	Tyr	Pro	Asn	Glu	Pro	His	Thr	Thr	Gly	Asp	
					245					250					255		
35	Glu	Ile	Leu	Arg	Ile	Glu	His	Leu	Thr	Ala	Trp	His	Pro	Val	Asn	Arg	
				260					265					270			
	His	Ile	Lys	Arg	Val	Asn	Asp	Val	Ser	Phe	Ser	Leu	Lys	Arg	Gly	Glu	
			275					280					285				
40	Ile	Leu	Gly	Ile	Ala	Gly	Leu	Val	Gly	Ala	Gly	Arg	Thr	Glu	Thr	Ile	
	290						295					300					
45	Gln	Cys	Leu	Phe	Gly	Val	Trp	Pro	Gly	Gln	Trp	Glu	Gly	Lys	Ile	Tyr	
	305					310					315					320	
	Ile	Asp	Gly	Lys	Gln	Val	Asp	Ile	Arg	Asn	Cys	Gln	Gln	Ala	Ile	Ala	
					325					330					335		
50	Gln	Gly	Ile	Ala	Met	Val	Pro	Glu	Asp	Arg	Lys	Arg	Asp	Gly	Ile	Val	
				340					345					350			
55	Pro	Val	Met	Ala	Val	Gly	Lys	Asn	Ile	Thr	Leu	Ala	Ala	Leu	Asn	Lys	
			355					360					365				

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Phe Thr Gly Gly Ile Ser Gln Leu Asp Asp Ala Ala Glu Gln Lys Cys
370 375 380

5 Ile Leu Glu Ser Ile Gln Gln Leu Lys Val Lys Thr Ser Ser Pro Asp
385 390 395 400

10 Leu Ala Ile Gly Arg Leu Ser Gly Gly Asn Gln Gln Lys Ala Ile Leu
405 410 415

15 Ala Arg Cys Leu Leu Leu Asn Pro Arg Ile Leu Ile Leu Asp Glu Pro
420 425 430

20 Thr Arg Gly Ile Asp Ile Gly Val Lys Tyr Glu Ile Tyr Lys Leu Ile
435 440 445

25 Asn Gln Leu Val Gln Gln Gly Ile Ala Val Ile Val Ile Ser Ser Glu
450 455 460

30 Leu Pro Glu Val Leu Gly Leu Ser Asp Arg Val Leu Val Met His Glu
465 470 475 480

Gly Lys Leu Lys Ala Asn Leu Ile Asn His Asn Leu Thr Gln Glu Gln
485 490 495

35 Val Met Glu Ala Ala Leu Arg Ser Glu His His Val Glu Lys Gln Ser
500 505 510

Val

<210> 51
<211> 513
<212> PRT
40 <213> Klebsiella pneumoniae

<400> 51

45 Met Ala Trp Leu Leu Glu Met Lys Asn Ile Thr Lys Thr Phe Gly Ala
1 5 10 15

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

Val Ser Leu Cys Gly Glu Asn Gly Ser Gly Lys Ser Thr Leu Met Lys
35 40 45

55 Val Leu Cys Gly Ile Tyr Pro His Gly Ser Tyr Glu Gly Glu Ile Ile
50 55 60

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	Phe	Ala	Gly	Glu	Thr	Leu	Gln	Ala	Asn	His	Ile	Arg	Asp	Thr	Glu	Arg	
	65					70					75					80	
5	Lys	Gly	Ile	Ala	Ile	Ile	His	Gln	Glu	Leu	Ala	Leu	Val	Lys	His	Leu	
					85					90					95		
	Thr	Val	Leu	Glu	Asn	Ile	Phe	Leu	Gly	Ala	Glu	Ile	Ser	Arg	His	Gly	
10				100					105					110			
	Leu	Leu	Asp	Tyr	Glu	Thr	Met	Thr	Leu	Arg	Cys	Gln	Lys	Leu	Leu	Ala	
			115					120					125				
15	Gln	Val	Asn	Leu	Pro	Ile	Ser	Pro	Asp	Thr	Arg	Val	Gly	Asp	Leu	Gly	
		130					135					140					
	Leu	Gly	Gln	Gln	Gln	Leu	Val	Glu	Ile	Ala	Lys	Ala	Leu	Asn	Lys	Gln	
20		145				150					155					160	
	Val	Arg	Leu	Leu	Ile	Leu	Asp	Glu	Pro	Thr	Ala	Ser	Leu	Thr	Glu	Gln	
					165					170					175		
25	Glu	Thr	Ala	Thr	Leu	Leu	Thr	Ile	Ile	Arg	Asp	Leu	Gln	Asn	His	Gly	
				180					185					190			
	Ile	Ala	Cys	Ile	Tyr	Ile	Ser	His	Lys	Leu	Asn	Glu	Val	Lys	Ala	Ile	
30			195					200					205				
	Ser	Asp	Thr	Ile	Cys	Val	Ile	Arg	Asp	Gly	Gln	His	Ile	Gly	Thr	Arg	
35		210					215					220					
	Asp	Ala	Ser	Gly	Met	Ser	Glu	Asp	Asp	Ile	Ile	Thr	Met	Met	Val	Gly	
		225				230					235					240	
40	Arg	Glu	Leu	Thr	Ala	Leu	Tyr	Pro	Ser	Glu	Pro	His	Ala	His	Gly	Glu	
					245					250					255		
	Glu	Ile	Leu	Arg	Val	Glu	His	Leu	Thr	Ala	Trp	His	Pro	Val	Asn	Arg	
45				260					265					270			
	His	Ile	Lys	Arg	Val	Asn	Asp	Val	Ser	Phe	Ser	Leu	Arg	Arg	Gly	Glu	
			275					280					285				
50	Ile	Leu	Gly	Ile	Ala	Gly	Leu	Val	Gly	Ala	Gly	Arg	Thr	Glu	Ala	Val	
		290					295					300					
	Gln	Cys	Leu	Phe	Gly	Val	Trp	Pro	Gly	Arg	Trp	Gln	Gly	Glu	Ile	Phe	
55		305				310					315					320	

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	Ile	Asp	Gly	Gln	Pro	Val	Ser	Ile	Ser	Asn	Cys	Gln	Gln	Ala	Ile	Ala	
					325					330					335		
5	His	Gly	Ile	Ala	Met	Val	Pro	Glu	Asp	Arg	Lys	Lys	Asp	Gly	Ile	Val	
				340					345					350			
10	Pro	Val	Met	Ala	Val	Gly	Lys	Asn	Ile	Thr	Leu	Ala	Ala	Leu	Asn	Gln	
			355					360					365				
	Phe	Thr	Gly	Ala	Met	Ser	Ser	Leu	Asp	Asp	Ala	Ala	Glu	Gln	His	Cys	
		370					375					380					
15	Ile	Gln	Gln	Ser	Ile	Gln	Arg	Leu	Lys	Ile	Lys	Thr	Ser	Ser	Pro	Glu	
	385					390					395					400	
20	Leu	Ala	Ile	Gly	Arg	Leu	Ser	Gly	Gly	Asn	Gln	Gln	Lys	Ala	Ile	Leu	
					405					410					415		
25	Ala	Arg	Cys	Leu	Leu	Leu	Asn	Pro	Arg	Ile	Leu	Ile	Leu	Asp	Glu	Pro	
				420					425					430			
30	Thr	Arg	Gly	Ile	Asp	Ile	Gly	Ala	Lys	Tyr	Glu	Ile	Tyr	Lys	Leu	Ile	
			435					440					445				
	Asn	Gln	Leu	Val	Gln	Gln	Gly	Ile	Ala	Val	Ile	Val	Ile	Ser	Ser	Glu	
	450						455					460					
35	Leu	Pro	Glu	Val	Leu	Gly	Leu	Ser	Asp	Arg	Val	Leu	Val	Met	His	Glu	
	465					470					475					480	
40	Gly	Arg	Leu	Lys	Ala	Asn	Leu	Val	Asn	Gln	His	Leu	Thr	Gln	Glu	Gln	
					485					490					495		
45	Val	Met	Glu	Ala	Ala	Leu	Arg	Ser	Glu	Arg	His	Val	Glu	Glu	His	Val	
				500					505					510			
50	Val																
	<210>	52															
	<211>	393															
	<212>	PRT															
	<213>	Citrobacter koseri															
55	<400>	52															
	Met	Ser	Lys	Asn	Asn	Pro	Ser	Glu	Val	Lys	Leu	Ser	Ala	Pro	Leu	Thr	
	1				5					10					15		

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Gly Gly Phe Pro Gly Leu Lys Ser Leu Asn Leu Gln Val Phe Val Met
 20 25 30
 5
 Ile Ala Ala Ile Ile Val Ile Met Leu Phe Phe Thr Trp Thr Thr Glu
 35 40 45
 10
 Gly Ala Tyr Leu Ser Ala Arg Asn Val Ser Asn Leu Leu Arg Gln Thr
 50 55 60
 15
 Ala Ile Thr Gly Ile Leu Ala Val Gly Met Val Phe Val Ile Ile Ser
 65 70 75 80
 20
 Ala Glu Ile Asp Leu Ser Val Gly Ser Met Met Gly Leu Leu Gly Gly
 85 90 95
 25
 Val Ala Ala Ile Cys Asp Val Trp Leu Gly Trp Pro Leu Pro Leu Thr
 100 105 110
 30
 Ile Val Val Thr Leu Ala Leu Gly Leu Leu Leu Gly Ala Trp Asn Gly
 115 120 125
 35
 Trp Trp Val Ala Tyr Arg Lys Val Pro Ser Phe Ile Val Thr Leu Ala
 130 135 140
 40
 Gly Met Leu Ala Phe Arg Gly Ile Leu Ile Gly Ile Thr Asn Gly Thr
 145 150 155 160
 45
 Thr Val Ser Pro Thr Ser Ser Ala Met Ser Gln Ile Gly Gln Ser Tyr
 165 170 175
 50
 Leu Ser Asn Gly Leu Gly Phe Thr Ile Gly Ala Ile Ser Leu Met Ala
 180 185 190
 55
 Phe Ile Gly Trp Gln Trp Arg Gly Arg Met Arg Arg Gln Ala Leu Ala
 195 200 205
 60
 Leu Ser Thr Pro Ala Ser Thr Ala Val Val Gly Arg Gln Ala Leu Thr
 210 215 220
 65
 Ala Val Ile Val Leu Gly Ala Ile Trp Leu Leu Asn Asp Tyr Arg Gly
 225 230 235 240
 70
 Val Pro Thr Pro Val Leu Leu Leu Val Phe Leu Leu Leu Gly Gly Met
 245 250 255
 75
 Phe Met Ala Thr Arg Thr Ala Phe Gly Arg Arg Ile Tyr Ala Ile Gly

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	260	265	270
5	Gly Asn Leu Glu Ala Ala Arg Leu Ser Gly Ile Asn Val Glu Arg Thr 275 280 285		
10	Lys Leu Ala Val Phe Ala Ile Asn Gly Leu Met Val Ala Ile Ala Gly 290 295 300		
15	Leu Ile Leu Ser Ser Arg Leu Gly Ala Gly Ser Pro Ser Ala Gly Asn 305 310 315 320		
20	Ile Ala Glu Leu Asp Ala Ile Ala Ala Cys Val Ile Gly Gly Thr Ser 325 330 335		
25	Leu Ala Gly Gly Val Gly Ser Val Ala Gly Ala Val Met Gly Ala Phe 340 345 350		
30	Ile Met Ala Ser Leu Asp Asn Gly Met Ser Met Met Asp Val Pro Thr 355 360 365		
35	Phe Trp Gln Tyr Ile Val Lys Gly Ala Ile Leu Leu Leu Ala Val Trp 370 375 380		
40	Met Asp Ser Ala Thr Lys Arg Arg Ser 385 390		

<210> 53

<211> 393

<212> PRT

<213> Shigella dysenteriae

<400> 53

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	Met	Ser	Lys	Ser	Asn	Pro	Ser	Glu	Val	Lys	Leu	Ala	Val	Pro	Thr	Ser
	1				5					10					15	
5	Gly	Gly	Phe	Ser	Gly	Leu	Lys	Ser	Leu	Asn	Leu	Gln	Val	Phe	Val	Met
				20					25					30		
	Ile	Ala	Ala	Ile	Ile	Ala	Ile	Met	Leu	Phe	Phe	Thr	Trp	Thr	Thr	Asp
10			35					40					45			
	Gly	Ala	Tyr	Leu	Ser	Ala	Arg	Asn	Val	Ser	Asn	Leu	Leu	His	Gln	Thr
		50					55					60				
15																
	Ala	Ile	Thr	Gly	Ile	Leu	Ala	Val	Gly	Met	Val	Phe	Val	Ile	Ile	Ser
	65					70					75					80
20																
	Ala	Glu	Ile	Asp	Leu	Ser	Val	Gly	Ser	Met	Met	Gly	Leu	Leu	Gly	Gly
25																
30																
35																
40																
45																
50																
55																

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	85	90	95
5	Val Ala Ala Ile Cys Asp Val Trp Leu Gly Trp Pro Leu Pro Leu Thr 100 105 110		
10	Ile Ile Val Thr Leu Val Leu Gly Leu Leu Leu Gly Ala Trp Asn Gly 115 120 125		
15	Trp Trp Val Ala Tyr Arg Lys Val Pro Ser Phe Ile Val Thr Leu Ala 130 135 140		
20	Gly Met Leu Ala Phe Arg Gly Ile Leu Ile Gly Ile Thr Asn Gly Thr 145 150 155 160		
25	Thr Val Ser Pro Thr Ser Ala Ala Met Ser Gln Ile Gly Gln Ser Tyr 165 170 175		
30	Leu Pro Ala Ser Thr Gly Phe Ile Ile Gly Ala Leu Gly Leu Met Ala 180 185 190		
35	Phe Val Gly Trp Gln Trp Arg Gly Arg Met Arg Arg Gln Ala Leu Gly 195 200 205		
40	Leu Gln Ser Pro Ala Ser Thr Ala Val Val Gly Arg Gln Ala Leu Thr 210 215 220		
45	Ala Ile Ile Val Leu Gly Ala Ile Trp Leu Leu Asn Asp Tyr Arg Gly 225 230 235 240		
50	Val Pro Thr Pro Val Leu Leu Leu Thr Leu Leu Leu Leu Gly Gly Met 245 250 255		
55	Phe Met Ala Thr Arg Thr Ala Phe Gly Arg Arg Ile Tyr Ala Ile Gly 260 265 270		
	Gly Asn Leu Glu Ala Ala Arg Leu Ser Gly Ile Asn Val Glu Arg Thr 275 280 285		
	Lys Leu Ala Val Phe Ala Ile Asn Gly Leu Met Val Ala Ile Ala Gly 290 295 300		
	Leu Ile Leu Ser Ser Arg Leu Gly Ala Gly Ser Pro Ser Ala Gly Asn 305 310 315 320		
	Ile Ala Glu Leu Asp Ala Ile Ala Ala Cys Val Ile Gly Gly Thr Ser 325 330 335		

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Leu Ala Gly Gly Val Gly Ser Val Ala Gly Ala Val Met Gly Ala Phe
340 345 350

5 Ile Met Ala Ser Leu Asp Asn Gly Met Ser Met Met Asp Val Pro Thr
355 360 365

10 Phe Trp Gln Tyr Ile Val Lys Gly Ala Ile Leu Leu Leu Ala Val Trp
370 375 380

Met Asp Ser Ala Thr Lys Arg Arg Ser
385 390

15 <210> 54
<211> 393
<212> PRT
<213> Enterobacter cloacae

20 <400> 54

25 Met Ser Lys Ser Asn Pro Ser Asp Ile Lys Val Ala Val Gln Thr Pro
1 5 10 15

Gly Ala Phe Ala Gly Leu Lys Ala Leu Asn Leu Gln Val Phe Val Met
20 25 30

30 Ile Ala Ala Ile Ile Ala Ile Met Leu Phe Phe Thr Trp Met Thr Asp
35 40 45

35 Gly Ser Tyr Leu Ser Ala Arg Asn Val Ser Asn Leu Leu Arg Gln Thr
50 55 60

40 Ala Ile Thr Gly Ile Leu Ala Val Gly Met Val Phe Val Ile Ile Ser
65 70 75 80

Ala Glu Ile Asp Leu Ser Val Gly Ser Met Met Gly Leu Leu Gly Gly
85 90 95

45 Val Ala Ala Ile Phe Asp Val Trp Leu Gly Trp Pro Leu Pro Leu Thr
100 105 110

50 Val Ala Val Thr Leu Val Leu Gly Leu Val Leu Gly Ala Trp Asn Gly
115 120 125

Trp Trp Val Ala Tyr Arg Lys Val Pro Ser Phe Ile Val Thr Leu Ala
130 135 140

55 Gly Met Leu Ala Phe Arg Gly Val Leu Ile Gly Ile Thr Asn Gly Thr
145 150 155 160

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Thr Val Ser Pro Thr Ser Ala Ala Met Ser Gln Ile Gly Gln Ser Tyr
165 170 175

5 Leu Ser Asp Ser Met Gly Phe Thr Ile Gly Val Val Gly Leu Leu Ala
180 185 190

10 Phe Val Val Trp Gln Trp Arg Gly Arg Met Arg Arg Gln Ser Leu Gly
195 200 205

15 Leu Ala Ser Ser Pro Ser Thr Ser Val Val Gly Arg Gln Ala Leu Thr
210 215 220

Ala Val Ile Val Leu Gly Ala Ile Trp Leu Leu Asn Asp Tyr Arg Gly
225 230 235 240

20 Val Pro Thr Pro Val Leu Leu Leu Ala Leu Leu Leu Leu Gly Gly Met
245 250 255

25 Phe Met Ala Thr Arg Thr Ala Phe Gly Arg Arg Ile Tyr Ala Ile Gly
260 265 270

Gly Asn Leu Glu Ala Ala Arg Leu Ser Gly Ile Asn Val Glu Arg Thr
275 280 285

30 Lys Leu Ala Val Phe Ala Ile Asn Gly Leu Met Val Ala Ile Ala Gly
290 295 300

35 Leu Ile Leu Ser Ser Arg Leu Gly Ala Gly Ser Pro Ser Ala Gly Asn
305 310 315 320

40 Ile Ala Glu Leu Asp Ala Ile Ala Ala Cys Val Ile Gly Gly Thr Ser
325 330 335

Leu Ala Gly Gly Val Gly Ser Val Ala Gly Ala Val Met Gly Ala Phe
340 345 350

45 Ile Met Ala Ser Leu Asp Asn Gly Met Ser Met Met Asp Val Pro Thr
355 360 365

50 Phe Trp Gln Tyr Ile Val Lys Gly Ala Ile Leu Leu Leu Ala Val Trp
370 375 380

55 Met Asp Ser Ala Thr Lys Arg Arg Thr
385 390

<210> 55

<211> 321

<212> PRT

<213> Shigella dysenteriae

<400> 55

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EP 2 593 540 B1

Met Thr Lys Tyr Ala Leu Val Gly Asp Val Gly Gly Thr Asn Ala Arg
 1 5 10 15
 5
 Leu Ala Leu Cys Asp Ile Ala Ser Gly Glu Ile Ser Gln Ala Lys Thr
 20 25 30
 10
 Tyr Ser Gly Leu Asp Tyr Pro Ser Leu Glu Ala Val Ile Arg Val Tyr
 35 40 45
 15
 Leu Glu Glu His Lys Val Glu Val Lys Asp Gly Cys Ile Ala Ile Ala
 50 55 60
 Cys Pro Ile Thr Gly Asp Trp Val Ala Met Thr Asn His Thr Trp Ala
 65 70 75 80
 20
 Phe Ser Ile Ala Glu Met Lys Lys Asn Leu Gly Phe Ser His Leu Glu
 85 90 95
 25
 Ile Ile Asn Asp Phe Thr Ala Val Ser Met Ala Val Pro Met Leu Lys
 100 105 110
 30
 Lys Glu His Leu Ile Gln Phe Gly Gly Ala Glu Pro Ile Glu Gly Lys
 115 120 125
 Pro Ile Ala Val Tyr Gly Ala Gly Thr Gly Leu Gly Val Ala His Leu
 130 135 140
 35
 Val His Val Asp Lys Arg Trp Val Ser Leu Pro Gly Glu Gly Gly His
 145 150 155 160
 40
 Val Asp Phe Ala Pro Asn Ser Glu Glu Glu Ala Ile Ile Leu Glu Ile
 165 170 175
 45
 Leu Arg Ala Glu Ile Gly His Val Ser Ala Glu Arg Val Leu Ser Gly
 180 185 190
 Pro Gly Leu Val Asn Leu Tyr Arg Ala Ile Val Lys Ala Asp Asn Arg
 195 200 205
 50
 Leu Pro Glu Asn Leu Lys Pro Lys Asp Ile Thr Glu Arg Ala Leu Ala
 210 215 220
 55
 Asp Ser Cys Thr Asp Cys Arg Arg Ala Leu Ser Leu Phe Cys Val Ile
 225 230 235 240

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Met Gly Arg Phe Gly Gly Asn Leu Ala Leu Asn Leu Gly Thr Phe Gly
245 250 255

5 Gly Val Phe Ile Ala Gly Gly Ile Val Pro Arg Phe Leu Glu Phe Phe
260 265 270

10 Lys Ala Ser Gly Phe Arg Ala Ala Phe Glu Asp Lys Gly Arg Phe Lys
275 280 285

Glu Tyr Val His Asp Ile Pro Val Tyr Leu Ile Val His Asp Asn Pro
290 295 300

15 Gly Leu Leu Gly Ser Gly Ala His Leu Arg Gln Thr Leu Gly His Ile
305 310 315 320

20 Leu

<210> 56
<211> 321
<212> PRT
25 <213> Escherichia fergusonii

<400> 56

30 Met Thr Lys Tyr Ala Leu Val Gly Asp Val Gly Gly Thr Asn Ala Arg
1 5 10 15

Leu Ala Leu Cys Asp Ile Ala Ser Gly Glu Ile Ser Gln Ala Lys Thr
20 25 30

35 Tyr Ser Gly Leu Asp Tyr Pro Ser Leu Glu Ala Val Ile Arg Val Tyr
35 40 45

40 Leu Glu Glu His Asn Val Glu Val Gln Asp Gly Cys Ile Ala Ile Ala
50 55 60

45 Cys Pro Ile Thr Gly Asp Trp Val Ala Met Thr Asn His Thr Trp Ala
65 70 75 80

Phe Ser Ile Ala Glu Met Lys Lys Asn Leu Gly Phe Ser His Leu Glu
85 90 95

50 Ile Ile Asn Asp Phe Thr Ala Val Ser Met Ala Ile Pro Met Leu Lys
100 105 110

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

Pro Ile Ala Val Tyr Gly Ala Gly Thr Gly Leu Gly Val Ala His Leu
130 135 140

Val	His	Val	Asp	Lys	Arg	Trp	Val	Ser	Leu	Pro	Gly	Glu	Gly	Gly	His
145					150					155					160

Val Asp Phe Ala Pro Asn Ser Glu Glu Glu Gly Ile Ile Leu Glu Ile
165 170 175

Leu Arg Ala Glu Ile Gly His Val Ser Ala Glu Arg Val Leu Ser Gly
180 185 190

Pro Gly Leu Val Asn Leu Tyr Arg Ala Ile Val Lys Ala Asp Asn Arg
195 200 205

Leu Pro Glu Asn Leu Lys Pro Lys Asp Ile Thr Glu Arg Ala Leu Ala
210 215 220

Asp Ser Cys Thr Asp Cys Arg Arg Ala Leu Ser Leu Phe Cys Val Ile
225 230 235 240

Met Gly Arg Phe Gly Gly Asn Leu Ala Leu Thr Leu Gly Thr Phe Gly
245 250 255

Gly Val Tyr Ile Ala Gly Gly Ile Val Pro Arg Phe Leu Glu Phe Phe
260 265 270

Lys Ala Ser Gly Phe Arg Ala Ala Phe Glu Asp Lys Gly Arg Phe Lys
275 280 285

Glu Tyr Val His Asp Ile Pro Val Tyr Leu Ile Val His Asp Asn Pro
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Gly Leu Leu Gly Ser Gly Ala His Leu Arg Gln Thr Leu Gly His Ile
305 310 315 320

Leu

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<211> 371

<212> PRT

<213> *Shigella dysenteriae*

<400> 57

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Gln Ser Arg Leu Ala Ile Thr Val Leu Ser Ala Val Ala Leu Thr Leu
 20 25 30

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Arg Arg Asp Ile Ile Arg Ala Asp Arg Leu His Pro Leu Thr Ser Cys
 35 40 45

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Leu Lys Arg Asn Thr Ile Met Thr Asp Lys Leu Thr Ser Leu Arg Gln
 50 55 60

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Tyr Thr Thr Val Val Ala Asp Thr Gly Asp Ile Ala Ala Met Lys Leu
 65 70 75 80

Tyr Gln Pro Gln Asp Ala Thr Thr Asn Pro Ser Leu Ile Leu Asn Ala
 85 90 95

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Ala Gln Ile Pro Glu Tyr Arg Lys Leu Ile Asp Asp Ala Val Ala Trp
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Ala Lys Gln Gln Ser Asn Asp Arg Ala Gln Gln Ile Val Asp Ala Thr
 115 120 125

Asp Lys Leu Ala Val Asn Ile Gly Leu Glu Ile Leu Lys Leu Val Pro
 130 135 140

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Gly Arg Ile Ser Thr Glu Val Asp Ala Arg Leu Ser Tyr Asp Thr Glu
 145 150 155 160

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Ala Ser Ile Ala Lys Ala Lys Arg Leu Ile Lys Leu Tyr Asn Asp Ala
 165 170 175

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Gly Ile Ser Asn Asp Arg Ile Leu Ile Lys Leu Ala Ser Thr Trp Gln
 180 185 190

Gly Ile Arg Ala Ala Glu Gln Leu Glu Lys Glu Gly Ile Asn Cys Asn
 195 200 205

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Leu Thr Leu Leu Phe Ser Phe Ala Gln Ala Arg Ala Cys Ala Glu Ala
 210 215 220

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Gly Val Phe Leu Ile Ser Pro Phe Val Gly Arg Ile Leu Asp Trp Tyr
 225 230 235 240

Lys Ala Asn Thr Asp Lys Lys Glu Tyr Ala Pro Ala Glu Asp Pro Gly
 245 250 255

55

Val Val Ser Val Ser Glu Ile Tyr Gln Tyr Tyr Lys Glu His Gly Tyr
 260 265 270

5 Glu Thr Val Val Met Gly Ala Ser Phe Arg Asn Ile Gly Glu Ile Leu
 275 280 285

 Glu Leu Ala Gly Cys Asp Arg Leu Thr Ile Ala Pro Ala Leu Leu Lys
 290 295 300

10 Glu Leu Ala Glu Ser Glu Gly Ala Ile Glu Arg Lys Leu Ser Tyr Thr
 305 310 315 320

15 Gly Glu Val Lys Ala Arg Pro Ala Arg Ile Thr Glu Ser Glu Phe Leu
 325 330 335

20 Trp Gln His Asn Gln Asp Pro Met Ala Val Asp Lys Leu Ala Glu Gly
 340 345 350

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 355 360 365

25 Asp Leu Leu
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 <212> PRT
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5	Asp	Thr	Gly	Asp	Ile	Ala	Ala	Met	Lys	Leu	Tyr	Gln	Pro	Gln	Asp	Ala	
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10	Thr	Thr	Asn	Pro	Ser	Leu	Ile	Leu	Asn	Ala	Ala	Gln	Ile	Pro	Glu	Tyr	
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15	Arg	Lys	Leu	Ile	Asp	Asp	Ala	Val	Ala	Trp	Ala	Lys	Gln	Gln	Ser	Ser	
		50					55					60					
20	Asp	Arg	Ala	Gln	Gln	Ile	Val	Asp	Ala	Thr	Asp	Lys	Leu	Ala	Val	Asn	
	65					70				75						80	
25	Ile	Gly	Leu	Glu	Ile	Leu	Lys	Leu	Val	Pro	Gly	Arg	Ile	Ser	Thr	Glu	
					85					90					95		
30	Val	Asp	Ala	Arg	Leu	Ser	Tyr	Asp	Thr	Glu	Ala	Ser	Ile	Ala	Lys	Ala	
				100					105					110			
35																	
40																	
45																	
50																	
55																	

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Lys Arg Leu Ile Lys Leu Tyr Asn Asp Ala Gly Ile Ser Asn Asp Arg
 115 120 125
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 Ile Leu Ile Lys Leu Ala Ser Thr Trp Gln Gly Ile Arg Ala Ala Glu
 130 135 140
 10
 Gln Leu Glu Lys Glu Gly Ile Asn Cys Asn Leu Thr Leu Leu Phe Ser
 145 150 155 160
 15
 Phe Ala Gln Ala Arg Ala Cys Ala Glu Ala Gly Val Tyr Leu Ile Ser
 165 170 175
 Pro Phe Val Gly Arg Ile Leu Asp Trp Tyr Lys Ala Asn Thr Asp Lys
 180 185 190
 20
 Lys Glu Tyr Ala Pro Ala Glu Asp Pro Gly Val Val Ser Val Ser Glu
 195 200 205
 25
 Ile Tyr Gln Tyr Tyr Lys Glu His Gly Tyr Glu Thr Val Val Met Gly
 210 215 220
 30
 Ala Ser Phe Arg Asn Met Gly Glu Ile Leu Glu Leu Ala Gly Cys Asp
 225 230 235 240
 Arg Leu Thr Ile Ala Pro Ala Leu Leu Lys Glu Leu Ala Glu Ser Glu
 245 250 255
 35
 Gly Ala Val Glu Arg Lys Leu Ser Phe Ser Gly Glu Val Lys Ala Arg
 260 265 270
 40
 Pro Glu Arg Ile Thr Glu Ser Glu Phe Leu Trp Gln His Asn Gln Asp
 275 280 285
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				20					25					30			
5	Lys	Ser	Gly	His	Pro	Gly	Ala	Pro	Met	Gly	Met	Ala	Asp	Ile	Ala	Glu	
			35					40					45				
10	Val	Leu	Trp	Arg	Asp	Phe	Leu	Lys	His	Asn	Pro	Gln	Asn	Pro	Ser	Trp	
		50					55					60					
	Ala	Asp	Arg	Asp	Arg	Phe	Val	Leu	Ser	Asn	Gly	His	Gly	Ser	Met	Leu	
	65					70					75					80	
15	Ile	Tyr	Ser	Leu	Leu	His	Leu	Thr	Gly	Tyr	Asp	Leu	Pro	Met	Glu	Glu	
					85					90					95		
20	Leu	Lys	Asn	Phe	Arg	Gln	Leu	His	Ser	Lys	Thr	Pro	Gly	His	Pro	Glu	
				100					105					110			
25	Val	Gly	Tyr	Thr	Ala	Gly	Val	Glu	Thr	Thr	Thr	Gly	Pro	Leu	Gly	Gln	
			115					120					125				
	Gly	Ile	Ala	Asn	Ala	Val	Gly	Met	Ala	Ile	Ala	Glu	Lys	Thr	Leu	Ala	
		130					135					140					
30	Ala	Gln	Phe	Asn	Arg	Pro	Gly	His	Asp	Ile	Val	Asp	His	Tyr	Thr	Tyr	
	145					150					155					160	
35	Ala	Phe	Met	Gly	Asp	Gly	Cys	Met	Met	Glu	Gly	Ile	Ser	His	Glu	Val	
					165					170					175		
40	Cys	Ser	Leu	Ala	Gly	Thr	Leu	Lys	Leu	Gly	Lys	Leu	Ile	Ala	Phe	Tyr	
				180					185					190			
	Asp	Asp	Asn	Gly	Ile	Ser	Ile	Asp	Gly	His	Val	Glu	Gly	Trp	Phe	Thr	
			195					200					205				
45	Asp	Asp	Thr	Ala	Met	Arg	Phe	Glu	Ala	Tyr	Gly	Trp	His	Val	Ile	Arg	
		210					215					220					
50	Asp	Ile	Asp	Gly	His	Asp	Ala	Ala	Ser	Ile	Lys	Arg	Ala	Val	Glu	Glu	
	225					230					235					240	
	Ala	Arg	Ala	Val	Thr	Asp	Lys	Pro	Ser	Leu	Leu	Met	Cys	Lys	Thr	Ile	
					245					250					255		
55	Ile	Gly	Phe	Gly	Ser	Pro	Asn	Lys	Ala	Gly	Thr	His	Asp	Ser	His	Gly	

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	260	265	270
5	Ala Pro Leu Gly Asp Ala Glu Ile Ala Leu Thr Arg Glu Gln Leu Gly 275 280 285		
10	Trp Lys Tyr Ala Pro Phe Glu Ile Pro Ser Glu Ile Tyr Ala Gln Trp 290 295 300		
15	Asp Ala Lys Glu Ala Gly Gln Ala Lys Glu Ser Ala Trp Asn Glu Lys 305 310 315 320		
20	Phe Ala Ala Tyr Ala Lys Ala Tyr Pro Gln Glu Ala Ala Glu Phe Thr 325 330 335		
25	Arg Arg Met Lys Gly Glu Met Pro Ser Asp Phe Asp Ala Lys Ala Lys 340 345 350		
30	Glu Phe Ile Ala Lys Leu Gln Ala Asn Pro Ala Lys Ile Ala Ser Arg 355 360 365		
35	Lys Ala Ser Gln Asn Ala Ile Glu Ala Phe Gly Pro Leu Leu Pro Glu 370 375 380		
40	Phe Leu Gly Gly Ser Ala Asp Leu Ala Pro Ser Asn Leu Thr Leu Trp 385 390 395 400		
45	Ser Gly Ser Lys Ala Ile Asn Glu Asp Ala Ala Gly Asn Tyr Ile His 405 410 415		
50	Tyr Gly Val Arg Glu Phe Gly Met Thr Ala Ile Ala Asn Gly Ile Ser 420 425 430		
55	Leu His Gly Gly Phe Leu Pro Tyr Thr Ser Thr Phe Leu Met Phe Val 435 440 445		
	Glu Tyr Ala Arg Asn Ala Val Arg Met Ala Ala Leu Met Lys Gln Arg 450 455 460		
	Gln Val Met Val Tyr Thr His Asp Ser Ile Gly Leu Gly Glu Asp Gly 465 470 475 480		
	Pro Thr His Gln Pro Val Glu Gln Val Ala Ser Leu Arg Val Thr Pro 485 490 495		
	Asn Met Ser Thr Trp Arg Pro Cys Asp Gln Val Glu Ser Ala Val Ala 500 505 510		

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Trp Lys Tyr Gly Val Glu Arg Gln Asp Gly Pro Thr Ala Leu Ile Leu
515 520 525

5 Ser Arg Gln Asn Leu Ala Gln Gln Glu Arg Thr Glu Glu Gln Leu Ala
530 535 540

10 Asn Ile Ala Arg Gly Gly Tyr Val Leu Lys Asp Cys Ala Gly Gln Pro
545 550 555 560

Glu Leu Ile Phe Ile Ala Thr Gly Ser Glu Val Glu Leu Ala Val Ala
565 570 575

15 Ala Tyr Glu Lys Leu Thr Ala Glu Gly Val Lys Ala Arg Val Val Ser
580 585 590

20 Met Pro Ser Thr Asp Ala Phe Asp Lys Gln Asp Ala Ala Tyr Arg Glu
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25 Ser Val Leu Pro Lys Ala Val Thr Ala Arg Val Ala Val Glu Ala Gly
610 615 620

Ile Ala Asp Tyr Trp Tyr Lys Tyr Val Gly Leu Asn Gly Ala Ile Val
625 630 635 640

30 Gly Met Thr Thr Phe Gly Glu Ser Ala Pro Ala Glu Leu Leu Phe Glu
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40 Leu

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55 Met Ala Asp Ile Ala Glu Val Leu Trp Arg Asp Phe Leu Lys His Asn
35 40 45

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	Pro	Gln	Asn	Pro	Ser	Trp	Ala	Asp	Arg	Asp	Arg	Phe	Val	Leu	Ser	Asn	
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	65					70					75					80	
	Asp	Leu	Pro	Met	Glu	Glu	Leu	Lys	Asn	Phe	Arg	Gln	Leu	His	Ser	Lys	
10					85					90					95		
	Thr	Pro	Gly	His	Pro	Glu	Val	Gly	Tyr	Thr	Ala	Gly	Val	Glu	Thr	Thr	
				100					105					110			
15	Thr	Gly	Pro	Leu	Gly	Gln	Gly	Ile	Ala	Asn	Ala	Val	Gly	Met	Ala	Ile	
			115					120					125				
	Ala	Glu	Lys	Thr	Leu	Ala	Ala	Gln	Phe	Asn	Arg	Pro	Gly	His	Asp	Ile	
20		130					135					140					
	Val	Asp	His	Tyr	Thr	Tyr	Ala	Phe	Met	Gly	Asp	Gly	Cys	Met	Met	Glu	
25		145				150					155					160	
	Gly	Ile	Ser	His	Glu	Val	Cys	Ser	Leu	Ala	Gly	Thr	Leu	Lys	Leu	Gly	
					165					170					175		
30	Lys	Leu	Ile	Ala	Phe	Tyr	Asp	Asp	Asn	Gly	Ile	Ser	Ile	Asp	Gly	His	
				180					185					190			
	Val	Glu	Gly	Trp	Phe	Thr	Asp	Asp	Thr	Ala	Met	Arg	Phe	Glu	Ala	Tyr	
35			195					200					205				
	Gly	Trp	His	Val	Ile	Arg	Gly	Ile	Asp	Gly	His	Asp	Ala	Ala	Ser	Ile	
		210					215					220					
40	Lys	Arg	Ala	Val	Glu	Glu	Ala	Arg	Ala	Val	Thr	Asp	Lys	Pro	Ser	Leu	
		225				230					235					240	
	Leu	Met	Cys	Lys	Thr	Ile	Ile	Gly	Phe	Gly	Ser	Pro	Asn	Lys	Ala	Gly	
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50				260					265					270			
	Thr	Arg	Glu	Gln	Leu	Gly	Trp	Lys	Tyr	Ala	Pro	Phe	Glu	Ile	Pro	Ser	
			275					280					285				
55	Glu	Ile	Tyr	Ala	Gln	Trp	Asp	Ala	Lys	Glu	Ala	Gly	Gln	Ala	Lys	Glu	
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5	Glu	Ala	Ala	Glu	Phe	Thr	Arg	Arg	Met	Lys	Gly	Glu	Met	Pro	Ser	Asp	
					325					330					335		
	Phe	Asp	Ala	Lys	Ala	Lys	Glu	Phe	Ile	Ala	Lys	Leu	Gln	Ala	Asn	Pro	
10				340					345					350			
	Ala	Lys	Ile	Ala	Ser	Arg	Lys	Ala	Ser	Gln	Asn	Ala	Ile	Glu	Ala	Phe	
			355					360					365				
15	Gly	Pro	Leu	Leu	Pro	Glu	Phe	Leu	Gly	Gly	Ser	Ala	Asp	Leu	Ala	Pro	
		370					375					380					
	Ser	Asn	Leu	Thr	Leu	Trp	Ser	Gly	Ser	Lys	Ala	Ile	Asn	Glu	Asp	Ala	
20		385				390					395					400	
	Ala	Gly	Asn	Tyr	Ile	His	Tyr	Gly	Val	Arg	Glu	Phe	Gly	Met	Thr	Ala	
				405					410						415		
25	Ile	Ala	Asn	Gly	Ile	Ser	Leu	His	Gly	Gly	Phe	Leu	Pro	Tyr	Thr	Ser	
				420					425					430			
	Thr	Phe	Leu	Met	Phe	Val	Glu	Tyr	Ala	Arg	Asn	Ala	Val	Arg	Met	Ala	
30			435					440					445				
	Ala	Leu	Met	Lys	Gln	Arg	Gln	Val	Met	Val	Tyr	Thr	His	Asp	Ser	Ile	
35		450				455						460					
	Gly	Leu	Gly	Glu	Asp	Gly	Pro	Thr	His	Gln	Pro	Val	Glu	Gln	Val	Ala	
		465				470					475					480	
40	Ser	Leu	Arg	Val	Thr	Pro	Asn	Met	Ser	Thr	Trp	Arg	Pro	Cys	Asp	Gln	
				485					490					495			
	Val	Glu	Ser	Ala	Val	Ala	Trp	Lys	Tyr	Gly	Val	Glu	Arg	Gln	Asp	Gly	
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	Pro	Thr	Ala	Leu	Ile	Leu	Ser	Arg	Gln	Asn	Leu	Ala	Gln	Gln	Glu	Arg	
50			515					520					525				
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		530				535					540						
55	Asp	Cys	Ala	Gly	Gln	Pro	Glu	Leu	Ile	Phe	Ile	Ala	Thr	Gly	Ser	Glu	
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Lys Ala Arg Val Val Ser Met Pro Ser Thr Asp Ala Phe Asp Lys Gln
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15 Val Ala Val Glu Ala Gly Ile Ala Asp Tyr Trp Tyr Lys Tyr Val Gly
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Claims

- 10 1. A microorganism of the genus *Cupriavidus* or *Ralstonia* genetically modified to express phosphomannose isomerase (EC 5.3.1.8) and facilitated diffusion protein for mannose uptake and optionally mannofructokinase (EC 2.7.1.4), wherein said microorganism is capable of growing on mannose and optionally also on glucose as the carbon source.
- 15 2. The microorganism according to claim 1, wherein the microorganism is genetically modified to express xylose isomerase (EC 5.3.1.5), xylulokinase (EC 2.7.1.17) and a high affinity ABC- transporter and/or xylose proton symporter E, wherein said microorganism is capable of growing on mannose, xylose, or xylose and arabinose, and optionally also on glucose and/or galactose as the carbon source.
- 20 3. The microorganism according to claim 1 or 2, wherein the microorganism is genetically modified to express transaldolase (EC 2.2.1.2) and/or transketolase (EC 2.2.1.1).
4. The microorganism according to any one of claims 1- 3, wherein the microorganism is genetically modified to express glucokinase (EC 2.7.1.2).
- 25 5. The microorganism according to any one of claims 1 to 4, wherein the microorganism belongs to *R. eutropha* (*C. necator*) species.
- 30 6. A vector comprising a nucleotide sequence encoding phosphomannose isomerase (EC 5.3.1.8) and a nucleotide sequence encoding facilitated diffusion protein for mannose uptake and optionally a nucleotide sequence encoding mannofructokinase (EC 2.7.1.4), and regulatory elements for regulating expression of said genes in *Cupriavidus* or *Ralstonia*.
- 35 7. A vector according to claim 6 comprising a nucleotide sequence encoding xylose isomerase (EC 5.3.1.5), a nucleotide sequence encoding xylulokinase (EC 2.7.1.17), and a nucleotide sequence encoding a high affinity ABC- transporter and/or a nucleotide sequence encoding xylose proton symporter E, and regulatory elements for regulating expression of said genes in *Cupriavidus* or *Ralstonia*.
- 40 8. A vector according to claim 6 or 7 comprising a nucleotide sequence encoding transaldolase (EC 2.2.1.2) and/or transketolase (EC 2.2.1.1).
9. A vector according to any one of claims 6, 7 or 8 comprising a gene encoding glucokinase (EC 2.7.1.2).
- 45 10. The vector according to any one of claims 6 to 9, wherein the regulatory element comprises a strong constitutive promoter.
- 50 11. The vector according to any one of claims 6 to 10, wherein the vector is a broad host range vector.
12. The vector according to any one of claims 6 to 11, wherein the promoter is *neokanamycin*-promoter, *lac*-promoter, glyceraldehyde-3-phosphate dehydrogenase promoter, P₂, hydrogenase (SH) promoter or P_L.
- 55 13. A vector system comprising
 - the vector of claim 6 and
 - a vector comprising a nucleotide sequence encoding xylose isomerase (EC 5.3.1.5), a nucleotide sequence encoding xylulokinase (EC 2.7.1.17), and a nucleotide sequence encoding a high affinity ABC-transporter and/or a nucleotide sequence encoding xylose proton symporter E, and regulatory elements for regulating expression of said genes in *Cupriavidus* or *Ralstonia*
 - and optionally one of said vectors comprises a gene encoding glucokinase (EC 2.7.1.2) or a nucleotide se-

quence encoding transaldolase (EC 2.2.1.2) and/or transketolase (EC 2.2.1.1).

14. A method for cultivating the microorganism according to any one of claims 1 to 5, or a *Cupriavidus* or *Ralstonia* microorganism comprising the vector according to any one of claims 6 to 13, which comprises that the cultivation medium comprises mannose, glucose, xylose, arabinose, or galactose, or a combination thereof as carbon source.
15. The method according to claim 14, which comprises that the microorganism is allowed to synthesize, optionally after genetical modification, a chemical, such as a lipid and the chemical is recovered from the cultivation medium or from the cells.
16. The method according to claim 14 or 15, which comprises that the microorganism is allowed to synthesize polyhydroxyalkanoates, such as PHB or fatty acids and/or fatty acid derivatives.
17. Use of the microorganism according to any one of claims 1 to 5, possibly after a genetic modification, for producing a desired chemical.

Patentansprüche

1. Mikroorganismus der Gattung *Cupriavidus* oder *Ralstonia*, der genetisch modifiziert ist, um Phosphomannoseisomerase (EC 5.3.1.8) und Diffusionserleichterungsprotein für die Mannoseaufnahme und optional Mannofructokinase (EC 2.7.1.4) zu exprimieren, wobei der Mikroorganismus zum Wachsen auf Mannose und optional auch auf Glucose als Kohlenstoffquelle imstande ist.
2. Mikroorganismus nach Anspruch 1, wobei der Mikroorganismus genetisch modifiziert ist, um Xyloseisomerase (EC 5.3.1.5), Xylulokinase (EC 2.7.1.17) und einen Hochaffinitäts-ABC-Transporter und/oder Xylose-Proton-Symporter E zu exprimieren, wobei der Mikroorganismus zum Wachsen auf Mannose, Xylose oder Xylose und Arabinose, und optional auch auf Glucose und/oder Galactose als Kohlenstoffquelle imstande ist.
3. Mikroorganismus nach Anspruch 1 oder 2, wobei der Mikroorganismus genetisch modifiziert ist, um Transaldolase (EC 2.2.1.2) und/oder Transketolase (EC 2.2.1.1) zu exprimieren.
4. Mikroorganismus nach einem der Ansprüche 1 bis 3, wobei der Mikroorganismus genetisch modifiziert ist, um Glucokinase (EC 2.7.1.2) zu exprimieren.
5. Mikroorganismus nach einem der Ansprüche 1 bis 4, wobei der Mikroorganismus zur Spezies *R. eutropha* (*C. necator*) gehört.
6. Vektor, umfassend eine für Phosphomannoseisomerase (EC 5.3.1.8) kodierende Nukleotidsequenz und eine für Diffusionserleichterungsprotein für die Mannoseaufnahme kodierende Nukleotidsequenz und optional eine für Mannofructokinase (EC 2.7.1.4) kodierende Nukleotidsequenz, und regulatorische Elemente zur Regulation der Expression dieser Gene in *Cupriavidus* oder *Ralstonia*.
7. Vektor nach Anspruch 6, umfassend eine für Xyloseisomerase (EC 5.3.1.5) kodierende Nukleotidsequenz, eine für Xylulokinase (EC 2.7.1.17) kodierende Nukleotidsequenz und eine für einen Hochaffinitäts-ABC-Transporter kodierende Nukleotidsequenz und/oder eine für Xylose-Proton-Symporter E kodierende Nukleotidsequenz, und regulatorische Elemente zur Regulation der Expression dieser Gene in *Cupriavidus* oder *Ralstonia*.
8. Vektor nach Anspruch 6 oder 7, umfassend eine für Transaldolase (EC 2.2.1.2) und/oder Transketolase (EC 2.2.1.1) kodierende Nukleotidsequenz.
9. Vektor nach einem der Ansprüche 6, 7 oder 8, umfassend ein für Glucokinase (EC 2.7.1.2) kodierendes Gen.
10. Vektor nach einem der Ansprüche 6 bis 9, wobei das regulatorische Element einen starken konstitutiven Promoter umfasst.
11. Vektor nach einem der Ansprüche 6 bis 10, wobei der Vektor ein Vektor mit breitem Wirtsbereich ist.

12. Vektor nach einem der Ansprüche 6 bis 11, wobei der Promoter ein *Neokanamycin*-Promoter, *lac*-Promoter, Glyceraldehyd-3-phosphatdehydrogenase-Promoter, P2, Hydrogenase (SH)-Promoter oder P_L ist.

13. Vektorsystem, umfassend

- den Vektor nach Anspruch 6 und
- einen Vektor umfassend eine für Xyloseisomerase (EC 5.3.1.5) kodierende Nukleotidsequenz, eine für Xylulokinase (EC 2.7.1.17) kodierende Nukleotidsequenz und eine für einen Hochaffinitäts-ABC-Transporter kodierende Nukleotidsequenz und/oder eine für Xylose-Proton-Symporter E kodierende Nukleotidsequenz, und regulatorische Elemente zur Regulation der Expression dieser Gene in *Cupriavidus* oder *Ralstonia*
- und optional umfasst einer der Vektoren ein für Glucokinase (EC 2.7.1.2) kodierendes Gen oder eine für Transaldolase (EC 2.2.1.2) und/oder Transketolase (EC 2.2.1.1) kodierende Nukleotidsequenz.

14. Verfahren zum Züchten des Mikroorganismus nach einem der Ansprüche 1 bis 5, oder eines *Cupriavidus*- oder *Ralstonia*-Mikroorganismus, der den Vektor nach einem der Ansprüche 6 bis 13 umfasst, umfassend, dass das Zuchtmedium Mannose, Glucose, Xylose, Arabinose oder Galactose oder eine Kombination daraus als Kohlenstoffquelle umfasst.

15. Verfahren nach Anspruch 14, das umfasst, dass der Mikroorganismus, optional nach genetischer Modifikation, einen chemischen Stoff, wie etwa ein Lipid, synthetisieren gelassen wird und der chemische Stoff aus dem Zuchtmedium oder den Zellen gewonnen wird.

16. Verfahren nach einem der Ansprüche 14 oder 15, das umfasst, dass der Mikroorganismus Polyhydroxyalkanoate wie etwa PHB oder Fettsäuren und/oder Fettsäurederivate synthetisieren gelassen wird.

17. Anwendung des Mikroorganismus nach einem der Ansprüche 1 bis 5, möglicherweise nach einer genetischen Modifikation, zur Herstellung eines gewünschten chemischen Stoffs.

Revendications

1. Micro-organisme du genre *Cupriavidus* ou *Ralstonia* génétiquement modifié pour exprimer :

la phosphomannose isomérase (EC 5.3.1.8) et la protéine de diffusion facilitée pour l'absorption du mannose et éventuellement la mannofructokinase (EC 2.7.1.4), ledit micro-organisme étant capable de se développer sur du mannose et éventuellement aussi sur du glucose en tant que source de carbone.

2. Micro-organisme selon la revendication 1, le micro-organisme étant génétiquement modifié pour exprimer la xylose isomérase (EC 5.3.1.5), la xylulokinase (EC 2.7.1.17) et un transporteur ABC à forte affinité et/ou un symport proton xylose E, le micro-organisme étant capable de se développer sur du mannose, du xylose, ou du xylose et de l'arabinose, et éventuellement aussi sur du glucose et/ou du galactose en tant que source de carbone.

3. Micro-organisme selon la revendication 1 ou la revendication 2, le micro-organisme étant génétiquement modifié pour exprimer la transaldolase (EC 2.2.1.2) et/ou la transcétolase (EC 2.2.1.1).

4. Micro-organisme selon la revendication 1 ou la revendication 3, le micro-organisme étant génétiquement modifié pour exprimer la glucokinase (EC 2.7.1.2).

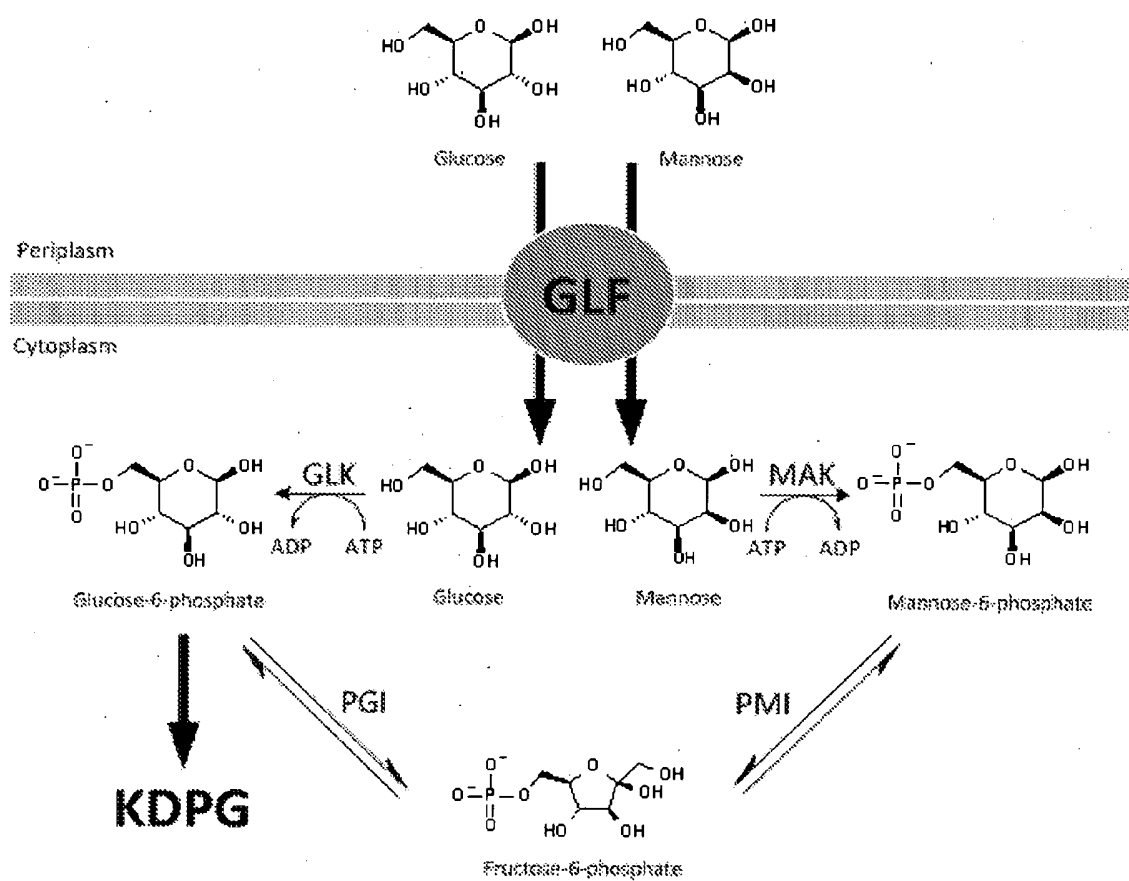
5. Micro-organisme selon la revendication 1 ou la revendication 4, le micro-organisme appartenant à l'espèce *R. eutropha* (*C. necator*).

6. Vecteur comprenant une séquence nucléotidique codant pour la phosphomannose isomérase (EC 5.3.1.8) et une séquence nucléotidique codant pour la protéine de diffusion facilitée pour l'absorption du mannose et éventuellement une séquence nucléotidique codant pour la mannofructokinase (EC 2.7.1.4), et des éléments de régulation pour réguler l'expression desdits gènes dans *Cupriavidus* ou *Ralstonia*.

7. Vecteur selon la revendication 6, comprenant une séquence nucléotidique codant pour la xylose isomérase (EC 5.3.1.5), une séquence nucléotidique codant pour la xylulokinase (EC 2.7.1.17) et une séquence nucléotidique

codant pour un transporteur ABC à forte affinité et/ou une séquence nucléotidique codant pour le symport proton xylose E, et des éléments de régulation pour la régulation de l'expression desdits gènes dans *Cupriavidus* ou *Ralstonia*.

- 5 **8.** Vecteur selon la revendication 6 ou la revendication 7, comprenant une séquence nucléotidique codant pour la transaldolase (EC 2.2.1.2) et/ou la transcétolase (EC 2.2.1.1).
9. Vecteur selon l'une quelconque des revendications 6, 7 ou 8, comprenant un gène codant pour la glucokinase (EC 2.7.1.2).
- 10 **10.** Vecteur selon l'une quelconque des revendications 6 à 9, l'élément de régulation comprenant un promoteur constitutif puissant.
11. Vecteur selon l'une quelconque des revendications 6 à 10, le vecteur étant un vecteur à large gamme d'hôtes.
- 15 **12.** Vecteur selon l'une quelconque des revendications 6 à 11, le promoteur étant un promoteur *néokanamycine*, le promoteur *lac*, un promoteur de glyceraldéhyde-3-phosphate déshydrogénase, P2, un promoteur d'hydrogénase (SH) ou P_L.
- 20 **13.** Système de vecteur comprenant
 - le vecteur selon la revendication 6 et
 - un vecteur comprenant une séquence nucléotidique codant pour la xylose isomérase (EC 5.3.1.5), une sé-
 - 25 quence nucléotidique codant pour la xylulokinase (EC 2.7.1.17) et une séquence nucléotidique codant pour un transporteur ABC à forte affinité et/ou une séquence nucléotidique codant pour le symport proton xylose E, et des éléments de régulation pour la régulation de l'expression desdits gènes dans *Cupriavidus* ou *Ralstonia*.
 - et éventuellement l'un desdits vecteurs comprenant un gène codant pour la glucokinase (EC 2.7.1.2), ou une séquence nucléotidique codant pour la transaldolase (EC 2.2.1.2) et/ou la transcétolase (EC 2.2.1.1).
- 30 **14.** Procédé de culture de micro-organisme selon l'une quelconque des revendications 1 à 5, ou micro-organisme *Cupriavidus* ou *Ralstonia* comprenant le vecteur selon l'une quelconque des revendications 6 à 13, qui comprend le fait que le milieu de culture contient le mannose, le glucose, le xylose, l'arabinose ou le galactose, ou une combinaison de ceux-ci en tant que source de carbone.
- 35 **15.** Procédé selon la revendication 14, qui comprend le fait qu'on laisse le micro-organisme synthétiser, éventuellement après modification génétique, un produit chimique, tel qu'un lipide et que le produit chimique est récupéré du milieu de culture ou des cellules.
- 40 **16.** Procédé selon la revendication 14 ou la revendication 15, qui comprend le fait qu'on laisse le micro-organisme synthétiser des polyhydroxyalcanoates, tels que le PHB ou des acides gras et/ou des dérivés d'acide gras.
- 45 **17.** Utilisation du micro-organisme selon l'une quelconque des revendications 1 à 5, éventuellement après une modification génétique, pour produire un produit chimique souhaité.

**Fig. 1**

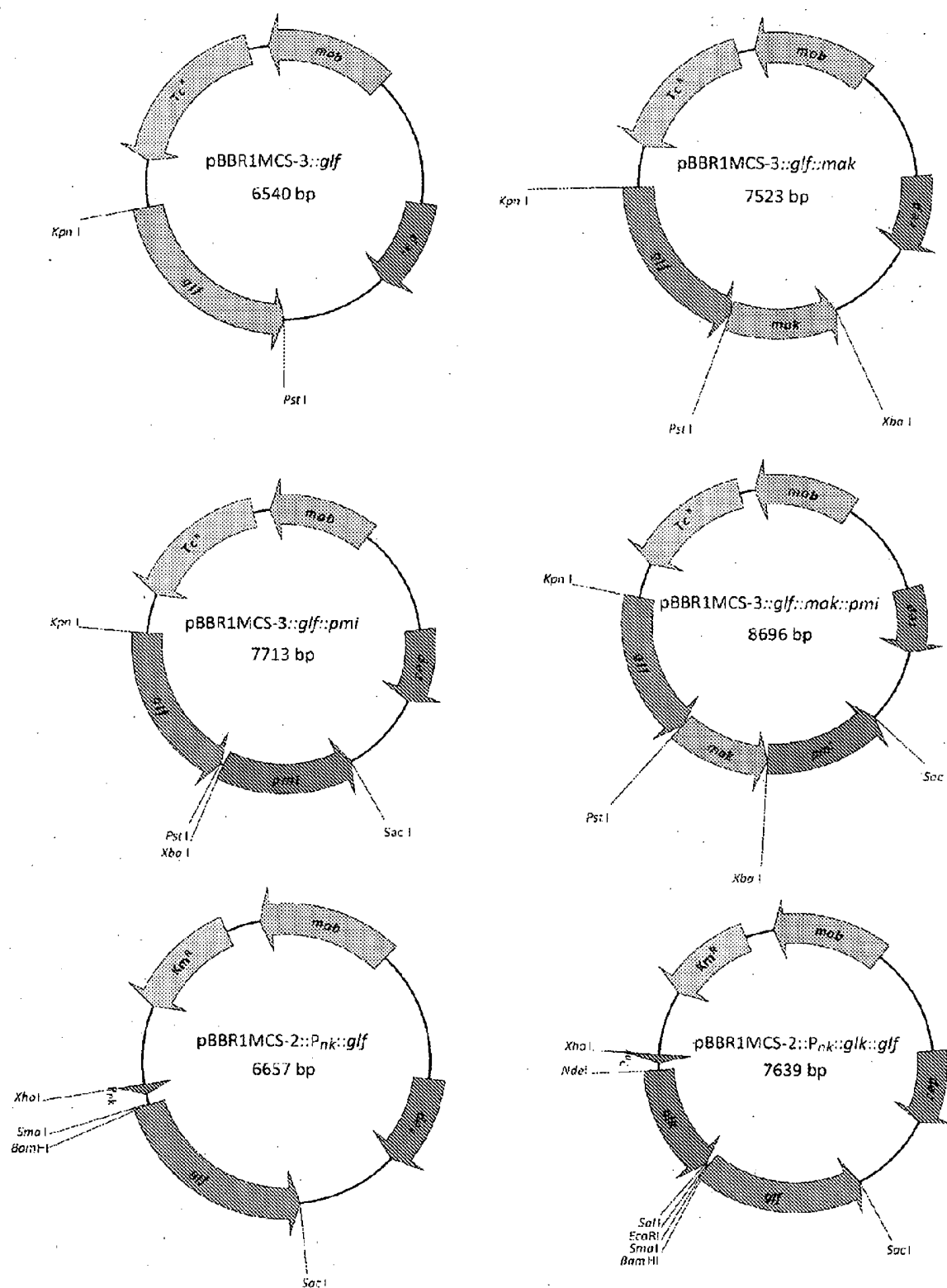


Fig. 2

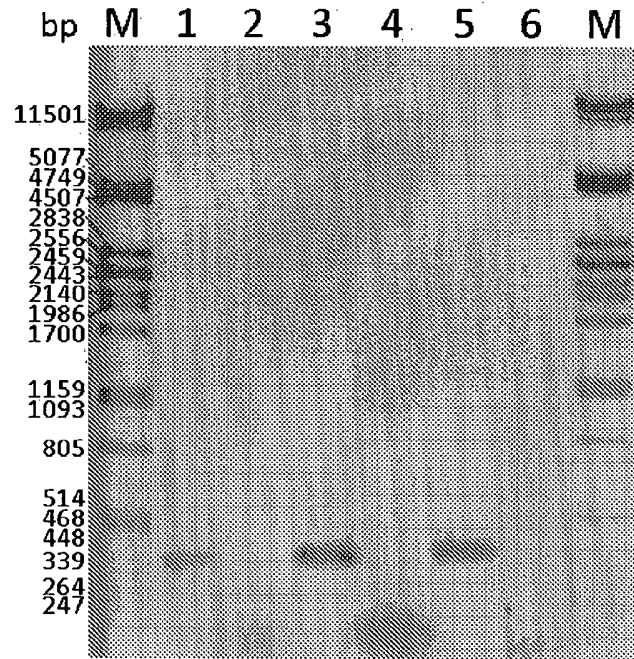


Fig. 3

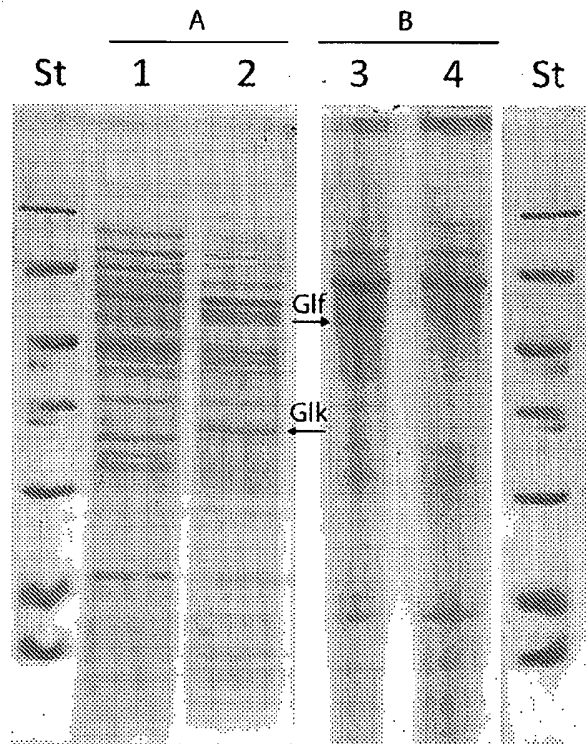
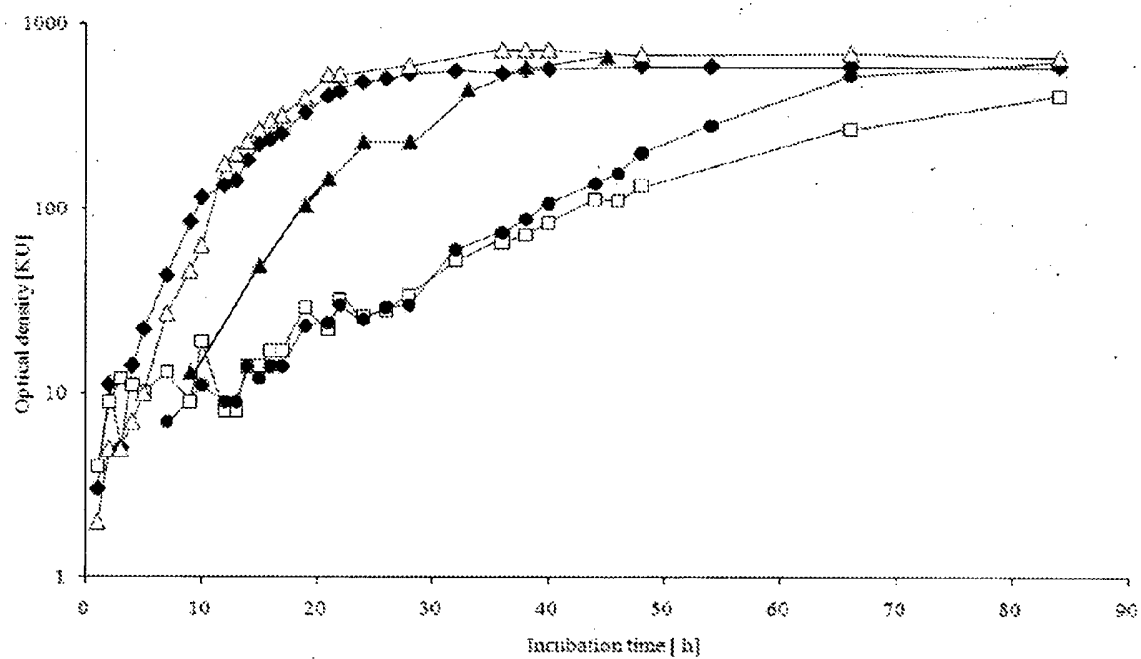
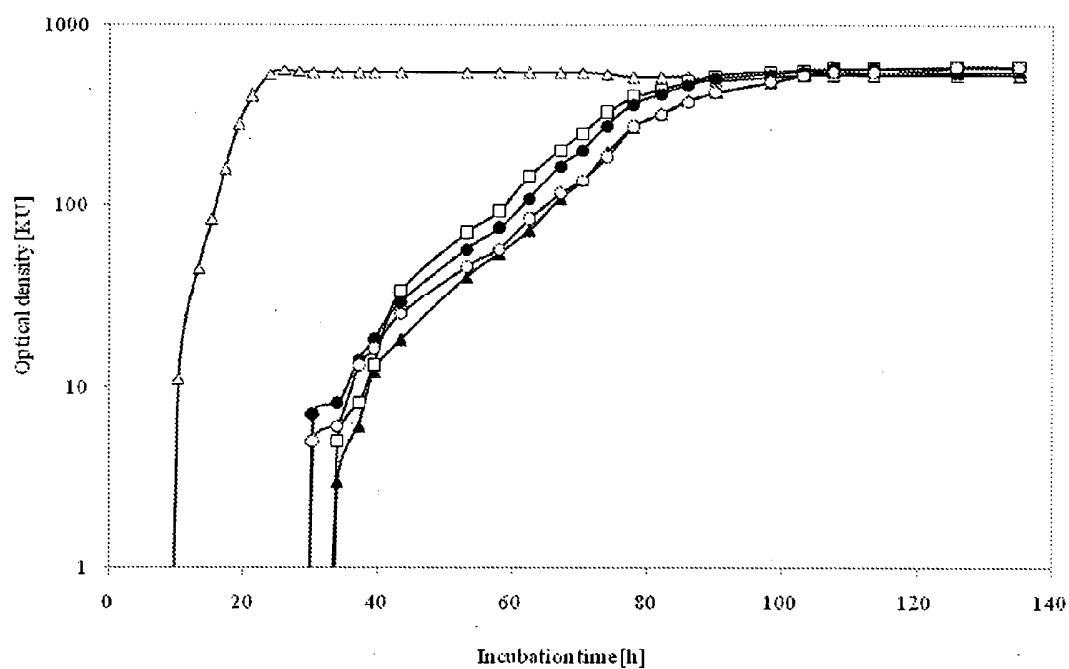


Fig. 4

**Fig. 5****Fig. 6**

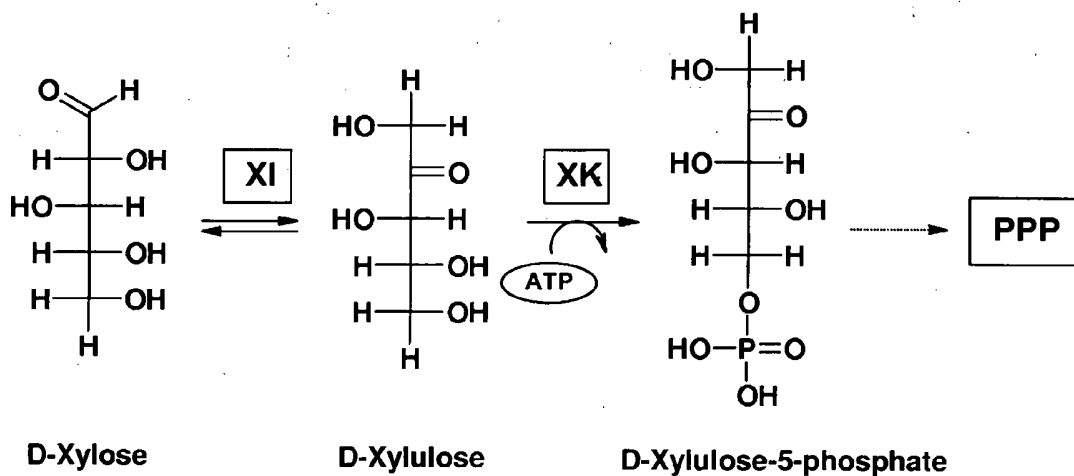


Fig. 7

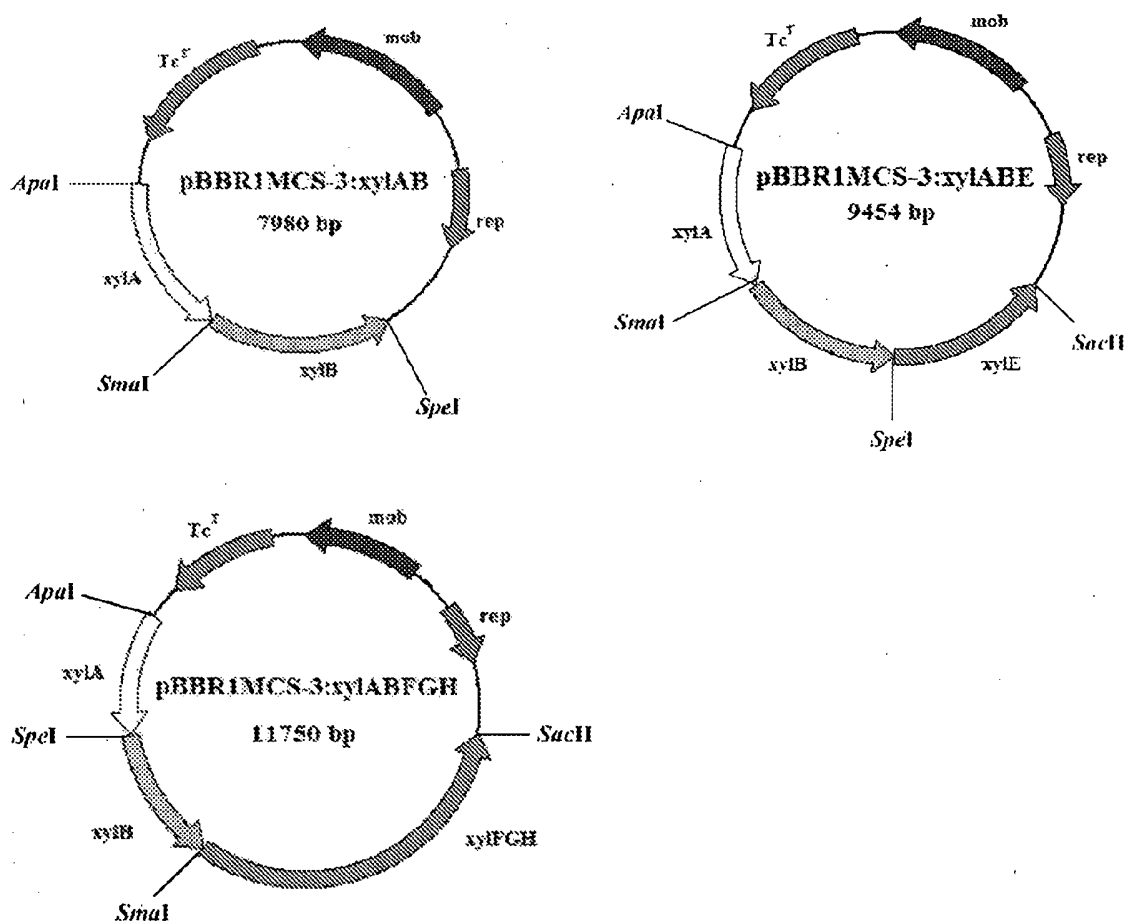
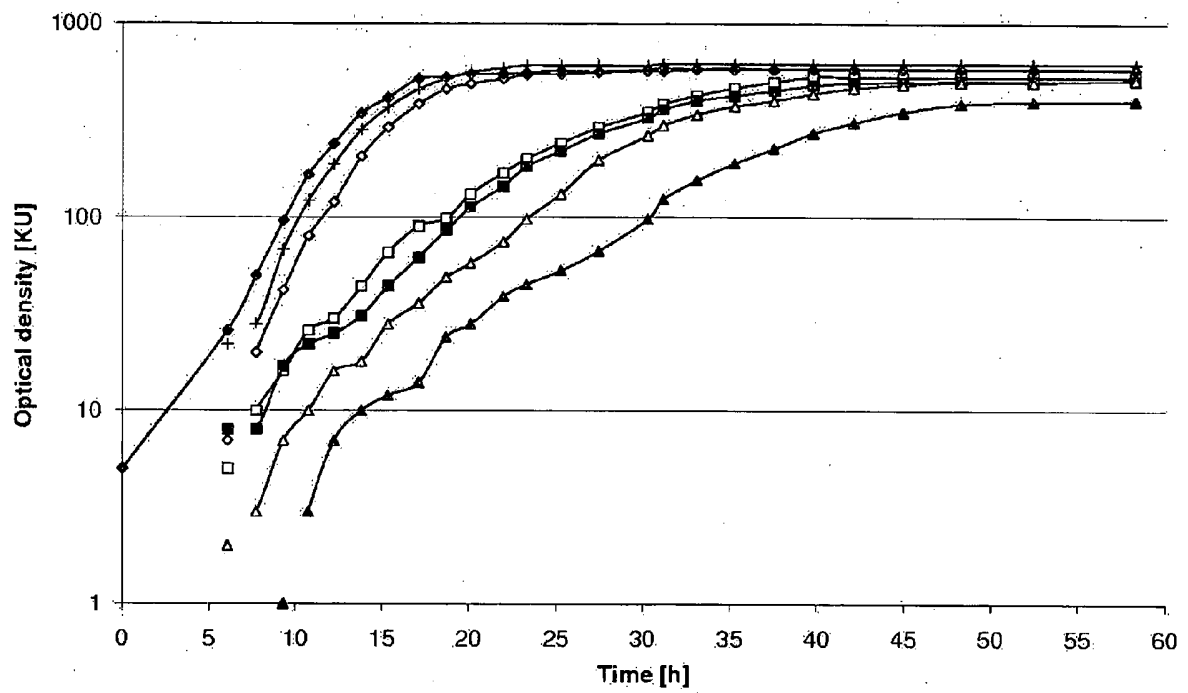


Fig. 8

**Fig. 9**

REFERENCES CITED IN THE DESCRIPTION

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KITERJESZTETT SZUBSZTRÁTHASZNOSÍTÁSI TARTOMÁNYÚ MIKROORGANIZMUSOK

Szabadalmi igénypontok

1. A *Cupriavidus* vagy *Ralstonia* nemzetségbe tartozó mikroorganizmus, amely genetikailag módosított foszfomannóz-izomeráz (EC 5.3.1.8) és mannózfelvételre alkalmas facilitált diffúziós fehérje, és adott esetben mannofruktokináz expresszáására (EC 2.7.1.4) módosított, ahol a mikroorganizmus képes szénforrásként mannózon szaporodni és adott esetben glükózon is szaporodni.
2. Az 1. igénypont szerinti mikroorganizmus, ahol a mikroorganizmus genetikailag módosított xilóz-izomeráz (EC 5.3.1.5), xilulokináz (EC 2.7.1.17) és nagy affinitású ABC-transzporter és/vagy xilóz-proton-szimporter-E expresszáására, ahol a mikroorganizmus képes szénforrásként mannózon, xilózon vagy xilózon és arabinózon, és adott esetben glükózon és/vagy galaktózon is szaporodni.
3. Az 1. vagy 2. igénypont szerinti mikroorganizmus, ahol a mikroorganizmus genetikailag módosított transzaldoláz (EC 2.2.1.2) és/vagy transzketoláz (EC 2.2.1.1) expresszáására.
4. Az 1-3. igénypontok bármelyike szerinti mikroorganizmus, ahol a mikroorganizmus genetikailag módosított (EC 2.7.1.2) glükokináz expresszáására.
5. Az 1-4. igénypontok bármelyike szerinti mikroorganizmus, ahol a mikroorganizmus *R. eutropha* (*C. necator*) fajba tartozik.
6. Vektor, amely foszfomannóz-izomerázt (EC 5.3.1.8) kódoló nukleotidszekvenciát és mannózfelvételre alkalmas facilitált diffúziós fehérjét kódoló nukleotidszekvenciát, és adott esetben mannofruktokinázt (EC 2.7.1.4) kódoló nukleotidszekvenciát, valamint a gének *Cupriavidusban* vagy *Ralstoniában* történő expresszióját szabályozó szabályozó elemeket tartalmaz.
7. A 6. igénypont szerinti vektor, amely xilóz-izomerázt (EC 5.3.1.5) kódoló nukleotidszekvenciát, xilulokinázt (EC 2.7.1.17) kódoló nukleotidszekvenciát és nagy affinitású ABC-transzportert kódoló nukleotidszekvenciát és/vagy xilóz-proton-szimporter-E-t kódoló nukleotidszekvenciát, valamint a gének *Cupriavidusban* vagy *Ralstoniában* történő expresszióját szabályozó szabályozó elemeket tartalmaz.
8. A 6. vagy 7. igénypont szerinti vektor, amely transzaldolázt (EC 2.2.1.2) és/vagy transzketolázt (EC 2.2.1.1) kódoló nukleotidszekvenciát tartalmaz.
9. A 6., 7. vagy 8. igénypontok bármelyike szerinti vektor, amely glükokinázt (EC 2.7.1.2) kódoló gént tartalmaz.
10. A 6-9. igénypontok bármelyike szerinti vektor, amelyben a szabályozó elemek erős konstitutív promotert tartalmaznak.
11. A 6-10. igénypontok bármelyike szerinti vektor, ahol a vektor széles gazdaspecifitású vektor.

12. A 6-11. igénypontok bármelyike szerinti vektor, amelyben a promóter *nekanamycin*-promóter, *lac*-promóter, gliceraldehid-3-foszfát dehidrogenáz promóter, P2, hidrogenáz (SH) promóter vagy P_L.

13. Vektorrendszer, amely tartalmaz

- 6. igénypont szerinti vektort, és
- vektort, amely xilóz-izomerázt (EC 5.3.1.5) kódoló nukleotidszekvenciát, xilulokinázt (EC 2.7.1.17) kódoló nukleotidszekvenciát és nagy affinitású ABC-transzportert kódoló nukleotidszekvenciát és/vagy xilóz-proton-szinporter-E-t kódoló nukleotidszekvenciát, valamint a gének *Cupriavidus*-ban vagy *Ralstonia*-ban történő expresszióját szabályozó szabályozó elemeket tartalmaz,
- és adott esetben a vektorok egyike glükokinázt (EC 2.7.1.2) kódoló gént vagy transzaldolázt (EC 2.2.1.2) és/vagy transzketolázt (EC 2.2.1.1) kódoló nukleotidszekvenciát tartalmaz.

14. Eljárás az 1-5. igénypontok bármelyike szerinti mikroorganizmus vagy *Cupriavidus* or *Ralstonia* mikroorganizmus tenyésztésére, amely mikroorganizmus 6-13. igénypontok bármelyike szerinti vektort tartalmaz, és amely magában foglalja, hogy szénforrásként a tenyésztő tápközeg mannózt, glükózt, xilózt, arabinózt vagy galaktózt tartalmaz vagy ezek kombinációját tartalmazza.

15. A 14. igénypont szerinti eljárás, amely magában foglalja, hogy a mikroorganizmus számára biztosítjuk, hogy szintetizáljon, adott esetben genetikai módosítás után egy vegyületet, például lipidet, és a vegyületet kinyerjük a tenyésztő tápközegből vagy a sejtekből.

16. A 14. vagy 15. igénypont szerinti eljárás, amely tartalmazza, hogy a mikroorganizmus számára biztosítjuk, hogy polihidroxi-alkanoátokat, például PHB-t vagy zsírsavakat és/vagy zsírsavszármazékokat szintetizáljon.

17. Az 1-5. igénypontok bármelyike szerinti mikroorganizmus alkalmazása, adott esetben genetikai módosítás után, kívánt vegyület előállítására.