(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
27 June 2019 (27.06.2019)

WIPO | PCT

(10) International Publication Number
WO 2019/121983 A1

(51) International Patent Classification:
C07K 14/245 (2006.01) *C12N 15/70* (2006.01)

(21) International Application Number:
PCT/EP2018/085941

(22) International Filing Date:
19 December 2018 (19.12.2018)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
17208600.1 19 December 2017 (19.12.2017) EP

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(81) Designated States *(unless otherwise indicated, for every kind of national protection available)*: AE, AG, AL, AM, AO, AT, AU, AZ, BA, BB, BG, BH, BN, BR, BW, BY, BZ, CA, CH, CL, CN, CO, CR, CU, CZ, DE, DJ, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IR, IS, JO, JP, KE, KG, KH, KN, KP, KR, KW, KZ, LA, LC, LK, LR, LS, LU, LY, MA, MD, ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PA, PE, PG, PH, PL, PT, QA, RO, RS, RU, RW, SA, SC, SD, SE, SG, SK, SL, SM, ST, SV, SY, TH, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW.

(84) Designated States *(unless otherwise indicated, for every kind of regional protection available)*: ARIPO (BW, GH, GM, KE, LR, LS, MW, MZ, NA, RW, SD, SL, ST, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, RU, TJ, TM), European (AL, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV, MC, MK, MT, NL, NO, PL, PT, RO, RS, SE, SI, SK, SM, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, KM, ML, MR, NE, SN, TD, TG).

Published:
— *with international search report (Art. 21(3))*
— *with sequence listing part of description (Rule 5.2(a))*

(54) Title: FERMENTATION PROCESS

(57) Abstract: Some embodiments relate to a method for producing a product of interest with a microbial host using an auto-replicative extra-chromosomal nucleic acid molecule comprising a first nucleic acid sequence whose genetic activity confers an advantage to the host, optionally wherein the genetic activity of said first nucleic acid molecule is controlled.

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Fermentation process

Field

Embodiments herein relate to a method for producing a product of interest with a microbial host using an auto-replicative extra-chromosomal nucleic acid molecule comprising a first nucleic acid sequence whose genetic activity confers an advantage to the host, optionally wherein the genetic activity of said first nucleic acid molecule is controlled.

Background

Antibiotics are widely used as selection agents for the production of a product of interest in microbial cells. However, there are several drawbacks associated with the use of antibiotics such as large-scale spreading of antibiotics in the environment. In addition the sequence coding for the resistance of the antibiotic in the DNA constructs represent an energetic burden for the cell and therefore negatively affects the yield of the product. This energetic burden is particularly relevant when the resistance-conferring gene is a large gene, when it is expressed at a high level and/or when it is expressed constitutively.

Therefore, there is still a need for an alternative and even improved method, which does not have all the drawbacks of existing methods.

Description

In a first aspect, there is provided a method for producing a product of interest with a microbial host, said method comprising the steps of:

a) Providing the microbial host comprising an auto-replicative extra-chromosomal nucleic acid molecule comprising a first nucleic acid sequence, optionally wherein the genetic activity of said first nucleic acid sequence is controlled;

b) Optionally said auto-replicative extra-chromosomal nucleic acid molecule comprises a second nucleic acid sequence that is involved in the production of said product of interest, wherein the genetic activity of

said second nucleic acid sequence is controlled independently from the first sequence;

c) Culturing said microbial host under conditions allowing said microbial host to express the first nucleic acid sequence to a given level to maintain the auto-replicative extra-chromosomal molecule into the growing microbial population and simultaneously genetically controlling the second sequence coding for said product of interest.

Step a)

Step a) comprises providing a microbial host comprising an auto-replicative extra-chromosomal nucleic acid molecule comprising a first nucleic acid sequence whose genetic activity confers an advantage to the host, optionally wherein the genetic activity of said first nucleic acid sequence is controlled. The auto-replicative extra-chromosomal nucleic acid molecule can be provided in a microbial host (e.g., a microbial cell as described herein). For example, the host or a predecessor of the host may have been previously transformed with the auto-replicative extra-chromosomal nucleic acid molecule. As such, in some embodiments, step a) comprises providing a microbial cell host comprising an auto-replicative extra-chromosomal nucleic acid molecule comprising a first nucleic acid sequence whose genetic activity confers an advantage to the host, optionally wherein the genetic activity of said first nucleic acid sequence is controlled.

Optional transforming step

In some embodiments, the microbial host is transformed with the auto-replicative extra-chromosomal nucleic acid molecule under conditions allowing only host that has received said auto-replicative extra-chromosomal nucleic acid molecule to survive, thus providing a microbial host comprising an auto-replicative extra-chromosomal nucleic acid molecule. As such, in some embodiments, the method further comprises transforming the microbial host with said auto-replicative extra-chromosomal nucleic acid molecule prior to or during step a) under conditions allowing only host that has received said auto-replicative extra-chromosomal nucleic acid molecule to survive, thus providing the microbial host comprising the auto-replicative extra-chromosomal nucleic acid molecule.

The auto-replicative extra-chromosomal nucleic acid molecule transformed into the microbial host optionally comprises the second nucleic acid sequence of step b). The microbial host comprising the auto-replicative extra-chromosomal nucleic acid molecule can subsequently be cultured according to step c).

Within the context of methods, uses, compositions, hosts, and nucleic acids of embodiments herein, an auto-replicative extra-chromosomal nucleic acid molecule comprising a first nucleic acid sequence is provided. An auto-replicative extra-chromosomal nucleic acid molecule can exist free of the genome and may be derived from or comprise, consist essentially of, or consist of a plasmid, or episome, minichromosome, or alike. This feature is attractive as a higher number (from one to hundreds of copies or from 10 to 50 copies depending on the plasmid used) of copies of such nucleic acid molecule can be introduced and maintained into the microbial cell host. In addition, any host can be used in the methods of embodiments herein. In some embodiments, there is no need to modify the genome of the host. The genetic elements needed to carry out the methods of embodiments herein are present in the auto-replicative extra-chromosomal nucleic acid molecule. Such an auto-replicative extra-chromosomal nucleic acid molecule usually comprises an origin of replication, a first nucleic acid sequence which is of interestand a regulatory region. In some embodiments, without being limited by theory, a first nucleic acid sequence encoding an immunity modulator acts as a selectable marker to maintain the presence and function of the auto-replicative extra-chromosomal nucleic acid in the host cell. In some embodiments, the first nucleic acid sequence encoding the immunity modulator maintains the presence of the auto-replicative extra-chromosomal nucleic acid so that a product can be produced. The product can alter the environment in which the host is present, for example by fermenting a substance in the environment to produce one or more new substances. In some embodiments, genetic drift is minimized by providing selective pressure against auto-replicative extra-chromosomal nucleic acids that have acquired mutations, and do not produce a functional immunity modulator, produce an immunity modulator with reduced function, and/or produced lower levels of immunity modulator than an auto-replicative extra-chromosomal nucleic acid that has not acquired the mutation(s).

Within the context of methods, uses compositions, hosts, and nucleic acids of embodiments herein, the first nucleic acid molecule represented by the first nucleic acid

sequence is able to exhibit a genetic activity, said genetic activity confering an selective advantage to the microbial host cell wherein it is present and wherein this genetic activity is expressed. This genetic activity is provided by the product encoded by the first nucleic acid molecule. Moreover this genetic activity can be controlled or is expressed constitutively at a low level or is tunable or is under the control of a weak constitutive promoter. The control of said activity is believed to provide an advantage to limit the burden of energy for the host. Similarly, an advantage to limit the energy burden of the host may be obtained when the genetic activity is expressed constitutively at a low level or is tunable or is under the control of a weak constitutive promoter. Throughout the application text, the concept "conferring an advantage" may be replaced by "conferring immunity to a bacteriocin" or "conferring resistance to a bacteriocin". In some embodiments, the first nucleic acid sequence encodes an immunity modulator as described herein, and thereby confers an advantage to the host.

A second nucleic acid sequence encodes directly or indirectly for a product of interest. The same description holds for the genetic activity of the second nucleic acid molecule described herein. In some embodiments, the product of interest comprises an enzyme that is useful in an industrial process, for example a fermentation process. The fermentation process can ferment at least one compound in the culture medium. In some embodiments, the product of interest comprises an industrially useful molecule, for example a carbohydrate, a lipid, an organic molecule, a nutrient, a fertilizer, a biofuel, a cosmetic (or precursor thereof), a pharmaceutical or biopharmaceutical product (or precursor thereof), or two or more of any of the listed items.

Within the context of methods, uses, compositions, hosts, and nucleic acids of embodiments herein, a genetic activity may mean any activity that is caused by or linked with the presence of the first nucleic acid molecule in a microbial host. The advantage of said activity may be the ability to survive or survive and grow under given conditions (pH, temperature, presence of a given molecule such as a bacteriocin or combination of two or more bacteriocins as described herein,…). Accordingly the advantage of said activity may be assessed by determining the number of microbial cells/hosts comprising the auto-replicative extrachromosomal nucleic acid molecule. The assessment may be carried out at the end of and/or during the optional transforming step (but prior to

culturing step c), or prior to steps a) and culturing step c)) and/or prior to culturing step c). In an embodiment, the number of microbial host cells comprising the auto-replicative extra-chromosomal nucleic acid molecule present has not been decreased and may be increased by at least 5%, at least 10%, at least 15%, at least 20%, at least 25%, at least 30%, at least 35%, at least 40%, at least 45%, at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95% or more compared to the number of initial microbial cells/host when the cells are being cultured under conditions allowing the microbial host that has received said auto-replicative extra-chromosomal nucleic acid molecule to survive (e.g., by possessing immunity to one or more bacteriocins as described herein, and which are present in the given conditions). This assessment step may have a duration of at least 6 hours, 12 hours, 24 hours, 36 hours, 48 hours, 60 hours, 72 hours, 84 hours, 96 hours, 108 hours, 120 hours or more, including ranges between any two of the listed values.

Within the context of methods, uses, compositions, hosts, and nucleic acids of embodiments herein, the control of a genetic activity may mean either an increase or decrease of activity of a nucleic acid molecule (i.e. first and/or second nucleic acid molecule). Accordingly, the control of a genetic activity can be controlled or is expressed constitutively at a low level or is tunable or is under the control of a weak constitutive promoter. In some embodiments, the coding product for which genetic activity is regulated/controlled comprises, consists essentially of, or consists of an immunity modulator or is involved in the production of a product of interest. In some embodiments, genetic activity is regulated/controlled at the level of gene expression. In some embodiments, genetic activity is regulated at the transcriptional level, for example by activating or repressing a promoter. In some embodiments, promoters in this context are inducible promoters. In some embodiments, promoters in this context are weak promoters. Without being limited by theory, weak promoters of some embodiments can be amendable to up- or down- regulating the level of transcription so that the advantage conferred to the host (e.g. immunity modulator activity) is sensitive to changes in levels and/or activity of the gene product(s) under the control of the promoter. In some embodiments, the promoter comprises, consists of, or consists essentially of the P24 promoter represented by SEQ ID NO:707 and/or the ProC promoter represented by SEQ ID NO: 708 and/or the P24 LacO hybrid promoter. The P24LacO hybrid promoter is a

tunable/controlled promoter. In some embodiments, gene activity is regulated/controlled at the post-transcriptional level, for example through regulation of RNA stability. In some embodiments, genetic activity is regulated/controlled at the translational level, for example through regulation of initiation of translation. In some embodiments, genetic activity is regulated/controlled at the post-translational level, for example through regulation of polypeptide stability, post-translational modifications to the polypeptide, or binding of an inhibitor to the polypeptide.

In some embodiments, genetic activity is increased. In some embodiments, activity of at least one of an immunity modulator and/or the coding product of the second nucleic acid molecule is involved in the production of a product of interest is increased. Conceptually, genetic activity can be increased by directly activating genetic activity, or by decreasing the activity of an inhibitor of genetic activity. In some embodiments, genetic activity is activated by at least one of: inducing promoter activity, inhibiting a transcriptional repressor, increasing RNA stability, inhibiting a post-transcriptional inhibitor (for example, inhibiting a ribozyme or antisense oligonucleotide), inducing translation (for example, via a regulatable tRNA), making a desired post-translational modification, or inhibiting a post-translational inhibitor (for example a protease directed to a polypeptide encoded by the gene). In some embodiments, a compound present in a desired environment induces a promoter. For example, the presence of iron in culture medium can induce transcription by an iron-sensitive promoter as described herein. In some embodiments, a compound present in a desired culture medium inhibits a transcriptional repressor. For example, the presence of tetracycline in an environment can inhibit the tet repressor, and thus allow activity from the tetO promoter. In some embodiments, a compound found only outside of a desired culture medium induces transcription. In some embodiments, genetic activity is decreased. Conceptually, genetic activity can be decreased by directly inhibiting genetic activity, or by decreasing the activity of an activator of genetic activity. In some embodiments, genetic activity is reduced, but some level of activity remains. In some embodiments, genetic activity is fully inhibited. In some embodiments, genetic activity is decreased by at least one of inhibiting promoter activity, activating a transcriptional repressor, decreasing RNA stability, activating a post-transcriptional inhibitor (for example, expressing a ribozyme or antisense oligonucleotide), inhibiting translation (for example, via a regulatable tRNA), failing to

make a required post-translational modification, inactivating a polypeptide (for example by binding an inhibitor or via a polypeptide-specific protease), or failing to properly localize a polypeptide. In some embodiments, genetic activity is decreased by removing a gene from a desired location, for example by excising a gene using a FLP-FRT or cre-lox cassette, homologous recombination or CRIPR-CAS9 activity or through loss or degradation of a plasmid. In some embodiments, a gene product (e.g. a polypeptide) or a product produced by a gene product (e.g. the product of an enzymatic reaction) inhibits further gene activity (e.g. a negative feedback loop).

In some embodiments, the advantage conferred to a microbial host by the genetic activity of the first nucleic acid molecule is the ability to survive or survive and grow in a medium comprising a bacteriocin (or a mix of bacteriocins). As used herein, "bacteriocin" encompasses a cell-free or chemically synthesized version of such a polypeptide. A “bacteriocin," and variations of this root term, may also refer to a polypeptide that had been secreted by a host cell. A bacteriocin therefore encompasses a proteinaceous toxin produced by bacteria to inhibit the growth of similar or closely related bacterial strain(s). They are similar to yeast and paramecium killing factors, and are structurally, functionally, and ecologically diverse. A bacteriocin also encompasses a synthetic variant of a bacteriocin secreted by a host cell. Synthetic variant of a bacteriocin may be derived from the bacteriocin secreted by a host cell in any way as long as the synthetic variant still exhibits at least 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90% of the activity of the corresponding bacteriocin secreted by a host cell. A detailed description of an antibiotic is provided in the part dedicated to general descriptions at the end of the specification.

A “bacteriocin” can neutralize at least one cell other than the individual host cell in which the polypeptide is made, including cells clonally related to the host cell and other microbial cells.

A cell that expresses a particular "immunity modulator" (discussed in more detail herein) is immune to the neutralizing effects of a particular bacteriocin or group of bacteriocins. As such, bacteriocins can neutralize a cell producing the bacteriocin and/or other microbial cells, so long as these cells do not produce an appropriate immunity modulator. As such, a bacteriocin can exert cytotoxic or growth- inhibiting effects on a plurality of other microbial organisms. In an embodiment, a bacteriocin is

produced by the translational machinery (e.g. a ribosome, etc.) of a microbial cell. In another embodiment, a bacteriocin is chemically synthesized. Some bacteriocins can be derived from a polypeptide precursor. The polypeptide precursor can undergo cleavage (for example processing by a protease) to yield the polypeptide of the bacteriocin itself. As such, in some embodiments, a bacteriocin is produced from a precursor polypeptide. In some embodiments, a bacteriocin comprises, consists essentially of, or consists of a polypeptide that has undergone post -translational modifications, for example cleavage, or the addition of one or more functional groups.

Neutralizing activity of bacteriocins can include arrest of microbial reproduction, or cytotoxicity. Some bacteriocins have cytotoxic activity (e.g. "bacteriocide" effects), and thus can kill microbial organisms, for example bacteria, yeast, algae, synthetic micoorganisms, and the like. Some bacteriocins can inhibit the reproduction of microbial organisms (e.g. "bacteriostatic" effects), for example bacteria, yeast, algae, synthetic micoorganisms, and the like, for example by arresting the cell cycle.

A number of bacteriocins have been identified and characterized (see tables 1.1 and 1.2.). Without being limited by any particular theory, exemplary bacteriocins can be classified as "class I" bacteriocins, which typically undergo post -translational modification, and "class II" bacteriocins, which are typically unmodified. Additionally, exemplary bacteriocins in each class can be categorized into various subgroups, as summarized in Table 1.1 , which is adapted from Cotter, P.D. et al. "Bacteriocins- a viable alternative to antibiotics" Nature Reviews Microbiology 1 1 : 95-105, hereby incorporated by reference in its entirety.

Without being limited by any particular theory, bacteriocins can effect neutralization of a target microbial cell in a variety of ways. For example, a bacteriocin can permeabilize a cell wall, thus depolarizing the cell wall and interfering with respiration. Table 1.1 : Classification of Exemplary Bacteriocins.

Table 1.1: Classification of Exemplary Bacteriocins

Group	**Distinctive feature**	**Examples**
Class I (typically modified)		

Group	Distinctive feature	Examples
MccC7-C51-type bacteriocins	Is covalently attached to a carboxy-terminal aspartic acid	MccC7-C51
Lasso peptides	Have a lasso structure	MccJ25
Linear azole- or azoline-containing peptides	Possess heterocycles but not other modifications	MccB17
Lantibiotics	Possess lanthionine bridges	Nisin, planosporicin, mersacidin, actagardine, mutacin 1140
Linaridins	Have a linear structure and contain dehydrated amino acids	Cypemycin
Proteusins	Contain multiple hydroxylations, epimerizations and methylations	Polytheonamide A
Sactibiotics	Contain sulphur–α-carbon linkages	Subtilosin A, thuricin CD
Patellamide-like cyanobactins	Possess heterocycles and undergo macrocyclization	Patellamide A
Anacyclamide-like cyanobactins	Cyclic peptides consisting of proteinogenic amino acids with prenyl attachments	Anacyclamide A10
Thiopeptides	Contain a central pyridine, dihydropyridine or piperidine ring as well as heterocycles	Thiostrepton, nocathiacin I, GE2270 A, philipimycin
Bottromycins	Contain macrocyclic amidine, a decarboxylated carboxy-terminal thiazole and carbon-methylated amino acids	Bottromycin A2
Glycocins	Contain *S*-linked glycopeptides	Sublancin 168
Class II (typically unmodified or cyclic)		
IIa peptides (pediocin PA-1-like bacteriocins)	Possess a conserved YGNGV motif (in which N represents any amino acid)	Pediocin PA-1, enterocin CRL35, carnobacteriocin BM1
IIb peptides	Two unmodified peptides are required for activity	ABP118, lactacin F
IIc peptides	Cyclic peptides	Enterocin AS-48

Group	Distinctive feature	Examples
IId peptides	Unmodified, linear, non-pediocin-like, single-peptide bacteriocins	MccV, MccS, epidermicin NI01, lactococcin A
IIe peptides	Contain a serine-rich carboxy-terminal region with a non-ribosomal siderophore-type modification	MccE492, MccM

A number of bacteriocins can be used in accordance with embodiments herein. Exemplary bacteriocins are shown in Table 1.2. In some embodiments, at least one bacteriocin comprising, consisting essentially of, or consisting of a polypeptide sequence of Table 1.2 is provided. As shown in Table 1.2, some bacteriocins function as pairs of molecules. As such, it will be understood that unless explicity stated otherwise, when a functional "bacteriocin" or "providing a bacteriocin," or the like is discussed herein, functional bacteriocin pairs are included along with bacteriocins that function individually. With reference to Table 1.2, "organisms of origin" listed in parentheses indicate alternative names and/or strain information for organisms known to produce the indicated bacteriocin.

Embodiments herein also include peptides and proteins with identity to bacteriocins described in Table 1.2. The term "identity" is meant to include nucleic acid or protein sequence homology or three-dimensional homology. Several techniques exist to determine nucleic acid or polypeptide sequence homology and/or three-dimensional homology to polypeptides. These methods are routinely employed to discover the extent of identity that one sequence, domain, or model has to a target sequence, domain, or model. A vast range of functional bacteriocins can incorporate features of bacteriocins disclosed herein, thus providing for a vast degree of identity to the bacteriocins in Table 1.2. In some embodiments, a bacteriocin has at least 50% identity, for example, at least 50%, 51%, 52%, 53%, 54%, 55%, 56%, 57%, 58%, 59%, 60%, 61%, 62%, 63%, 64%, 65%, 66%, 67%, 68%, 69%, 70%, 71%, 72%, 73%, 74%, 75%, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identity to any one of the polypeptides of Table 1.2. Percent identity may be determined using the BLAST software (Altschul, S.F., et al. (1990) "Basic local alignment search tool." J. Mol. Biol.

215:403-410, accessible on the world wide web at blast.ncbi.nlm.nih.gov) with the default parameters.

While the bacteriocins in Table 1.2 are naturally-occurring, the skilled artisan will appreciate that variants of the bacteriocins of Table 1.2, naturally-occurring bacteriocins other than the bacteriocins of Table 1.2 or variants thereof, or synthetic bacteriocins can be used according to some embodiments herein. In some embodiments, such variants have enhanced or decreased levels of cytotoxic or growth inhibition activity on the same or a different microorganism or species of microorganism relative to the wild type protein. Several motifs have been recognized as characteristic of bacteriocins. For example, the motif YGXGV (SEQ ID NO: 2), wherein X is any amino acid residue, is a N-terminal consensus sequence characteristic of class IIa bacteriocins. Accordingly, in some embodiments, a synthetic bacteriocin comprises an N-terminal sequence with at least 50% identity to SEQ ID NO: 2, for example at least 50%, 51%, 52%, 53%, 54%, 55%, 56%, 57%, 58%, 59%, 60%, 61%, 62%, 63%, 64%, 65%, 66%, 67%, 68%, 69%, 70%, 71%, 72%, 73%, 74%, 75%, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identity to SEQ ID NO: 2. In some embodiments, a synthetic bacteriocin comprises a N-terminal sequence comprising SEQ ID NO: 2. Additionally, some class lib bacteriocins comprise a GxxxG motif (x means any amino acid). Without being limited by any particular theory, it is believed that the GxxxG motif can mediate association between helical proteins in the cell membrane, for example to facilitate bacteriocin-mediated neutralization through cell membrane interactions. As such, in some embodiments, the bacteriocin comprises a motif that facilitates interactions with the cell membrane. In some embodiments, the bacteriocin comprises a GxxxG motif. Optionally, the bacteriocin comprising a GxxxG motif can comprise a helical structure. In addition to structures described herein, "bacteriocin" as used herein also encompasses structures that have substantially the same effect on microbial cells as any of the bacteriocins explicitly provided herein.

It has been shown that fusion polypeptides comprising, consisting essentially of, or consisting of two or more bacteriocins or portions thereof can have neutralizing activity against a broader range of microbial organisms than either individual bacteriocin. For example, it has been shown that a hybrid bacteriocin, Ent35-MccV

(GKYYGNGVSCNKKGCSVDWGRAIGIIGNNSAANLATGGAAGWKSGGGASGR DIAMAIGTLSGQFVAGGIGAAAGGVAGGAIYDYASTHKPNPAMSPSGLGGTIK QKPEGIPSE AWNYAAGRLCNWSPNNLSDVCL, SEQ ID NO: 3), displays antimicrobial activity against pathogenic Gram-positive and Gram-negative bacteria (Acuna et al. (2012), FEBS Open Bio, 2: 12-19). It is noted that that Ent35-MccV fusion bacteriocin comprises, from N -terminus to C-terminus, an N-terminal glycine, Enterocin CRL35, a linker comprising three glycines, and a C-terminal Microcin V. It is contemplated herein that bacteriocins can comprise fusions of two or more polypeptides having bacteriocin activity. In some embodiments, a fusion polypeptide of two or more bacteriocins is provided. In some embodiments, the two or more bacteriocins comprise, consist essentially of, or consist of polypeptides from Table 1.2, or modifications thereof. In some embodiments, the fusion polypeptide comprising of two or more bacteriocins has a broader spectrum of activity than either individual bacteriocin, for example having neutralizing activity against more microbial organisms, neutralizing activity under a broader range of environmental conditions, and/or a higher efficiency of neutralization activity. In some embodiments, a fusion of two or more bacteriocins is provided, for example two, three, four, five, six, seven, eight, nine, or ten bacteriocins. In some embodiments, two or more bacteriocin polypeptides are fused to each other via a covalent bond, for example a peptide linkage. In some embodiments, a linker is positioned between the two bacteriocin polypeptides. In some embodiments, the linker comprises, consists essentially of, or consists of one or more glycines, for example about 1 , 2, 3, 4, 5, 6, 7, 8, 9, 10, 1 1 , 12, 13, 14, 15, 16, 17, 18, 19, or 20 glycines. In some embodiments, the linker is cleaved within the cell to produce the individual bacteriocins included in the fusion protein. In some embodiments, a bacteriocin as provided herein is modified to provide a desired spectrum of activity relative to the unmodified bacteriocin. For example, the modified bacteriocin may have enhanced or decreased activity against the same organisms as the unmodified bacteriocin. Alternatively, the modified bacteriocin may have enhanced activity against an organism against which the unmodified bacteriocin has less activity or no activity.

Table 1.2: Exemplary Bacteriocins

Poly-peptide SEQ ID NO:	Name	Class	Organism of origin	Poly-nucleotide SEQ ID NO:
4	Acidocin 8912	Unclassified	Lactobacillus acidophilus	5
6	Acidocin A	class IIA/YGNGV	Lactobacillus acidophilus	7
8	Acidocin B (AcdB)	Unclassified	Lactobacillus acidophilus	9
10	Acidocin LF221B (Gassericin K7 B)	Unclassified	Lactobacillus gasseri	11
12	Aureocin A53	Unclassified	Staphylococcus aureus	13
14	Avicin A	class IIA/YGNGV	Enterococcus avium (Streptococcus avium)	15
16	Bacteriocin 31	Unclassified	Enterococcus faecalis (Streptococcus faecalis)	17
18	Bacteriocin J46	Unclassified	Lactococcus lactis	19
20	Bacteriocin T8	class IIa	Enterococcus faecium (Streptococcus faecium)	21
22	Boticin B	Unclassified	Clostridium botulinum	23
24	Bovicin HJ50	Lantibiotic	Streptococcus equinus (Streptococcus bovis)	25
26	Brochocin-c	Unclassified	Brochothrix campestris	27
28	Butyrivibriocin AR10	Unclassified	Butyrivibrio fibrisolvens	29
30	Butyrivibriocin OR79	Lantibiotic	Butyrivibrio fibrisolvens	31
32	Carnobacteriocin B2 (Carnocin CP52)	class IIA/YGNGV	Carnobacterium maltaromaticum (Carnobacterium piscicola)	33
34	Carnobacteriocin BM1 (Carnobacteriocin B1)	class IIA/YGNGV	Carnobacterium maltaromaticum (Carnobacterium piscicola)	35

36	Carnobacteriocin-A (Piscicolin-61)	class IIc, non subgrouped bacteriocins (problematic)	Carnobacterium maltaromaticum (Carnobacterium piscicola)	37
38	Carnocyclin-A	Unclassified	Carnobacterium maltaromaticum (Carnobacterium piscicola)	39
40	Carocin D	Unclassified	Pectobacterium carotovorum subsp. carotovorum (Erwinia carotovora subsp. carotovora)	41
42	Cerein 7B	Unclassified	Bacillus cereus	43
44	Cinnamycin (Lanthiopeptin)	Lantibiotic	Streptoverticillium griseoverticillatum	45
46	Circularin A	Unclassified	Geobacillus kaustophilus (strain HTA426)	47
48	Closticin 574	Unclassified	Clostridium tyrobutyricum	49
50	Coagulin A	Unclassified	Bacillus coagulans	51
52	Colicin-10	Unclassified	Escherichia coli	53
54	Colicin-E1	Unclassified	Escherichia coli	55
56	Colicin-Ia	Unclassified	Escherichia coli	57
58	Colicin-Ib	Unclassified	Escherichia coli	59
60	Colicin-M	Unclassified	Escherichia coli	61
62	Colicin-N	Unclassified	Escherichia coli	63
64	Colicin-V (Microcin-V)	Unclassified	Escherichia coli	65
66	Columbicin A	Lantibiotic	Enterococcus columbae	69
68	Curvacin-A	class IIA/YGNGV	Lactobacillus curvatus	69
70	Cypemycin	Unclassified	Streptomyces sp.	71
72	Cytolysin	Lantibiotic	Bacillus halodurans (strain ATCC BAA-125 / DSM 18197 / FERM 7344 / JCM 9153 / C-125)	73
74	Divercin V41	class IIa/YGNGV	Carnobacterium divergens (Lactobacillus divergens)	75
76	Divergicin 750	Unclassified	Carnobacterium divergens (Lactobacillus divergens)	77
78	Divergicin A	Class IIc	Carnobacterium divergens (Lactobacillus divergens)	79
80	Durancin Q	Unclassified	Enterococcus durans	81
82	Durancin TW-49M	Unclassified	Enterococcus durans	83

84	Dysgalacticin	Unclassified	Streptococcus dysgalactiae subsp. equisimilis (Streptococcus equisimilis)	85
86	Enterocin 1071A	Unclassified	Enterococcus faecalis (Streptococcus faecalis)	87
88	Enterocin 7A (Enterocin L50A)	bacteriocins without sequence leader	Enterococcus faecalis (Streptococcus faecalis)	89
90	Enterocin 7B	Unclassified	Enterococcus faecalis (Streptococcus faecalis)	91
92	Enterocin 96	Class II	Enterococcus faecalis (strain ATCC 700802 / V583)	93
94	Enterocin A	Class IIa, IIc (problematic)	Enterococcus faecium (Streptococcus faecium)	95
96	Enterocin AS-48 (BACTERIOCIN AS-48)	Unclassified	Enterococcus faecalis (Streptococcus faecalis)	97
98	Enterocin B	class IIc, non subgrouped bacteriocins (problematic)	Enterococcus faecium (Streptococcus faecium)	99
100	Enterocin CRL35 (Mundticin KS)	Class IIa	Enterococcus mundtii	101
102	Enterocin EJ97	Unclassified	Enterococcus faecalis (Streptococcus faecalis)	103
104	Enterocin P	Class IIa, IIb and IIc (problematic)	Enterococcus faecium (Streptococcus faecium)	105
106	Enterocin Q	Class IIc	Enterococcus faecium (Streptococcus faecium)	107
108	Enterocin SE-K4	Class IIa	Enterococcus faecalis (Streptococcus faecalis)	109
110	Enterocin W alfa	Class IIb	Enterococcus faecalis (Streptococcus faecalis)	111
112	Enterocin W beta	Class IIb	Enterococcus faecalis (Streptococcus faecalis)	113
114	Enterocin Xalpha	Class IIb	Enterococcus faecium (Streptococcus faecium)	115

116	Enterocin Xbeta	Class IIb	Enterococcus faecium (Streptococcus faecium)	117
118	Enterolysin A	class III	Enterococcus faecalis (Streptococcus faecalis)	119
120	Epicidin 280	Lantibiotic	Staphylococcus epidermidis	121
122	Epidermicin NI01	Unclassified	Staphylococcus epidermidis	123
124	Epidermin	Lantibiotic	Staphylococcus epidermidis	125
126	Epilancin K7	Lantibiotic	Staphylococcus epidermidis	127
128	Gallidermin	Lantibiotic	Staphylococcus gallinarum	129
130	Garvicin A	IId	Lactococcus garvieae	131
132	Garvicin ML	Unclassified	Lactococcus garvieae	133
134	Gassericin A	Unclassified	Lactobacillus gasseri	135
136	Gassericin T (gassericin K7 B)	Unclassified	Lactobacillus gasseri	137
138	Glycocin F	Unclassified	Lactobacillus plantarum	139
140	Halocin H4	Unclassified	Haloferax mediterranei (strain ATCC 33500 / DSM 1411 / JCM 8866 / NBRC 14739 / NCIMB 2177 / R-4) (Halobacterium mediterranei)	141
142	Halocin-S8	Unclassified	Haloarchaeon S8a	143
144	Helveticin-J	Unclassified	Lactobacillus helveticus (Lactobacillus suntoryeus)	145
146	Hiracin JM79	Class II sec-dependent	Enterococcus hirae	147
148	Lactacin-F (lafA)	class IIB	Lactobacillus johnsonii (strain CNCM I-12250 / La1 / NCC 533)	149
150	Lactacin-F (lafX)	class IIB	Lactobacillus johnsonii (strain CNCM I-12250 / La1 / NCC 533)	151
152	Lacticin 3147 A1	Lantibiotic	Lactococcus lactis subsp. lactis (Streptococcus lactis)	153
154	Lacticin 3147 A2	Lantibiotic	Lactococcus lactis subsp. lactis (Streptococcus lactis)	155
156	Lacticin 481 (Lactococcin DR)	Lantibiotic	Lactococcus lactis subsp. lactis (Streptococcus lactis)	157
158	Lacticin Q	Unclassified	Lactococcus lactis	159

160	Lacticin Z	Unclassified	Lactococcus lactis	161
162	Lactobin-A (Amylovorin-L471)	class IIB	Lactobacillus amylovorus	163
164	Lactocin-S	Lantibiotic	Lactobacillus sakei L45	165
166	Lactococcin 972	Unclassified	Lactococcus lactis subsp. lactis (Streptococcus lactis)	167
168	Lactococcin-A	Unclassified	Lactococcus lactis subsp. cremoris (Streptococcus cremoris)	169
170	Lactococcin-B	Unclassified	Lactococcus lactis subsp. cremoris (Streptococcus cremoris)	171
172	Lactocyclicin Q	Unclassified	Lactococcus sp. QU 12	173
174	Laterosporulin	Unclassified	Brevibacillus sp. GI-9	175
176	Leucocin N	Class IId	Leuconostoc pseudomesenteroides	177
178	Leucocin Q	Class IId	Leuconostoc pseudomesenteroides	179
180	Leucocin-A (Leucocin A-UAL 187)	class IIA/YGNGV	Leuconostoc gelidum	181
182	Leucocin-B (Leucocin B-Ta11a)	class IIA/YGNGV	Leuconostoc carnosum	183
184	Leucocyclicin Q	Unclassified	Leuconostoc mesenteroides	185
186	Lichenicidin A1	Lantibiotic (two-peptide)	Bacillus licheniformis (strain DSM 13 / ATCC 14580)	187
188	Linocin M18	Unclassified	Brevibacterium linens	189
190	Listeriocin 743A	Class IIa	Listeria innocua	191
192	Mersacidin	Lantibiotic, type B	Bacillus sp. (strain HIL-Y85/54728)	193
194	Mesentericin Y105	class IIA/YGNGV	Leuconostoc mesenteroides	195
196	Michiganin-A	Lantibiotic	Clavibacter michiganensis subsp. michiganensis	197
198	Microcin B17 (MccB17)	Unclassified	Escherichia coli	199
200	Microcin C7	Unclassified	Escherichia coli	201
202	Microcin E492	Unclassified	Klebsiella pneumoniae	203
204	Microcin H47	Unclassified	Escherichia coli	205
206	Microcin J25	Unclassified	Escherichia coli	207

208	Microcin-24	Unclassified	Escherichia coli	209
210	Mundticin KS	Unclassified	Enterococcus mundtii	211
212	Mundticin L	class IIA/YGNGV	Enterococcus mundtii	213
214	Mutacin 1140 (Mutacin III)	Lantibiotic	Streptococcus mutans	215
216	Mutacin-2	Lantibiotic	Streptococcus mutans	217
218	Nisin A	Lantibiotic	Lactococcus lactis subsp. lactis (Streptococcus lactis)	219
220	Nisin F	Lantibiotic	Lactococcus lactis	221
222	Nisin Q	Lantibiotic	Lactococcus lactis	223
224	Nisin U	Lantibiotic	Streptococcus uberis	225
226	Nisin Z	Lantibiotic	Lactococcus lactis subsp. lactis (Streptococcus lactis)	227
228	Nukacin ISK-1	Lantibiotic	Staphylococcus warneri	229
230	Paenicidin A	Lantibiotic	Paenibacillus polymyxa (Bacillus polymyxa)	231
232	Pediocin PA-1 (Pediocin ACH)	class IIA/YGNGV	Pediococcus acidilactici	233
234	Penocin A	class IIA/YGNGV	Pediococcus pentosaceus (strain ATCC 25745 / 183-1w)	235
236	Pep5	Lantibiotic	Staphylococcus epidermidis	237
238	Piscicolin 126	class IIA/YGNGV	Carnobacterium maltaromaticum (Carnobacterium piscicola)	239
240	Plantaricin 1.25 β	Unclassified	Lactobacillus plantarum	241
242	Plantaricin 423	class IIa	Lactobacillus plantarum	243
244	Plantaricin ASM1	Unclassified	Lactobacillus plantarum	245
246	Plantaricin E	Unclassified	Lactobacillus plantarum	247
248	Plantaricin F	Class IIb	Lactobacillus plantarum	249
250	Plantaricin J	Class IIb	Lactobacillus plantarum	251
252	Plantaricin K	Unclassified	Lactobacillus plantarum	253
254	Plantaricin NC8 α	Unclassified	Lactobacillus plantarum	255
256	Plantaricin NC8 β	Unclassified	Lactobacillus plantarum	257
258	Plantaricin S α	Unclassified	Lactobacillus plantarum	259
260	Plantaricin S β	Unclassified	Lactobacillus plantarum	261

262	Plantaricin W α	Lantibiotic (two-peptide)	Lactobacillus plantarum	263
264	Plantaricin W β	Lantibiotic (two-peptide)	Lactobacillus plantarum	265
266	Plantaricin-A	Unclassified	Lactobacillus plantarum (strain ATCC BAA-793 / NCIMB 8826 / WCFS1)	267
268	Propionicin SM1	Unclassified	Propionibacterium jensenii	269
270	Propionicin T1	Unclassified	Propionibacterium thoenii	271
272	Propionicin-F	Unclassified	Propionibacterium freudenreichii subsp. freudenreichii	273
274	Pyocin S1	Unclassified	Pseudomonas aeruginosa	275
276	Pyocin S2	colicin/pyosin nuclease family	Pseudomonas aeruginosa (strain ATCC 15692 / PAO1 / 1C / PRS 101 / LMG 12228)	277
278	Ruminococcin-A	Lantibiotic	Ruminococcus gnavus	279
280	Sakacin G	Class IIa	Lactobacillus sakei	281
282	Sakacin-A	class IIA/YGNGV	Lactobacillus sakei	283
284	Sakacin-P (Sakacin 674)	class IIA/YGNGV	Lactobacillus sakei	285
286	Salivaricin 9	lantibiotic	Streptococcus salivarius	287
288	Salivaricin A	Lantibiotic	Streptococcus pyogenes serotype M28 (strain MGAS6180)	289
290	Salivaricin A3	Lantibiotic	Streptococcus salivarius	291
292	Salivaricin-A sa	Lantibiotic	Streptococcus salivarius	293
294	Staphylococcin C55 alpha	Lantibiotic (two-peptide)	Staphylococcus aureus	295
296	Staphylococcin C55 beta	Lantibiotic (two-peptide)	Staphylococcus aureus	297
298	Streptin	lantibiotic	Streptococcus pyogenes	299
300	Streptococcin A-FF22	Lantibiotic	Streptococcus pyogenes	301
302	Streptococcin A-M49	Lantibiotic	Streptococcus pyogenes serotype M49	303
304	Sublancin 168	Lantibiotic	Bacillus subtilis (strain 168)	305
306	Subtilin	Lantibiotic	Bacillus subtilis	307
308	Subtilosin	Unclassified	Bacillus subtilis (strain 168)	309

310	Subtilosin-A	Unclassified	Bacillus subtilis (strain 168)	311
312	Thermophilin 1277	Lantibiotic	Streptococcus thermophilus	313
314	Thermophilin 13	Unclassified	Streptococcus thermophilus	315
316	Thermophilin A	Unclassified	Streptococcus thermophilus	317
318	Thiocillin (Micrococcin P1) (Micrococcin P2) (Thiocillin I) (Thiocillin II) (Thiocillin III) (Thiocillin IV) (Antibiotic YM-266183) (Antibiotic YM-266184)	Unclassified	Bacillus cereus (strain ATCC 14579 / DSM 31)	319
320	Thuricin CD alpha	two-peptide lantibiotic	Bacillus cereus 95/8201	321
322	Thuricin CD beta	two-peptide lantibiotic	Bacillus cereus 95/8201	323
324	Thuricin-17	Class IId	Bacillus thuringiensis	325
326	Trifolitoxin	Unclassified	Rhizobium leguminosarum bv. trifolii	327
328	Ubericin A	Class IIa	Streptococcus uberis	329
330	Uberolysin	Unclassified	Streptococcus uberis	331
332	UviB	Unclassified	Clostridium perfringens	333
334	Variacin	Lantibiotic, Type A	Micrococcus varians	335
336	Zoocin A	Unclassified	Streptococcus equi subsp. zooepidemicus	337
338	Fulvocin-C	Unclassified	Myxococcus fulvus	339
340	Duramycin-C	Lantibiotic	Streptomyces griseoluteus	341
342	Duramycin (duramycin-B) (Leucopeptin)	Lantibiotic B	Streptoverticillium griseoverticillatum	343
344	Carnocin UI49	lantibiotic	Carnobacterium sp. (strain UI49)	345
346	Lactococcin-G α	Unclassified	Lactococcus lactis subsp. lactis (Streptococcus lactis)	347

348	Lactococcin-G β	Unclassified	Lactococcus lactis subsp. lactis (Streptococcus lactis)	349
350	Ancovenin	Lantibiotic	Streptomyces sp. (strain A647P-2)	351
352	Actagardine (Gardimycin)	Lantibiotic	Actinoplanes liguriae	353
354	Curvaticin FS47	Unclassified	Lactobacillus curvatus	355
356	Bavaricin-MN	class IIA/YGNGV	Lactobacillus sakei	357
358	Mutacin B-Ny266	Lantibiotic	Streptococcus mutans	359
360	Mundticin	class IIA/YGNGV	Enterococcus mundtii	361
362	Bavaricin-A	class IIA/YGNGV	Lactobacillus sakei	363
364	Lactocin-705	Class IIb	Lactobacillus paracasei	365
366	Leucocin-B	Unclassified	Leuconostoc mesenteroides	367
368	Leucocin C	class IIA/YGNGV	Leuconostoc mesenteroides	369
370	LCI	Unclassified	Bacillus subtilis	371
372	Lichenin	Unclassified	Bacillus licheniformis	373
374	Lactococcin MMFII	class IIA/YGNGV	Lactococcus lactis subsp. lactis (Streptococcus lactis)	375
376	Serracin-P	Phage-Tail-Like	Serratia plymuthica	377
378	Halocin-C8	Unclassified	Halobacterium sp. (strain AS7092)	379
380	Subpeptin JM4-B	Unclassified	Bacillus subtilis	381
382	Curvalicin-28a	Unclassified	Lactobacillus curvatus	383
384	Curvalicin-28b	Unclassified	Lactobacillus curvatus	385
386	Curvalicin-28c	Unclassified	Lactobacillus curvatus	387
388	Thuricin-S	Unclassified	Bacillus thuringiensis subsp. entomocidus	389
390	Curvaticin L442	Unclassified	Lactobacillus curvatus	391
392	Divergicin M35	class IIa/YGNGV	Carnobacterium divergens (Lactobacillus divergens)	393
394	Enterocin E-760	class IIb	Enterococcus sp.	395
396	Bacteriocin E50-52	Unclassified	Enterococcus faecium (Streptococcus faecium)	397
398	Paenibacillin	Unclassified	Paenibacillus polymyxa (Bacillus polymyxa)	399
400	Epilancin 15x	Unclassified	Staphylococcus epidermidis	401
402	Enterocin-HF	class IIa	Enterococcus faecium (Streptococcus faecium)	403

404	Bacillocin 602	Class IIa	Paenibacillus polymyxa (Bacillus polymyxa)	405
406	Bacillocin 1580	Class IIa	Bacillus circulans	407
408	Bacillocin B37	Unclassified	Paenibacillus polymyxa (Bacillus polymyxa)	409
410	Rhamnosin A	Unclassified	Lactobacillus rhamnosus	411
412	Lichenicidin A2	Lantibiotic (two-peptide)	Bacillus licheniformis (strain DSM 13 / ATCC 14580)	413
414	Plantaricin C19	Class IIa	Lactobacillus plantarum	415
416	Acidocin J1132 β	Class IIb	Lactobacillus acidophilus	417
418	factor with anti-*Candida* activity	Unclassified	Enterococcus faecalis	419
420	Ava_1098 (putative heterocyst differentiation protein)	Unclassified	Anabaena variabilis ATCC 29413	421
422	alr2818 (putative heterocyst differentiation protein)	Unclassified	Nostoc sp 7120	423
424	Aazo_0724 (putative heterocyst differentiation protein)	Unclassified	Nostoc azollae 0708	425
426	AM1_4010 (putative heterocyst differentiation protein)	Unclassified	Acaryochloris marina MBIC11017	427
428	PCC8801_3266 (putative heterocyst differentiation protein)	Unclassified	Cyanothece PCC 8801	429

430	Cyan8802_2855 (putative heterocyst differentiation protein)	Unclassified	Cyanothece PCC 8802	431
432	PCC7424_3517	Unclassified	Cyanothece PCC 7424	433
434	cce_2677(putative HetP protein)	Unclassified	Cyanothece ATCC 51142	435
436	CY0110_11572 (putative heterocyst differentiation protein)	Unclassified	Cyanothece CCY0110	437
438	MC7420_4637	Unclassified	Microcoleus chthonoplastes PCC 7420	439
440	asr1611 (putative DUF37 family protein)	Unclassified	Nostoc sp 7120	441
442	Ava_4222 (putative DUF37 family protein)	Unclassified	Anabaena variabilis ATCC 29413	443
444	N9414_07129 (putative DUF37 family protein)	Unclassified	Nodularia spumigena CCY9414	445
446	Aazo_0083 (putative DUF37 family protein)	Unclassified	Nostoc azollae 0708	447
448	S7335_3409 (putative DUF37 family protein)	Unclassified	Synechococcus PCC 7335	449
450	P9303_21151 (putative DUF37 family protein)	Unclassified	Prochlorococcus marinus MIT 9303	451

720	Curvalicin-28c	Unclassified	Lactobacillus curvatus	721
722	thruicin-S	Unclassified	Bacillus thuringiensis	723
724	curvaticin L442	Unclassified	Lactobacillus curvatus L442	725
726	Bacteriocin divergicin M35	P84962	Carnobacterium divergens (Lactobacillus divergens)	727
728	Lantibiotic 107891	P85065	Microbispora sp. (strain 107891)	729
730	Enterocin E-760 (Bacteriocin E-760)	P85147	Enterococcus sp.	731
732	Bacteriocin E50-52	P85148	Enterococcus faecium (Streptococcus faecium)	733
734	Lantibiotic paenibacillin	P86013	Paenibacillus polymyxa (Bacillus polymyxa)	735
736	Lantibiotic epilancin 15X	P86047	Staphylococcus epidermidis	737
738	Enterocin-HF	P86183	Enterococcus faecium (Streptococcus faecium)	739
740	Bacteriocin SRCAM 602	P86393	Paenibacillus polymyxa (Bacillus polymyxa)	741
742	Bacteriocin SRCAM 1580	P86394	Bacillus circulans	743
744	Bacteriocin SRCAM 37	P86395	Paenibacillus polymyxa (Bacillus polymyxa)	745
746	Bacteriocin rhamnosin A (Fragment)	P86526	Lactobacillus rhamnosus	747
748	Lantibiotic lichenicidin A2 (LchA2) (BliA2)	P86720	Bacillus licheniformis (strain ATCC 14580 / DSM 13 / JCM 2505 / NBRC 12200 / NCIMB 9375 / NRRL NRS-1264 / Gibson 46)	749
750	Pyocin-S2 (EC 3.1.-.-) (Killer protein)		Pseudomonas aeruginosa (strain ATCC 15692 / DSM 22644 / CIP 104116 / JCM 14847 / LMG 12228 / 1C / PRS 101 / PAO1)	751

752	Plantaricin C19 (Fragment)		Lactobacillus plantarum	753
754	LsbB		Lactococcus lactis subsp. lactis (Streptococcus lactis)	755
756	ACIDOCIN J1132 beta peptide (Fragment)		Lactobacillus acidophilus	757
758	Uncharacterized protein		Lactobacillus salivarius cp400	759

For example, in some embodiments, an anti-fungal activity (such as anti- yeast activity) is desired. A number of bacteriocins with anti- fungal activity have been identified. For example, bacteriocins from Bacillus have been shown to have neutralizing activity against yeast strains {see Adetunji and Olaoye (2013) Malaysian Journal of Microbiology 9: 130-13, hereby incorporated by reference in its entirety), an Enterococcus faecalis peptide (WLPPAGLLGRCGRWFRPWLLWLQ SGAQY KWLGNLFGLGPK, SEQ ID NO: 1) has been shown to have neutralizing activity against Candida species {see Shekh and Roy (2012) BMC Microbiology 12: 132, hereby incorporated by reference in its entirety), and bacteriocins from Pseudomonas have been shown to have neutralizing activity against fungi such as Curvularia lunata, Fusarium species, Helminthosporium species, and Biopolaris species (Shalani and Srivastava (2008) The Internet Journal of Microbiology. Volume 5 Number 2. DOI: 10.5580/27dd - accessible on the worldwide web at archive, ispub.com/journal/the-internet-journal-of-micro bio logy/volume-5-number-2/screening- for-antifungal-activity-of-pseudomonas-fluorescens-against-phytopathogenic-fungi.html#sthash.d0Ys03UO. lDKuTlUS.dpuf, hereby incorporated by reference in its entirety). By way of example, botrycidin AJ1316 {see Zuber, P et al. (1993) Peptide Antibiotics. In Bacillus subtilis and Other Gram-Positive Bacteria: Biochemistry, Physiology, and Molecular Genetics ed Sonenshein et al., pp. 897-916, American Society for Microbiology, hereby incorporated by reference in its entirety) and alirin Bl {see Shenin et al. (1995) Antibiot Khimioter 50: 3-7, hereby incorporated by reference in its entirity) from 5. subtilis have been shown to have antifungal activities. As such,

in some embodiments, for example embodiments in which neutralization of a fungal microbial organism is desired, a bacteriocin comprises at least one of botrycidin AJ1316 or alirin B 1.

For example, in some embodiments, bacteriocin activity in a culture of cyanobacteria is desirable. In some embodiments, bacteriocins are provided to neutralize cyanobacteria. In some embodiments, bacteriocins are provided to neutralize invading microbial organisms typically found in a cyanobacteria culture environment. Clusters of conserved bacteriocin polypetides have been identified in a wide variety of cyanobacteria species. For example, at least 145 putative bacteriocin gene clusters have been identified in at least 43 cyanobacteria species, as reported in Wang et al. (2011), Genome Mining Demonstrates the Widespread Occurrence of Gene Clusters Encoding Bacteriocins in Cyanobacteria. PLoS ONE 6(7): e22384, hereby incorporated by reference in its entirety. Exemplary cyanobacteria bacteriocins are shown in Table 1.2 as SEQ ID NO's 420, 422, 424, 426, 428, 30, 432, 434, 436, 438, 440, 442, 444, 446, 448, and 450.

Within the context of methods, uses, compositions, hosts, and nucleic acids of embodiments herein, although a bacteriocin may work via different mechanisms on a microbial cell as explained herein, a bacteriocin may be said to be active when the number of microbial host has decreased by at least 5%, at least 10%, at least 15%, at least 20%, at least 25%, at least 30%, at least 35%, at least 40%, at least 45%, at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95% or more compared to the number of initial microbial host when the microbial hosts are being cultured with a medium comprising a bacteriocin. This culture step may have a duration of at least 12 hours, 24 hours, 36 hours, 48 hours, 60 hours, 72 hours or more before assessing the activity of the bacteriocin by counting the number of microbial hosts present. The activity may be assessed by counting the cells under the microscope or by any known microbial techniques. In some embodiments, a bacteriocin is active when the growth has been arrested in at least a specified number or paercentage of microbial hosts, for example at least 5%, at least 10%, at least 15%, at least 20%, at least 25%, at least 30%, at least 35%, at least 40%, at least 45%, at least 50%, at least 55%, at least 60%, at least 65%,

at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, or at least 95% of the microbial hosts arrested compared to the initial popualtion of microbial hosts when the microbial hosts are being cultured with a medium comprising a bacteriocin.

Within the context of methods, uses, compositions, hosts, and nucleic acids of some embodiments herein, the bacteriocin is B17 or C7 represented by an amino acid sequence comprising or consisting of SEQ ID NO: 198 or 200 respectively. B17 and C7 have been experimentally confirmed to be selection agents simple to produce, easy to use and stable in culture medium in accordance with some embodiments herein (*See* **Example 1**).Some of methods, uses, compositions, hosts, and nucleic acids of embodiments herein also encompass the use a bacteriocin having at least 50% identity to SEQ ID NO: 198 or 200, for example at least 50%, 51%, 52%, 53%, 54%, 55%, 56%, 57%, 58%, 59%, 60%, 61%, 62%, 63%, 64%, 65%, 66%, 67%, 68%, 69%, 70%, 71%, 72%, 73%, 74%, 75%, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identity to SEQ ID NO: 198 or 200. Such variants of B17 or C7 may be used in methods, uses, compositions, hosts, and nucleic acids of embodiments herein as long as they exhibit at least a substantial activity of B17 or C7. In this context, "substantial" means, for example, at least 50%, at least 60%, at least 705, at least 80%, at least 90%, or at least 100% or more of the activity of B17 or C7 having SEQ ID NO: 198 or 200. The activity of a bacteriocin has been described earlier herein.

Within the context of methods, uses, compositions, hosts, and nucleic acids of embodiments herein, and depending on the microbial host targeted and the bacteriocin used, the skilled person will know which concentration of bacteriocin is to be used in a medium or in an agar petri plate. Using bacteriocin B17 or C7 inventors were able to prepare culture medium comprising said bacteriocin in a concentration which allows one to carry out the methods and uses of embodiments herein, i.e. to observe or visualize an advantage of the expression of said genetic activity. If an advantage of said activity is to allow the growth of the host comprising the auto-replicative extra-chromosomal nucleic acid molecule, then the quantity of bacteriocin in said medium or agar plate is such that the number of host that does not comprise said auto-replicative extra-chromosomal has been decreased by at least 5%, at least 10%, at least 15%, at

least 20%, at least 25%, at least 30%, at least 35%, at least 40%, at least 45%, at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95% or more compared to the number of initial microbial cells/host when the cells are being cultured under conditions allowing the microbial host that has received said auto-replicative extra-chromosomal nucleic acid molecule to survive and to grow. This assessment step may have a duration of at least 6 hours, 12 hours, 24 hours, 36 hours, 48 hours, 60 hours, 72 hours, 84 hours, 96 hours, 108 hours, 120 hours or more. Said culture medium may be sterilized without losing substantial bacteriocin activity. In this context "substantial" means, for example, more than 50%, more than 60%, more than 70%, more than 80%, more than 90% of the bacteriocin activity present in the culture medium before sterilization.

First nucleic acid sequences suitable for methods, uses, compositions, hosts, and nucleic acids of some embodiments herein, and whose product provides immunity to a bacteriocin are shown in Table 2.

Table 2: Exemplary nucleic acid sequences whose provide immunity to a bacteriocin

Poly-peptide SEQ ID NO:	**Name**	**Organism of origin**	**Poly-nucleotide SEQ ID NO:**
452	Microcin H47 immunity modulator MchI	Escherichia coli	453
454	Colicin-E3 immunity modulator (Colicin-E3 chain B) (ImmE3) (Microcin-E3 immunity modulator)	Escherichia coli	455
456	Colicin-E1 immunity modulator (ImmE1) (Microcin-E1 immunity modulator)	Escherichia coli	457
458	Cloacin immunity modulator	Escherichia coli	459
460	Colicin-E2 immunity modulator (ImmE2) (Microcin-E2 immunity modulator)	Escherichia coli	461
462	Colicin-A immunity modulator (Microcin-A immunity modulator)	Citrobacter freundii	463
464	Colicin-Ia immunity modulator	Escherichia coli	465
466	Colicin-Ib immunity modulator	Escherichia coli	467
468	Colicin-N immunity modulator (Microcin-N immunity modulator)	Escherichia coli	469
470	Colicin-E8 immunity modulator (ImmE8) (Microcin-E8 immunity modulator)	Escherichia coli	471

472	Lactococcin-A immunity modulator	Lactococcus lactis subsp. lactis (Streptococcus lactis)	473
474	Lactococcin-A immunity modulator	Lactococcus lactis subsp. cremoris (Streptococcus cremoris)	475
476	Colicin-D immunity modulator (Microcin-D immunity modulator)	Escherichia coli	477
478	Colicin-E5 immunity modulator (ImmE5) (Microcin-E5 immunity modulator)	Escherichia coli	479
480	Colicin-E6 immunity modulator (ImmE6) (Microcin-E6 immunity modulator)	Escherichia coli	481
482	Colicin-E8 immunity modulator in ColE6 (E8Imm[E6])	Escherichia coli	483
484	Colicin-E9 immunity modulator (ImmE9) (Microcin-E9 immunity modulator)	Escherichia coli	485
486	Colicin-M immunity modulator (Microcin-M immunity modulator)	Escherichia coli	487
488	Colicin-B immunity modulator (Microcin-B immunity modulator)	Escherichia coli	489
490	Colicin-V immunity modulator (Microcin-V immunity modulator)	Escherichia coli	491
492	Colicin-E1* immunity modulator (ImmE1) (Microcin-E1* immunity modulator)	Shigella sonnei	493
494	Colicin-E1 immunity modulator (ImmE1) (Microcin-E1 immunity modulator)	Escherichia coli	495
496	Probable leucocin-A immunity modulator	Leuconostoc gelidum	497
498	Lactococcin-B immunity modulator	Lactococcus lactis subsp. cremoris (Streptococcus cremoris)	499
500	Pediocin PA-1 immunity modulator (Pediocin ACH immunity modulator)	Pediococcus acidilactici	501

502	Putative carnobacteriocin-BM1 immunity modulator	Carnobacterium maltaromaticum (Carnobacterium piscicola)	503
504	Putative carnobacteriocin-B2 immunity modulator (Carnocin-CP52 immunity modulator)	Carnobacterium maltaromaticum (Carnobacterium piscicola)	505
506	Nisin immunity modulator	Lactococcus lactis subsp. lactis (Streptococcus lactis)	507
508	Trifolitoxin immunity modulator	Rhizobium leguminosarum bv. trifolii	509
510	Antilisterial bacteriocin subtilosin biosynthesis protein AlbD	Bacillus subtilis (strain 168)	511
512	Putative ABC transporter ATP-binding protein AlbC (Antilisterial bacteriocin subtilosin biosynthesis protein AlbC)	Bacillus subtilis (strain 168)	513
514	Antilisterial bacteriocin subtilosin biosynthesis protein AlbB	Bacillus subtilis (strain 168)	515
516	Colicin-E7 immunity modulator (ImmE7) (Microcin-E7 immunity modulator)	Escherichia coli	517
518	Pyocin-S1 immunity modulator	Pseudomonas aeruginosa	519
520	Pyocin-S2 immunity modulator	Pseudomonas aeruginosa (strain ATCC 15692 / PAO1 / 1C / PRS 101 / LMG 12228)	521
522	Hiracin-JM79 immunity factor	Enterococcus hirae	523
524	Probable mesentericin-Y105 immunity modulator	Leuconostoc mesenteroides	525
526	Microcin-24 immunity modulator	Escherichia coli	527
528	Colicin-K immunity modulator	Escherichia coli	529

530	Microcin C7 self-immunity modulator MccF	Escherichia coli	531
532	Sakacin-A immunity factor	Lactobacillus sakei	533
534	Colicin-E5 immunity modulator in ColE9 (E5Imm[E9])	Escherichia coli	535
536	Antilisterial bacteriocin subtilosin biosynthesis protein AlbD	Bacillus subtilis	537
538	Microcin-J25 export ATP-binding/permease protein McjD (Microcin-J25 immunity modulator) (Microcin-J25 secretion ATP-binding protein McjD)	Escherichia coli	539
540	Microcin E492 immunity modulator	Klebsiella pneumoniae	541
	McbG	Escherichia coli	699
	MccE	Escherichia coli	700
706	C-terminal part of MccE	Derived from E. coli and considered as artificial	701
	Cvi	Escherichia coli	709
	McbG-MccE	Derived from E. coli and considered as artificial	715
	McbG-Cter part MccE (Cter could be replaced by C-terminal)	Derived from E. coli and considered as artificial	716
	Cvi-MccE	Derived from E. coli and considered as artificial	717
	Cvi-Cter part MccE (Cter could be replaced by C-terminal)	Derived from E. coli and considered as artificial	718

While the sequence providing immunity to a bacteriocin of Table 2 are naturally-occurring, the skilled artisan will appreciate that variants of such molecules , naturally-occurring molecules other than the ones of Table 2, or synthetic ones can be used according to some embodiments herein. In some embodiments, a particular molecule conferring immunity or particular combination of molecules conferring immunity to a particular bacteriocin, particular class or category of bacteriocins, or particular combination of bacteriocins. Exemplary bacteriocins to which molecules can confer

immunity are identified in Table 2. While Table 2 identifies an "organism of origin" for a molecule conferring immunity, these molecules conferring immunity can readily be expressed in other naturally-occurring, genetically modified, or synthetic microorganisms to provide a desired bacteriocin immunity activity in accordance with some embodiments herein. As such, as used herein "immunity modulator" or "molecule conferring or providing immunity to a bacteriocin" encompasses not only to structures expressly provided herein, but also structures that have substantially the same effect as the "immunity modulator" structures described herein, including fully synthetic immunity modulators, and immunity modulators that provide immunity to bacteriocins that are functionally equivalent to the bacteriocins disclosed herein.

Exemplary polynucleotide sequences encoding the polypeptides of Table 2 are indicated in Table 2. The skilled artisan will readily understand that the genetic code is degenerate, and moreover, codon usage can vary based on the particular organism in which the gene product is being expressed, and as such, a particular polypeptide can be encoded by more than one polynucleotide. In some embodiments, a polynucleotide encoding a bacteriocin immunity modulator is selected based on the codon usage of the organism expressing the bacteriocin immunity modulator. In some embodiments, a polynucleotide encoding a bacteriocin immunity modulator is codon optimized based on the particular organism expressing the bacteriocin immunity modulator. A vast range of functional immunity modulators can incorporate features of immunity modulators disclosed herein, thus providing for a vast degree of identity to the immunity modulators in Table 2. In some embodiments, an immunity modulator has at least about 50% identity, for example, at least 50%, 51%, 52%, 53%, 54%, 55%, 56%, 57%, 58%, 59%, 60%, 61%, 62%, 63%, 64%, 65%, 66%, 67%, 68%, 69%, 70%, 71%, 72%, 73%, 74%, 75%, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identity to any one of the polypeptides of Table 2.

Within the context of methods, uses, compositions, hosts, and nucleic acids of embodiments herein, resistance or immunity to a bacteriocin may mean the number of microbial cells at the end of a culturing step with a bacteriocin has not been decreased, and in some embodiments has been increased of at least 5%, at least 10%, at least 15%,

at least 20%, at least 25%, at least 30%, at least 35%, at least 40%, at least 45%, at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95% or more compared to the number of initial microbial cells when the cells are being cultured with a medium comprising a bacteriocin. This culture step may have a duration of at least 12 hours, 24 hours, 36 hours, 48 hours, 60 hours, 72 hours, 84 hours, 96 hours, 108 hours, 120 hours or more before assessing the activity of the bacteriocin by counting the number of microbial cells present.

A nucleic acid molecule suitable for methods, uses, compositions, hosts, and nucleic acids of some embodiments herein and whose encoding product confers immunity is McbG (Immunity to the bacteriocin B17), which is represented by SEQ ID NO: 699. McbG has been experimentally confirmed to be useful as a selectable marker either constitutively or inducibly in accordance with some embodiments herien (*See* **Example 3**). Another suitable nucleic acid is the MccE (Immunity to the bacteriocin C7) which is represented by SEQ ID NO: 700 or its c-terminal portion, represented by SEQ ID NO: 701. MccE hsd been used as a vector selection marker in strains sensitive to microcines/bacteriocins (*See* **Example 2**). Methods, uses, compositions, hosts, and nucleic acids of some embodiments also encompass the use of a nucleic acid molecule whose encoding product confers immunity to bacteriocin B17 and/or C7 and having at least 50% identity to SEQ ID NO: 699, 700, or 701, for example at least 50%, 51%, 52%, 53%, 54%, 55%, 56%, 57%, 58%, 59%, 60%, 61%, 62%, 63%, 64%, 65%, 66%, 67%, 68%, 69%, 70%, 71%, 72%, 73%, 74%, 75%, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identity to SEQ ID NO: 699, 700 or 701. Such variants of McbG and/or MccE may be used in methods, uses, compositions, hosts, and nucleic acids of embodiments hereinas long as they exhibit at least a substantial activity of McbG (respectively MccE). In this context, "substantial" means, for example, at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, or at least 100% or more of the activity of McbG (respectively MccE) having SEQ ID NO: 699, 700 or 701. The immunity conferred by the encoding product of McbG (respectively MccE) has been described earlier herein.

Surprisingly it has been found that the C-terminal part of MccE which is represented by SEQ ID NO: 701 is sufficient to confer resistance to bacteriocin C7. Part means in this context, for example, at least 50%, at least 60%, at least 70%, at least 80%, at least 90% or more of the original nucleic acid molecule. This is quite attractive and surprising that such a short nucleic acid molecule can confer resistance to a bacteriocin. It is expected that an auto-replicative extra-chromosomal nucleic acid molecule comprising such short nucleic acid molecule does not form any burden for the microbial cell.

A further suitable nucleic acid molecule for methods, uses, compositions, hosts, and nucleic acids of some embodiments herein, and whose product provides immunity to a bacteriocin is a single nucleic acid molecule whose single product provides immunity to at least two distinct bacteriocins. In some embodiments, such product of such nucleic acid molecule provides immunity to B17 and C7 or to ColV and C7 or to ColV and B17 or to B17, C7 and ColV. A nucleic acid encoding ColV is identified as SEQ ID NO: 65 and a corresponding coding amino acid sequence is identified as SEQ ID NO: 64.

In some embodiments, a nucleic acid molecule whose product provides immunity to B17 and C7 is represented by a sequence having at least 50% identity to SEQ ID NO: 715 or 716 for example at least 50%, 51%, 52%, 53%, 54%, 55%, 56%, 57%, 58%, 59%, 60%, 61%, 62%, 63%, 64%, 65%, 66%, 67%, 68%, 69%, 70%, 71%, 72%, 73%, 74%, 75%, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identity to SEQ ID NO: 715 or 716. SEQ ID NO: 715 is a nucleic acid molecule of McbG fused to MccE. SEQ ID NO: 716 is a nucleic acid molecule of McbG fused to the C-terminal part of MccE as earlier described herein.

In some embodiments, a nucleic acid molecule whose product provides immunity to ColV and C7 is represented by a sequence having at least 50% identity to SEQ ID NO: 717 or 718 for example at least 50%, 51%, 52%, 53%, 54%, 55%, 56%, 57%, 58%, 59%, 60%, 61%, 62%, 63%, 64%, 65%, 66%, 67%, 68%, 69%, 70%, 71%, 72%, 73%, 74%, 75%, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identity to SEQ ID NO: 717 or 718. SEQ ID NO: 717 is a nucleic acid molecule of Cvi fused to

MccE. SEQ ID NO: 718 is a nucleic acid molecule of Cvi fused to the C-terminal part of MccE as earlier described herein.

Such identity variants of the core sequence may be used in methods, uses, compositions, hosts, and nucleic acids of embodiments herein as long as they exhibit at least a substantial activity of the molecule they derived from as earlier described herein.

In methods, uses, compositions, hosts, and nucleic acids of some embodiments herein, each of these nucleic acid molecules described herein whose product confers immunity to a single or to more than one or to at least two bacteriocins may be operably linked to a promoter as described herein. In some embodiments, the promoter is a weak promoter. In some embodiments, the weak promoter is the proC promoter represented by SEQ ID NO : 708 or the P24 promoter represented by SEQ ID NO : 707, which has been experimentally confirmed (*See, e.g.* **Example 3**).

Suitable constructs useful in methods, uses, compositions, hosts, and nucleic acids of embodiments herein can comprise a first nucleic acid molecule whose product confers immunity to a bacteriocin, and these constructs may comprise, consist essentially of, or consist of SEQ ID NO : 702, 703, 710, 711, 704, 705, 712, 713 or 714. Each of these constructs has been extensively described in the experimental part of the application, which notes that each of these constructs was actually constructed and confirmed to be suitable in accordance with some embodiments herein (*See, e.g.,* **Examples 1** and **2** and **3**).

In a method of some embodiments, the bacteriocin added to the culture medium is a B17 and/or a C7 and/or a ColV as identified herein

The method may allow the production of any product of interest. In a method of some embodiments, the product of interest is a microbial biomass, the auto-replicative extra-chromosomal nucleic acid molecule, the transcript of said second nucleic sequence, a polypeptide encoded by said second sequence or a metabolite produced directly or indirectly by said polypeptide.

In a method of some embodiments, the product of interest is purified at the end of the culturing step c). This may be carried out using techniques known to the skilled person. Since the energetic burden associated with the presence of the auto-replicative extra-chromosomal nucleic acid molecule has been minimized, the yield of the product of interest is expected to be optimal.

The method may use any suitable microbial cells, for example as hosts. Suitable microbial cells are listed in the part of the specification entitled general descriptions. Suitable microbial cells per se and for use in methods, uses, compositions, and hosts of embodiments herein include, but are not limited to: a bacterium (for example, a Gram negative bacterium, for example an E. coli species), a yeast, a filamentous fungus or an algae. In some embodiments, the microbial cell is a synthetic microbial cell.

In a method, the first nucleic acid sequence present on the auto-replicative extra-chromosomal nucleic acid molecule may be operably linked to a promoter. In some embodiments, said promoter is a weak promoter. In some embodiments, said promoter is a constitutive promoter. In some embodiments, said promoter is inducible. In some embodiments, said promoter is a weak constitutive promoter. In some embodiments, said promoter is a weak inducible promoter. The inducibility of said promoter is a way of controlling the presence of the genetic activity of the first nucleic acid sequence. Promoters are well known in the art. A detailed description is provided in the part of the specification dedicated to the general descriptions. A promoter can be used to drive the transcription of one or more coding sequences. Optionally said auto-replicative extra-chromosomal nucleic acid molecule comprises a second nucleic acid sequence that is involved in the production of a product of interest, wherein the genetic activity of said second nucleic acid sequence is controlled independently from the one of the first sequence.

In an embodiment, the control of the genetic activity of said second nucleic acid sequence is not independent from the control of the genetic activity of the first sequence.

In some embodiments, a second promoter drives expression of said second nucleic acid sequence being involved in the production of a product of interest as described herein. In an embodiment, a first promoter drives expression of an immunity modulator polynucleotide as described herein.

A promoter that could be used herein may be not native to a nucleic acid molecule to which it is operably linked, i.e. a promoter that is heterologous to the nucleic acid molecule (coding sequence) to which it is operably linked. Although a promoter of some embodiments is heterologous to a coding sequence to which it is operably linked, in some

embodiments, a promoter is homologous, e.g., endogenous to a microbial cell. In some embodiments, a heterologous promoter (to the nucleotide sequence) is capable of producing a higher steady state level of a transcript comprising a coding sequence (or is capable of producing more transcript molecules, i.e. mRNA molecules, per unit of time) than is a promoter that is native to a coding sequence. Some promoters can drive transcription at all times ("constitutive promoters"). Some promoters can drive transcription under only select circumstances ("conditional promoters" or "inducible promoter"), for example depending on the presence or absence of an environmental condition, chemical compound, gene product, stage of the cell cycle, or the like.

The skilled artisan will appreciate that depending on the desired expression activity, an appropriate promoter can be selected, and placed in cis (i.e. or is operably linked with) with a sequence to be expressed. Exemplary promoters with exemplary activities are provided in Table 3.1-3.11 herein. The skilled artisan will appreciate that some promoters are compatible with particular transcriptional machinery (e.g. RNA polymerases, general transcription factors, and the like). As such, while compatible "species" are identified for some promoters described herein, it is contemplated that according to some embodiments herein, these promoters can readily function in microorganisms other than the identified species, for example in species with compatible endogenous transcriptional machinery, genetically modified species comprising compatible transcriptional machinery, or fully synthetic microbial organisms comprising compatible transcriptional machinery.

The promoters of Tables 3.1-3.11 herein are publicly available from the Biobricks foundation. It is noted that the Biobricks foundation encourages use of these promoters in accordance with BioBrick™ Public Agreement (BPA).

It should be appreciated that any of the "coding" polynucleotides described herein (for example a first nucleic acid sequence and/or a second nucleic acid sequence involved in the production of a product of itnerest) is generally amenable to being expressed under the control of a desired promoter. In an embodiment, a first nucleic acid sequence is under the control of a first promoter. In an embodiment, a second nucleic acid sequence involved in the production of a product of interest is under the control of a second promoter.

Generally, translation initiation for a particular transcript is regulated by particular sequences at 5 ' end of the coding sequence of a transcript. For example, a coding sequence can begin with a start codon configured to pair with an initiator tRNA. While naturally-occurring translation systems typically use Met (AUG) as a start codon, it will be readily appreciated that an initiator tRNA can be engineered to bind to any desired triplet or triplets, and accordingly, triplets other than AUG can also function as start codons in certain embodiments. Additionally, sequences near the start codon can facilitate ribosomal assembly, for example a Kozak sequence ((gcc)gccNccAUGG, SEQ ID NO: 542, in which N represents "A" or "G") or Internal Ribosome Entry Site (IRES) in typical eukaryotic translational systems, or a Shine-Dalgarno sequence (GGAGGU, SEQ ID NO: 543) in typical prokaryotic translation systems. As such in some embodiments, a transcript comprising a "coding" polynucleotide sequence, for example a first nucleic acid sequence, or second nucleic acid sequence involved in the production of a fermentation product, comprises an appropriate start codon and translational initiation sequence. In some embodiments, for example if two or more "coding" polynucleotide sequences are positioned in cis on a transcript, each polynucleotide sequence comprises an appropriate start codon and translational initiation sequence(s).

In some embodiments, for example if two or more "coding" polynucleotide sequences are positioned in cis on a transcript, the two sequences are under control of a single translation initiation sequence, and either provide a single polypeptide that can function with both encoded polypeptides in cis, or provide a means for separating two polypeptides encoded in cis, for example a 2A sequence or the like. In some embodiments, a translational intiator tRNA is regulatable, so as to regulate initiation of translation of an immunity modulator or industrially useful molecule.

Table 3.1: Exemplary Metal-Sensitive Promoters

SEQ ID NO:	Name	Description
544	BBa_I721001	Lead Promoter
545	BBa_I731004	FecA promoter
546	BBa_I760005	Cu-sensitive promoter
547	BBa_I765000	Fe promoter
548	BBa_I765007	Fe and UV promoters

549	BBa_J3902	PrFe (PI + PII rus operon)

Table 3.2: Exemplary Cell-Signaling-Responsive Promoters

SEQ ID NO:	Name	Description
550	BBa_I1051	Lux cassette right promoter
551	BBa_I14015	P(Las) TetO
552	BBa_I14016	P(Las) CIO
553	BBa_I14017	P(Rhl)
554	BBa_I739105	Double Promoter (LuxR/HSL, positive / cI, negative)
555	BBa_I746104	P2 promoter in agr operon from S. aureus
556	BBa_I751501	plux-cI hybrid promoter
557	BBa_I751502	plux-lac hybrid promoter
558	BBa_I761011	CinR, CinL and glucose controlled promotor
559	BBa_J06403	RhIR promoter repressible by CI
560	BBa_J102001	Reverse Lux Promoter
561	BBa_J64000	rhlI promoter
562	BBa_J64010	lasI promoter
563	BBa_J64067	LuxR+3OC6HSL independent R0065
564	BBa_J64712	LasR/LasI Inducible & RHLR/RHLI repressible Promoter
565	BBa_K091107	pLux/cI Hybrid Promoter
566	BBa_K091117	pLas promoter
567	BBa_K091143	pLas/cI Hybrid Promoter
568	BBa_K091146	pLas/Lux Hybrid Promoter
569	BBa_K091156	pLux
570	BBa_K091157	pLux/Las Hybrid Promoter
571	BBa_K145150	Hybrid promoter: HSL-LuxR activated, P22 C2 repressed
572	BBa_K266000	PAI+LasR -> LuxI (AI)
573	BBa_K266005	PAI+LasR -> LasI & AI+LuxR --| LasI
574	BBa_K266006	PAI+LasR -> LasI+GFP & AI+LuxR --| LasI+GFP
575	BBa_K266007	Complex QS -> LuxI & LasI circuit
576	BBa_K658006	position 3 mutated promoter lux pR-3 (luxR & HSL regulated)
577	BBa_K658007	position 5 mutated promoter lux pR-5 (luxR & HSL regulated)
578	BBa_K658008	position 3&5 mutated promoter lux pR-3/5 (luxR & HSL regulated)
579	BBa_R0061	Promoter (HSL-mediated luxR repressor)
580	BBa_R0062	Promoter (luxR & HSL regulated -- lux pR)
581	BBa_R0063	Promoter (luxR & HSL regulated -- lux pL)

582	BBa_R0071	Promoter (RhlR & C4-HSL regulated)
583	BBa_R0078	Promoter (cinR and HSL regulated)
584	BBa_R0079	Promoter (LasR & PAI regulated)
585	BBa_R1062	Promoter, Standard (luxR and HSL regulated -- lux pR)

Table 3.3: Exemplary Constitutive *E. coli* σ^{70} Promoters

SEQ ID NO:	**Name**	**Description**
586	BBa_I14018	P(Bla)
587	BBa_I14033	P(Cat)
588	BBa_I14034	P(Kat)
589	BBa_I732021	Template for Building Primer Family Member
590	BBa_I742126	Reverse lambda cI-regulated promoter
591	BBa_J01006	Key Promoter absorbs 3
592	BBa_J23100	constitutive promoter family member
593	BBa_J23101	constitutive promoter family member
594	BBa_J23102	constitutive promoter family member
595	BBa_J23103	constitutive promoter family member
596	BBa_J23104	constitutive promoter family member
597	BBa_J23105	constitutive promoter family member
598	BBa_J23106	constitutive promoter family member
599	BBa_J23107	constitutive promoter family member
600	BBa_J23108	constitutive promoter family member
601	BBa_J23109	constitutive promoter family member
602	BBa_J23110	constitutive promoter family member
603	BBa_J23111	constitutive promoter family member
604	BBa_J23112	constitutive promoter family member
605	BBa_J23113	constitutive promoter family member
606	BBa_J23114	constitutive promoter family member
607	BBa_J23115	constitutive promoter family member
608	BBa_J23116	constitutive promoter family member
609	BBa_J23117	constitutive promoter family member
610	BBa_J23118	constitutive promoter family member
611	BBa_J23119	constitutive promoter family member
612	BBa_J23150	1bp mutant from J23107
613	BBa_J23151	1bp mutant from J23114
614	BBa_J44002	pBAD reverse

615	BBa_J48104	NikR promoter, a protein of the ribbon helix-helix family of trancription factors that repress expre
616	BBa_J54200	lacq_Promoter
617	BBa_J56015	lacIQ - promoter sequence
618	BBa_J64951	E. Coli CreABCD phosphate sensing operon promoter
619	BBa_K088007	GlnRS promoter
620	BBa_K119000	Constitutive weak promoter of lacZ
621	BBa_K119001	Mutated LacZ promoter
622	BBa_K137029	constitutive promoter with (TA)10 between -10 and -35 elements
623	BBa_K137030	constitutive promoter with (TA)9 between -10 and -35 elements
624	BBa_K137031	constitutive promoter with (C)10 between -10 and -35 elements
625	BBa_K137032	constitutive promoter with (C)12 between -10 and -35 elements
626	BBa_K137085	optimized (TA) repeat constitutive promoter with 13 bp between -10 and -35 elements
627	BBa_K137086	optimized (TA) repeat constitutive promoter with 15 bp between -10 and -35 elements
628	BBa_K137087	optimized (TA) repeat constitutive promoter with 17 bp between -10 and -35 elements
629	BBa_K137088	optimized (TA) repeat constitutive promoter with 19 bp between -10 and -35 elements
630	BBa_K137089	optimized (TA) repeat constitutive promoter with 21 bp between -10 and -35 elements
631	BBa_K137090	optimized (A) repeat constitutive promoter with 17 bp between -10 and -35 elements
632	BBa_K137091	optimized (A) repeat constitutive promoter with 18 bp between -10 and -35 elements
633	BBa_K256002	J23101:GFP
634	BBa_K256018	J23119:IFP
635	BBa_K256020	J23119:HO1
636	BBa_K256033	Infrared signal reporter (J23119:IFP:J23119:HO1)
637	BBa_K292000	Double terminator + constitutive promoter
638	BBa_K292001	Double terminator + Constitutive promoter + Strong RBS
639	BBa_K418000	IPTG inducible Lac promoter cassette
640	BBa_K418002	IPTG inducible Lac promoter cassette
641	BBa_K418003	IPTG inducible Lac promoter cassette
642	BBa_M13101	M13K07 gene I promoter
643	BBa_M13102	M13K07 gene II promoter
644	BBa_M13103	M13K07 gene III promoter
645	BBa_M13104	M13K07 gene IV promoter

646	BBa_M13105	M13K07 gene V promoter
647	BBa_M13106	M13K07 gene VI promoter
648	BBa_M13108	M13K07 gene VIII promoter
649	BBa_M13110	M13110
650	BBa_M31519	Modified promoter sequence of g3.
651	BBa_R1074	Constitutive Promoter I
652	BBa_R1075	Constitutive Promoter II
653	BBa_S03331	—Constitutive promoter

Table 3.4: Exemplary Constitutive *E. coli* σ^8 Promoters

SEQ ID NO:	**Name**	**Description**
654	BBa_J45992	Full-length stationary phase osmY promoter
655	BBa_J45993	Minimal stationary phase osmY promoter

Table 3.5: Exemplary Constitutive *E. coli* σ^{32} Promoters

SEQ ID NO:	**Name**	**Description**
656	BBa_J45504	htpG Heat Shock Promoter

Table 3.6: Exemplary Constitutive *B. subtilis* σ^A Promoters

SEQ ID NO:	**Name**	**Description**
657	BBa_K143012	Promoter veg a constitutive promoter for B. subtilis
658	BBa_K143013	Promoter 43 a constitutive promoter for B. subtilis
659	BBa_K780003	Strong constitutive promoter for Bacillus subtilis
660	BBa_K823000	PliaG
661	BBa_K823002	PlepA
662	BBa_K823003	Pveg

Table 3.7: Exemplary Constitutive *B. subtilis* σ^B Promoters

SEQ ID NO:	**Name**	**Description**
663	BBa_K143010	Promoter ctc for B. subtilis
664	BBa_K143011	Promoter gsiB for B. subtilis
665	BBa_K143013	Promoter 43 a constitutive promoter for B. subtilis

Table 3.8: Exemplary Constitutive Promoters from miscellaneous prokaryotes

SEQ ID NO:	Name	Description
666	a_K112706	Pspv2 from *Salmonella*
667	BBa_K112707	Pspv from *Salmonella*

Table 3.9: Exemplary Constitutive Promoters from bacteriophage T7

SEQ ID NO:	Name	Description
668	BBa_I712074	T7 promoter (strong promoter from T7 bacteriophage)
669	BBa_I719005	T7 Promoter
670	BBa_J34814	T7 Promoter
671	BBa_J64997	T7 consensus -10 and rest
672	BBa_K113010	overlapping T7 promoter
673	BBa_K113011	more overlapping T7 promoter
674	BBa_K113012	weaken overlapping T7 promoter
675	BBa_R0085	T7 Consensus Promoter Sequence
676	BBa_R0180	T7 RNAP promoter
677	BBa_R0181	T7 RNAP promoter
678	BBa_R0182	T7 RNAP promoter
679	BBa_R0183	T7 RNAP promoter
680	BBa_Z0251	T7 strong promoter
681	BBa_Z0252	T7 weak binding and processivity
682	BBa_Z0253	T7 weak binding promoter

Table 3.10: Exemplary Constitutive Promoters from yeast

SEQ ID NO:	Name	Description
683	BBa_I766555	pCyc (Medium) Promoter
684	BBa_I766556	pAdh (Strong) Promoter
685	BBa_I766557	pSte5 (Weak) Promoter
686	BBa_J63005	yeast ADH1 promoter
687	BBa_K105027	cyc100 minimal promoter
688	BBa_K105028	cyc70 minimal promoter
689	BBa_K105029	cyc43 minimal promoter
690	BBa_K105030	cyc28 minimal promoter
691	BBa_K105031	cyc16 minimal promoter
692	BBa_K122000	pPGK1

693	BBa_K124000	pCYC Yeast Promoter
694	BBa_K124002	Yeast GPD (TDH3) Promoter
695	BBa_K319005	yeast mid-length ADH1 promoter
696	BBa_M31201	Yeast CLB1 promoter region, G2/M cell cycle specific

Table 3.11: Exemplary Constitutive Promoters from miscellaneous eukaryotes

SEQ ID NO:	**Name**	**Description**
697	BBa_I712004	CMV promoter
698	BBa_K076017	Ubc Promoter

The above-referenced promoters are provided by way of non-limiting example only. A promoter may be a synthetic promoter. Suitable promoters for methods, uses, compositions, hosts, and nucleic acids of some embodiments herein have been described earlier herein e.g., proC represented by SEQ ID NO: 708 which has been experimentally confirmed in accordance with some embodiments herein (*See* **Example 2**) and P24 represented by SEQ ID NO: 707 which has been experimentally confirmed in accordance with some embodiments herein (*See* **Example 3**). In some embodiments, a suitable inducible promoter is the P24 LacO hybrid promoter, which is repressed in the presence of LacI and active in presence of IPTG. This promoter has been experimentally confirmed in accordance with some embodiments herein (*See* **Example 3**).

The skilled artisan will readily recognize that many variants of the above- referenced promoters, and many other promoters (including promoters isolated from naturally existing organisms, variations thereof, and fully synthetic or engineered promoters) can readily be used in accordance with some embodiments herein. A variant, fully synthetic or synthetic or engineer promoter is said to be active or functional and can therefore be used in methods, uses, compositions, hosts, and nucleic acids of embodiments herein when tested in a control or reference plasmid being operably linked with a nucleic acid molecule encoding a transcript, a detectable amount of said transcript molecule is present when said plasmid is present in a cell. A variant, fully synthetic or synthetic or engineer promoter may have at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, or at least 100% of the activity of the promoter it derives from.

Optionally, the method comprises transforming said microbial host with said auto-replicative extra-chromosomal nucleic acid molecule under conditions allowing the host that has received said auto-replicative extra-chromosomal nucleic acid molecule to survive. It is noted that in some embodiments, the auto-replicative extra-chromosomal nucleic acid molecule can be provided in a microbial cell (e.g., if the microbial cell, or a predecessor thereof was transformed with the auto-replicative extra-chromosomal nucleic acid molecule), and as such, in some embodiments, the transformation step is not needed in the method. The transforming step can be performed prior to the culturing of step c). In some embodiments, the transforming step is provided prior to step a) so as to provide the host cell comprising the auto-replicative extra-chromosomal nucleic acid molecule. In some embodiments, the auto-replicative extra-chromosomal nucleic acid molecule used in the transforming step further comprises the second nucleic acid of optional step b).

Techniques of genetically modifying microbial organisms are well known in the art (for example see Molecular Cloning Fourth edition, 2012 Cold Spring Harbor Laboratory Press, A laboratory manual, by M.R. Green and J Sambrook, which is herein incorporated by reference in its entirety). In some embodiments, a microorganism is genetically modified to comprise said auto-replicative extra-chromosomal nucleic acid molecule comprising a first nucleic acid sequence and optionally a molecule involved in the production of a product of interest. Polynucleotides or nucleic acid molecules can be delivered to microorganisms.

In an embodiment a microbial cell is positively selected for by the genetic activity of the first nucleic acid sequence corresponding to at least one given condition allowing the cell that has received the said auto-replicative extra-chromosomal nucleic acid molecule to survive and said conditions can be environmental conditions. Environmental conditions may be a culture medium.

It can be useful to flexibly genetically modify a microbial cell, for example to engineer or reengineer a microbial cell to have a desired type and/or spectrum of genetic activities. In some embodiments, a cassette for inserting one or more desired distinct

first nucleic acid sequences is provided. Exemplary cassettes include, but are not limited to, a Cre/lox cassette or FLP/FRT cassette.

In an embodiment, a microbial cell comprises more than one (more than two, more than three, ...) different auto-replicative extra-chromosomal nucleic acid molecule comprising a first nucleic acid sequence as described herein, meaning that said cell can exhibit more than one (more than two, more than three,...) genetic activity, each genetic activity conferring an advantage to the cell. If a first promoter is present in each of the different auto-replicative extra-chromosomal nucleic acid molecule, each of said first promoters may be different or identical. It is therefore within the scope of the of methods, uses, compositions, hosts, and nucleic acids of embodiments herein to use one, two, three, four or more distinct bacteriocins in a method for producing a product of interest wherein the microbial host comprises one, two, three, four or more distinct extra-chromosomal nucleic acid molecule, each conferring a distinct genetic activity to said microbial host. Alternatively, it is within the scope of methods, uses, compositions, hosts, and nucleic acids of embodiments herein that a single nucleic acid molecule whose product provides immunity to at least distinct bacteriocins is used. Such a nucleic acid molecule has been described herein.

In some embodiments, plasmid conjugation can be used to introduce a desired plasmid from a "donor" microbial cell to a recipient microbial cell. Goni-Moreno, et al. (2013) Multicellular Computing Using Conjugation for Wiring. PLoS ONE 8(6): e65986, hereby incorporated by reference in its entirety. In some embodiments, plasmid conjugation can genetically modify a recipient microbial cell by introducing a conjugation plasmid from a donor microbial cell to a recipient microbial cell. Without being limited by any particular theory, conjugation plasmids that comprise the same or functionally same set of replication genes typically cannot coexist in the same microbial cell. As such, in some embodiments, plasmid conjugation "reprograms" a recipient microbial cell by introducing a new conjugation plasmid to supplant another conjugation plasmid that was present in the recipient cell. In some embodiments, plasmid conjugation is used to engineer (or reengineer) a microbial cell with a particular combination of first nucleic acid molecules (which can code for immunity modulators in some embodiments). According to some embodiments, a variety of

conjugation plasmids comprising different combinations of first acid sequence (which can code for immunity modulators in some embodiments) is provided. The plasmids can comprise additional genetic elements as described herein, for example promoters, translational initiation sites, and the like. In some embodiments the variety of conjugation plasmids is provided in a collection of donor cells, so that a donor cell comprising the desired plasmid can be selected for plasmid conjugation. In some embodiments, a particular combination of immunity modulators is selected, and an appropriate donor cell is conjugated with a microbial cell of interest to introduce a conjugation plasmid comprising that combination into a recipient cell. In some embodiments, the recipient cell is a "newly engineered" cell, for example to be introduced into or for initiating a culture.

Step b)

In addition to step a), in some embodiments the method further comprises optional step b) wherein said auto-replicative extra-chromosomal nucleic acid molecule comprises a second nucleic acid sequence that is involved in the production of said product of interest, wherein the genetic activity of said second nucleic acid sequence is controlled independently from the one of the first sequence.

In the context of methods, uses, compositions, hosts, and nucleic acids of embodiments herein, the expression “controlled independently” has its customary and ordinary meanings as understood by one of skill in the art in view of this disclosure, including meaning that distinct ways are used for controlling the genetic activity of the first and the second nucleic acid sequences. Ways of controlling the genetic activity of a nucleic acid sequence have been already described in detail herein.

Step c)

In some embodiments, step a) (which optionally includes transforming as described herein) and optional step b) is followed by step c), which comprises culturing said transformed microbial host under conditions allowing said transformed microbial host to express the first nucleic acid sequence to a given level to maintain the auto-replicative extra-chromosomal molecule into the growing microbial population. In some embodiments, optionally controlling the second sequence coding for said product of interest.

In a method of some embodiments, at least part of step c) conditions are such that the first nucleic acid sequence does not exhibit said genetic activity. “Part of step c)” means, for example, at least 20%, 30%, 40%, 50%, 60%, 70%, 80% or 90% or up to 100% of the duration of step c). This embodiment of the method is quite attractive as part of step c) is carried out without the presence of the genetic activity of the first nucleic acid sequence. The presence of said genetic activity forms an energetic burden for the microbial host cell and is not always needed in order to keep a suitable production level of a product of interest. It is envisaged in some embodiments to have part of step c) without genetic activity of the first nucleic acid sequence followed by a part with said activity. These two parts may be repeated one or more time during step c).

A microbial cell may be cultured in any suitable microbial culture environment. Microbial culture environments can comprise a wide variety of culture media, for example feedstocks. The selection of a particular culture medium can depend upon the desired application. Conditions of a culture medium include not only chemical composition, but also temperature, amounts of light, pH, CO_2 levels, and the like. The culture medium can comprise a bacteriocin. In an embodiment, a compound that induces the activity of the bacteriocin is present outside of the feedstock, but not in the feedstock.

In an embodiment, a genetically engineered or transformed microorganism as described herein is added to a culture medium that comprises at least one feedstock. In an embodiment, the culture medium comprises a compound that induces the activity or expression of an immunity modulator.

The term "feedstock" has is customary and ordinary meaning as understood by one of skill in the art in view of this disclosure, and encompasses material that can be consumed, fermented, purified, modified, or otherwise processed by microbial organisms, for example in the context of industrial processes. As such, "feedstock" is not limited to food or food products. As used herein a "feedstock" is a category of culture medium. Accordingly, as used herein "culture medium" includes, but it is not limited to feedstock. As such, whenever a "culture medium" is referred to herein, feedstocks are also expressly contemplated.

Before culturing a transformed microbial cell, it can be useful to determine the effects, if any, or optimize the conditions allowing the host that has received said auto-replicative extra-chromosomal nucleic acid molecule to survive and optionally to grow.

In some embodiments, a microbial cell or microbial host or microbial host cell or synthetic microbial host cell comprising an auto-replicative extra-chromosomal nucleic acid molecule is provided, comprising a first nucleic acid sequence whose genetic activity confers an advantage to a microbial host wherein the genetic activity of said first nucleic acid sequence is controlled, and optionally comprising a second nucleic acid sequence that is directly or indirectly involved in the production of a product of interest.

In some embodiments, there is provided an auto-replicative extra-chromosomal nucleic acid molecule, comprising a first nucleic acid sequence whose genetic activity confers an advantage to a microbial host wherein the genetic activity of said first nucleic acid sequence is controlled, and optionally comprising a second nucleic acid sequence that is directly or indirectly involved in the production of a product of interest.

Each feature of this microbial host and of this auto-replicative extra-chromosomal nucleic acid molecule have already been described herein.

General Descriptions

The terms used herein have the customary and ordinary meaning understood by one of skill in the art when read in view fo this disclosure, and can include the following general descriptions.

Microorganism

As used herein, "microbial organism," "microorganism," “microbial cell” or “microbial host” and variations of these root terms (such as pluralizations and the like) have their customary and ordinary meanings as understood by one of skill in the art in view of this disclosure, including any naturally-occurring species or synthetic or fully synthetic prokaryotic or eukaryotic unicellular organism, as well as Archae species. Thus, this expression can refer to cells of bacterial species, fungal species, and algae. Exemplary microorganisms that can be used in accordance with embodiments herein include, but

are not limited to, bacteria, yeast, filamentous fungi, and algae, for example photosynthetic microalgae. Furthermore, fully synthetic microorganism genomes can be synthesized and transplanted into single microbial cells, to produce synthetic microorganisms capable of continuous self-replication (see Gibson et al. (2010), "Creation of a Bacterial Cell Controlled by a Chemically Synthesized Genome," Science 329: 52-56, hereby incorporated by reference in its entirety). As such, in some embodiments, the microorganism is fully synthetic. A desired combination of genetic elements, including elements that regulate gene expression, and elements encoding gene products (for example immunity modulators, poison, antidote, and industrially useful molecules also called product of interest) can be assembled on a desired chassis into a partially or fully synthetic microorganism. Description of genetically engineered microbial organisms for industrial applications can also be found in Wright, et al. (2013) "Building-in biosafety for synthetic biology" Microbiology 159: 1221-1235. Suitable embodiments of genetic elements will be described later herein.

A variety of bacterial species and strains can be used in accordance with embodiments herein, and genetically modified variants, or synthetic bacteria based on a "chassis" of a known species can be provided. Exemplary bacteria with industrially applicable characteristics, which can be used in accordance with embodiments herein include, but are not limited to, Bacillus species (for example Bacillus coagulans, Bacillus subtilis, and Bacillus licheniformis), Paenibacillus species, Streptomyces species, Micrococcus species, Corynebacterium species, Acetobacter species, Cyanobacteria species, Salmonella species, Rhodococcus species, Pseudomonas species, Lactobacillus species, Enterococcus species, Alcaligenes species, Klebsiella species, Paenibacillus species, Arthrobacter species, Corynebacterium species, Brevibacterium species, Thermus aquaticus, Pseudomonas stutzeri, Clostridium thermocellus, and Escherichia coli.

A variety of yeast species and strains can be used in accordance with embodiments herein, and genetically modified variants, or synthetic yeast based on a "chassis" of a known species can be provided. Exemplary yeast with industrially applicable characteristics, which can be used in accordance with embodiments herein include, but are not limited to Saccharomyces species (for example, Saccharomyces cerevisiae, Saccharomyces bayanus, Saccharomyces boulardii), Candida species (for example, Candida utilis, Candida krusei), Schizosaccharomyces species (for example Schizosaccharomyces pombe, Schizosaccharomyces japonicas), Pichia or Hansenula

species (for example, Pichia pastoris or Hansenula polymorphd) species, and Brettanomyces species (for example, Brettanomyces claussenii).

A variety of algae species and strains can be used in accordance with embodiments herein, and genetically modified variants, or synthetic algae based on a "chassis" of a known species can be created. In some embodiments, the algae comprises, consists essentially of, or consists of photosynthetic microalgae. Exemplary algae species that can be useful for biofuels, and can be used in accordance with embodiments herein, include Botryococcus braunii, Chlorella species, Dunaliella tertiolecta, Gracilaria species, Pleurochrysis carterae, and Sargassum species. Additionally, many algaes can be useful for food products, fertilizer products, waste neutralization, environmental remediation, and carbohydrate manufacturing (for example, biofuels).

A variety of filamentous fungal species and strains can be used in accordance with embodiments herein, and genetically modified variants, or synthetic filamentous fungi based on a "chassis" of a known species can be provided. Exemplary filamentous fungi with industrially applicable characteristics, which can be used in accordance with embodiments herein include, but are not limited to an Acremonium, Agaricus, Alternaria, Aspergillus, Aureobasidium, Botryospaeria, Ceriporiopsis, Chaetomidium, Chrysosporium, Claviceps, Cochliobolus, Coprinopsis, Coptotermes, Corynascus, Cryphonectria, Cryptococcus, Diplodia, Exidia, Filibasidium, Fusarium, Gibberella, Holomastigotoides, Humicola, lrpex, Lentinula, Leptospaeria, Magnaporthe, Melanocarpus, Meripilus, Mucor, Myceliophthora, Neocaffimastix, Neurospora, Paecilomyces, Peniciffium, Phanerochaete, Piromyces, Poitrasia, Pseudoplectania, Pseudotrichonympha, Rhizomucor, Schizophyllum, Scytalidium, Talaromyces, Thermoascus, Thielavia, Tolypocladium, Trichoderma, Trichophaea, Verticillium, Volvariella, or Xylaria.

Species include Acremonium cellulolyticus, Aspergillus aculeatus, Aspergillus awamori, Aspergillus foetidus, Aspergillus fumigatus, Aspergillus japonicus, Aspergillus nidulans, Aspergillus niger, Aspergillus oryzae, Chrysosporium inops, Chrysosporium keratinophilum, Chrysosporium lucknowense, Chrysosporium merdarium, Chrysosporium pannicola, Chrysosporium queenslandicum, Chrysosporium tropicum, Chrysosporium zona turn, Fusarium bactridioides, Fusarium cerealis, Fusarium crookwellense, Fusarium culmorum, Fusarium graminearum, Fusarium graminum, Fusarium heterosporum, Fusarium negundi, Fusarium oxysporum,

Fusarium reticulatum, Fusarium roseum, Fusarium sambucinum, Fusarium sarcochroum, Fusarium sporotrichioides, Fusarium sulphureum, Fusarium torulosum, Fusarium trichothecioides, Fusarium venenaturn, Humicola grisea, Humicola insolens, Humicola lanuginosa, Irpex lacteus, Mucor miehei, Myceliophthora thermophila, Neurospora crassa, Penicillium funiculosum, Penicillium purpurogenum, Phanerochaete chrysosporium, Thielavia achromatica, Thielavia albomyces, Thielavia albopilosa, Thielavia australeinsis, Thielavia fimeti, Thielavia microspora, Thielavia ovispora, Thielavia peruviana, Thielavia setosa, Thielavia spededonium, Thielavia subthermophila, Thielavia terrestris, Trichoderma harzianum, Trichoderma koningii, Trichoderma longibrachiatum, Trichoderma reesei, or Trichoderma viride.

Antibiotic

"Antibiotic," and variations of this root term, have their customary and ordinary meanings as understood by one of skill in the art in view of this disclosure, including a metabolite, or an intermediate of a metabolic pathway which can kill or arrest the growth of at least one microbial cell. Some antibiotics can be produced by microbial cells, for example bacteria. Some antibiotics can be synthesized chemically. It is understood that bacteriocins are distinct from antibiotics, at least in that bacteriocins refer to gene products (which, in some embodiments, undergo additional post-translational processing) or synthetic analogs of the same, while antibiotics refer to intermediates or products of metabolic pathways or synthetic analogs of the same.

Sequence identity and similarity

Sequence identity has its customary and ordinary meanings as understood by one of skill in the art in view of this disclosure, including a relationship between two or more amino acid (polypeptide or protein) sequences or two or more nucleic acid (polynucleotide) sequences, as determined by comparing the sequences. Usually, sequence identities or similarities are compared over the whole length of the sequences compared. In the art, "identity" can also refer to the degree of sequence relatedness between amino acid or nucleic acid sequences, as the case may be, as determined by the match between strings of such sequences. "Similarity" between two amino acid sequences can be determined by comparing the amino acid sequence and its conserved amino acid substitutes of one polypeptide to the sequence of a second polypeptide.

"Identity" and "similarity" can be readily calculated by various methods, known to those skilled in the art. In someembodiments, sequence identity is determined by comparing the whole length of the sequences as identified herein.

Some methods to determine identity are designed to give the largest match between the sequences tested. Methods to determine identity and similarity are codified in publicly available computer programs. Computer program methods to determine identity and similarity between two sequences include e.g. the BestFit, BLASTP, BLASTN, and FASTA (Altschul, S. F. et al., J. Mol. Biol. 215:403-410 (1990), publicly available from NCBI and other sources (BLAST Manual, Altschul, S., et al., NCBI NLM NIH Bethesda, MD 20894). An algorithm used can be EMBOSS (accessible on the world wide web at www.ebi.ac.uk/emboss/align). Parameters for amino acid sequences comparison using EMBOSS can include gap open 10.0, gap extend 0.5, Blosum 62 matrix. Parameters for nucleic acid sequences comparison using EMBOSS can include gap open 10.0, gap extend 0.5, DNA full matrix (DNA identity matrix).

Optionally, in determining the degree of amino acid similarity, the skilled person may also take into account so-called "conservative" amino acid substitutions, as will be clear to the skilled person. Conservative amino acid substitutions refer to the interchangeability of residues having similar side chains. For example, a group of amino acids having aliphatic side chains is glycine, alanine, valine, leucine, and isoleucine; a group of amino acids having aliphatic-hydroxyl side chains is serine and threonine; a group of amino acids having amide-containing side chains is asparagine and glutamine; a group of amino acids having aromatic side chains is phenylalanine, tyrosine, and tryptophan; a group of amino acids having basic side chains is lysine, arginine, and histidine; and a group of amino acids having sulphur-containing side chains is cysteine and methionine. Suitable conservative amino acids substitution groups include: valine-leucine-isoleucine, phenylalanine-tyrosine, lysine-arginine, alanine-valine, and asparagine-glutamine. Substitutional variants of the amino acid sequence disclosed herein are those in which at least one residue in the disclosed sequences has been removed and a different residue inserted in its place. In some embodiments, the amino acid change is conservative. Suitable conservative substitutions for each of the naturally occurring amino acids include: Ala to ser; Arg to lys; Asn to gln or his; Asp to glu; Cys to ser or ala; Gln to asn; Glu to asp; Gly to pro; His to asn or gln; Ile to leu or val; Leu to

ile or val; Lys to arg; gln or glu; Met to leu or ile; Phe to met, leu or tyr; Ser to thr; Thr to ser; Trp to tyr; Tyr to trp or phe; and, Val to ile or leu.

Homologous

The term "homologous" has its customary and ordinary meanings as understood by one of skill in the art in view of this disclosure, including when used to indicate the relation between a given (recombinant) nucleic acid or polypeptide molecule and a given host organism or host cell, it can be understood to mean that in nature the nucleic acid or polypeptide molecule is produced by a host cell or organisms of the same species, optionally of the same variety or strain. If homologous to a host cell, a nucleic acid sequence encoding a polypeptide will typically be operably linked to another promoter sequence than in its natural environment. When used to indicate the relatedness of two nucleic acid sequences the term "homologous" has its customary and ordinary meanings as understood by one of skill in the art in view of this disclosure, and can refer to one single-stranded nucleic acid sequence that may hybridize to a complementary single-stranded nucleic acid sequence. The degree of hybridization may depend on a number of factors including the amount of identity between the sequences and the hybridization conditions such as temperature and salt concentration as earlier presented. The region of identity can be greater than about 5 bp, the region of identity can be greater than 10 bp. In some embodiments, two nucleic acid or polypeptides sequences are said to be homologous when they have more than 80% identity.

Heterologous

The term "heterologous" has its customary and ordinary meanings as understood by one of skill in the art in view of this disclosure, including when used with respect to a nucleic acid (DNA or RNA) or protein, it can refer to a nucleic acid or protein (also named polypeptide or enzyme) that does not occur naturally as part of the organism, cell, genome or DNA or RNA sequence in which it is present, or that is found in a cell or location or locations in the genome or DNA or RNA sequence that differ from that in which it is found in nature. Heterologous nucleic acids or proteins are not endogenous to the cell into which it is introduced, but has been obtained from another cell or synthetically or recombinantly produced. Generally, though not necessarily, such nucleic acids encode proteins that are not normally produced by the cell in which the DNA is

transcribed or expressed. Similarly exogenous RNA encodes for proteins not normally expressed in the cell in which the exogenous RNA is present. Heterologous nucleic acids and proteins may also be referred to as foreign nucleic acids or proteins. Any nucleic acid or protein that one of skill in the art would recognize as heterologous or foreign to the cell in which it is expressed is herein encompassed by the term heterologous nucleic acid or protein. The term heterologous also applies to non-natural combinations of nucleic acid or amino acid sequences, i.e. combinations where at least two of the combined sequences are foreign with respect to each other.

Operably linked

As used herein, the term "operably linked" has its customary and ordinary meanings as understood by one of skill in the art in view of this disclosure, and can refer to a linkage of polynucleotide elements (or coding sequences or nucleic acid sequence or nucleic acid molecule) in a functional relationship. A nucleic acid sequence is "operably linked" when it is placed into a functional relationship with another nucleic acid sequence. For instance, a promoter or enhancer is operably linked to a coding sequence if it affects the transcription of the coding sequence. Operably linked means that the nucleic acid sequences being linked are typically contiguous and, where necessary to join two protein coding regions, contiguous and in reading frame.

Promoter

As used herein, the term "promoter" has its customary and ordinary meanings as understood by one of skill in the art in view of this disclosure, and can refer to a nucleic acid fragment that functions to control the transcription of one or more nucleic acid molecules, located upstream with respect to the direction of transcription of the transcription initiation site of the nucleic acid molecule, and is structurally identified by the presence of a binding site for DNA-dependent RNA polymerase, transcription initiation sites and any other DNA sequences, including, but not limited to transcription factor binding sites, repressor and activator protein binding sites, and any other sequences of nucleotides known to one of skill in the art to act directly or indirectly to regulate/control the amount of transcription from the promoter. A "constitutive" promoter is a promoter that is active under most environmental and developmental

conditions. An "inducible" promoter is a promoter that is active under environmental or developmental regulation.

In this document and in its claims, the verb "to comprise" and its conjugations is used in its non-limiting sense to mean that items following the word are included, but items not specifically mentioned are not excluded. In addition the verb “to consist” may be replaced by “to consist essentially of” meaning that an auto-replicative extra-chromosomal nucleic acid molecule, a microbial host (or a method) as defined herein may comprise additional component(s) (or additional steps) than the ones specifically identified, said additional component(s) (or additional steps) not altering the unique characteristic of the invention. In addition, reference to an element by the indefinite article "a" or "an" does not exclude the possibility that more than one of the element is present, unless the context clearly requires that there be one and only one of the elements. The indefinite article "a" or "an" thus usually means "at least one".

All patent and literature references cited in the present specification are hereby incorporated by reference in their entirety. The following examples are offered for illustrative purposes only, and are not intended to limit the scope of the present invention in any way

Sequence listing (DNA unless otherwise indicated)

SEQ ID NO: 1 Enterococcus faecalis peptide

SEQ ID NO: 2 motif characteristic of bacteriocin

SEQ ID NO: 3 hybrid bacteriocin, Ent35-MccV

SEQ ID NO: 4-698, 720-759: Sequences in tables 1-3 (half DNA/half protein as indicated in the tables)

SEQ ID NO: 699 McbG

SEQ ID NO: 700 MccE

SEQ ID NO: 701 C terminal part of MccE

SEQ ID NO: 702 pSyn2-McbG

SEQ ID NO: 703 pSyn2-McbE/F

SEQ ID NO: 704 pMcbG1.0

SEQ ID NO: 705 pMcbG.1.1.

SEQ ID NO: 706 C-terminal part of MccE (amino acid)

SEQ ID NO: 707 P24 promoter

SEQ ID NO: 708 proC promoter

SEQ ID NO: 709 Cvi

SEQ ID NO: 710 proC-McbG-CterMccE(proc) (Cter could be replaced by C-terminal)

SEQ ID NO: 711 proC-Cvi-Cter-MccE (proc) (Cter could be replaced by C-terminal)

SEQ ID NO: 712 pBACT5.0

SEQ ID NO: 713 pBACT2.0

SEQ ID NO: 714 pBACT5.0-mcherry

SEQ ID NO: 715 McbG-MccE

SEQ ID NO: 716 McbG-Cter part MccE (Cter could be replaced by C-terminal)

SEQ ID NO: 717 Cvi-MccE

SEQ ID NO: 718 Cvi-Cter part MccE (Cter could be replaced by C-terminal)

SEQ ID NO: 719: vector pUC-ColV

Description of the figures

Figure 1: Construction: pSyn2-McbE/F: containing the gene McbE and F under Ptac.

Figure 2: Construction: pSyn2-McbG : containing the gene McbG under Ptac.

Figure 3: Construction: pMcbG 1.1: containing the gene McbG under P24.

Figure 4: Construction: pMcbG 1.0: containing the gene McbG under P24 LacO.

Figure 5: pBACT5.0 vector

Figure 6: pBACT2.0 vector

Figure 7: pBACT5.0-mcherry vector

Figure 8: Tuning promoter. In the absence of inductor (upper part), repressor can bind to operator and prevent expression of selection gene. In the presence of inductor (lower part) , repressor cannot bind to operator allowing expression of selection gene.

Figure 9: Comparison of overexpression of protein X in E. coli with KanR (pKan-pLac) and with 2 immunities against microcines C7 and ColV (pBACT6.0-pLac). 5 mg of total extract was analysed in SDS-PAGE.

Figure 10: Comparison of overexpression of iota-carrageenase protein in E. coli with KanR (pKan-T7prom) and with 2 immunities against microcines C7 and ColV (pBACT5.0-T7prom). 5 mg of total extract was analysed in SDS-PAGE.

Figure 11: Comparison of overexpression of lambda-carrageenase protein in E. coli with KanR (pKan-T7prom) and with 2 immunities against microcines C7 and ColV (pBACT5.0-T7prom). 5 mg of total extract was analysed in SDS-PAGE.

Examples

Example 1: Use of bacteriocin B17 and C7 as selection agent

1. Production of bacteriocin B17, C7 and ColV

Strain used: C600: F^- tonA21 thi-1 thr-1 leuB6 lacY1 glnV44 rfbC1 fhuA1 λ^-

Described in Appleyard Genetics 39 (1954), 440-452.

The vector used for producing Mic B17 is described in the table below.

Table X: Vector used for producing Mic B17

Construct used pCID909	pACYC184 containing the *mccB17*-producing genes (*mcbABCDEFG*), chloramphenicol resistance

These constructs were described in detail in Rodriguez-Sainz, M. C., C. et al .**1990**. Mol. Microbiol. **4:**1921-1932.

The vector used for producing Mic C7 is Pp70. This vector is based on pBR322 and bears a ~6000 bp DNA fragment with the *mcc* gene cluster (as described in Zukher I et al, *Nucleic Acids Research, 2014, Vol. 42, No. 19 11891–11902).*

The vector used for producing ColV is pUC-ColV (SEQ ID NO: 719). This vector is based on pUC57 and bear a ~5000 bp DNA fragment with the *ColV* gene cluster. The strains harbouring these recombinant vectors were grown in LB medium at 37°C. After an overnight culture the fermented medium was centrifuged and the supernatant flit red on a 0,2 micron filter.
The bacteriocin activity present in the supernatant was estimated by the size of the diffusion inhibition growth on a plate containing a sensitive strain.

2. Results

We demonstrated that we can use the supernatants that exhibit B17, C7 or ColV activities as classical antibiotics such as Amp, Kan or Chlo added in culture medium. Supernatant presenting such a bacteriocin activity were stored for several months (at least 12 months) at -20°C and we did not observe a significant decrease of activity. Petri plates containing medium with such a bacteriocin activity were stored at +4oC for several weeks (at least 4 weeks). We did not observe a decrease of activity. Therefore we demonstrated that such B17, C7 or ColV activities as present in culture medium are stable.

3. Conclusion

Bacteriocins B17, C7 and ColV produced by fermentation in laboratory are selection agents simple to produce, easy to use and stable in culture medium. These properties are similar to the ones of antibiotics used as classical selection agent.

Example 2: Identification of the minimum genetic elements necessary to confer resistance to C7 and B17

1.Construction of needed vectors

The literature has made it possible to determine the elements necessary for the production of the host against the production of its own bacteriocin, also in the case of B17 bacteriocin: McbG for B17, represented by SEQ ID NO: 699 and pumps (McbE and McbF for B17, represented by SEQ ID NO: 703). These genes are known to be necessary (or more precisely involved in protection against the action of bacteriocin B17). The literature for the B17 locus does not identify which is or is the sufficient element to give resistance.

We have separated genes from B17 immunity structures and cloned these into vectors behind an inducible promoter (Ptac).

Construction: pSyn2-McbG (Figure 2, SEQ ID NO: 702): containing the gene McbG under Ptac
Construction: pSyn2-McbE/F (Figure 1, SEQ ID NO: 703): containing the gene McbE and F under Ptac

We have separated the genes from B17 immunity structures and cloned them into vectors behind an inducible promoter (Ptac).

We have shown that low McbG expression (Ptac not induced) is sufficient to give the phenotype of resistance to the strain on the other hand the presence of McbE / F is toxic and did not allow to give a response As to the protection provided in relation to the presence of B17.

2. Results

Surprisingly it has been found that the C-terminal part of MccE which is represented by SEQ ID NO: 701 is sufficient to confer resistance to bacteriocin C7.

We have demonstrated that expression from a plasmid of the McbG and MccE genes (or truncated MccE, represented by SEQ ID NO: 701) are capable when cloned into a vector to give resistance to B17 and C7 respectively and that these proteins can be used as a vector selection marker in strains sensitive to these microcines/bacteriocins. The vector used is pBACT2.0 (Figure 6, SEQ ID NO: 713).

SEQ ID NO: 710 represents the construct proC-McbG-CterMccE

We have demonstrated that expression from a plasmid of the Cvi and C-terminal part of MccE genes are capable when cloned into a vector to give resistance to ColV and C7 respectively and that these proteins can be used as a vector selection marker in strains sensitive to these microcines/bacteriocins. The vector used is pBACT5.0 (Figure 5, SEQ ID NO: 712).

SEQ ID NO: 711 represents the construct proC- Cvi-CterMccE

3.Conclusion

It is therefore possible to use a single segment of small size represented by SEQ ID NO: 701 as a selection marker against C7.

Example 3: Can we generate a selectable marker using little or no energy from the bacteria from McbG?

1. Vectors constructed

To answer this question the McbG gene was cloned under a weak promoter P24 (SEQ ID NO: 707). The P24 promoter was described in Braatsch S et al, Biotechniques. 2008 Sep;45(3):335-7.

We inserted the B17 McbG immunity structure gene and cloned the latter into vectors behind the weak constitutive promoter (P24). A second construct was generated with a P24 LacO hybrid promoter which is an inducible promoter repressed in the presence of lacI and active in presence of IPTG.

Construction: pMcbG 1.0 (Figure 4, SEQ ID NO: 704) containing the gene McbG under P24 LacO.

Construction: pMcbG 1.1 (Figure 3, SEQ ID NO: 705) containing the gene McbG under P24.

The strains used are the following:

BL21(DE3): *fhuA2 [lon] ompT gal (λ DE3) [dcm] ΔhsdS*

λ DE3 = λ sBamHIo ΔEcoRI-B int::(lacI::PlacUV5::T7 gene1) i21 Δnin5

The BL21(DE3) strain was transformed with vector pMcbG1.0 or pMcbG1.1 (see figure 3 or 4). After transformation, transformants were selected on plates containing B17. Isolated colonies were re-grown and the plasmid they contained was analyzed by gel electrophoresis after treatment with relevant restriction enzymes.

2.Results

We showed that a weak transcription of McbG is sufficient to give the resistance to B17. In addition, we have shown that this selection marker is inducible via the P24 LacO promoter and that the vectors containing this gene gives the phenotype of resistance only in presence of IPTG.

3.Conclusions

It is possible to use the McbG gene as a selectable marker either constitutively or inducibly. Thus, it is shown that constitutive expression at a low level and inducible expression according to the need during the process, allows to reduce the energy burden for the producing cell, without loss of the plasmid from the producing cell.

Example 4 : production of m-cherry protein

SEQ ID NO : 714 represents the construct used for producing the m-cherry protein. This construct is depicted in figure 7.

The m-cherry protein was produced and visualised as the bacterial colony turns red on petri dish in the presence of IPTG.

Example 5 : tuning promoter

We have prepared vectors with immunity selection that is tunable (see FIG 8). Tuning the promoter allows us to switch the selection "on" or "off". For the first time this allows to adapt the selective pressure to the need during the fermentation process, for example according to the loss by the host of the recombinant plasmid. This will provide an advantage by limiting the burden of energy for the host. Thus, such vectors improve the industrial outcome (recombinant product). Moreover, they are easy to use in any strain (no requirement for a special feature in the host genome) and require no antibiotics.

Example 6: comparison of antibiotic (kanamycin) selection with immunity selection

We have applied immunity selection on different recombinant proteins.

Figure 9 shows the comparison of overexpression of protein X in E. coli with KanR (pKan-pLac) and with 2 immunities against microcines C7 and ColV (pBACT6.0-pLac). 5 mg of total extract was analysed in SDS-PAGE.

Figure 10 shows the comparison of overexpression of iota-carrageenase protein in E. coli with KanR (pKan-T7prom) and with 2 immunities against microcines C7 and ColV (pBACT5.0-T7prom). 5 mg of total extract was analysed in SDS-PAGE. The vector used is based on the pBACT5.0 vector (SEQ ID NO: 712).

Figure 11 shows the comparison of overexpression of lambda-carrageenase protein in E. coli with KanR (pKan-T7prom) and with 2 immunities against microcines C7 and ColV (pBACT5.0-T7prom). 5 mg of total extract was analysed in SDS-PAGE. The vector used is based on the pBACT5.0 vector (SEQ ID NO: 712).

The weak constitutive proC promoter used in this example allows to reduce the energy burden for the host.

Claims

1. Method for producing a product of interest with a microbial host, said method comprising the steps of:
a) Providing the microbial host comprising an auto-replicative extra-chromosomal nucleic acid molecule comprising a first nucleic acid sequence whose genetic activity confers an advantage to the host, wherein the genetic activity of said first nucleic acid sequence is controlled;
b) Optionally said auto-replicative extra-chromosomal nucleic acid molecule comprises a second nucleic acid sequence that is involved in the production of said product of interest, wherein the genetic activity of said second nucleic acid sequence is controlled independently from the one of the first sequence;
c) Culturing said transformed microbial host under conditions allowing said transformed microbial host to express the first nucleic acid sequence to a given level to maintain the auto-replicative extra-chromosomal molecule into the growing microbial population and simultaneously genetically controlling the second sequence coding for said product of interest.

2. The method according to claim 1, further comprising transforming the microbial host with said auto-replicative extra-chromosomal nucleic acid molecule prior to or during step a), wherein the auto-replicative extra-chromosomal nucleic acid molecule optionally comprises the second nucleic acid sequence of step b), thereby providing the microbial host comprising the auto-replicative extra-chromosomal nucleic acid molecule.

3. A method according to claim 1 or 2, wherein at least in part of step c) conditions are such that the first nucleic acid sequence does not exhibit said genetic activity.

4. A method according to any one of claims 1 to 3, wherein the product of interest is purified at the end of the culturing step c).

5. A method according to any one of claims 1 to 4, wherein the product of interest is a microbial biomass, the auto-replicative extra-chromosomal nucleic acid molecule, the transcript of said second nucleic sequence, a polypeptide encoded by said second sequence or a metabolite produced directly or indirectly by said polypeptide.

6. A method according to any one of claims 1 to 5, wherein the microbial host is a bacterium, yeast, filamentous fungus or an algae

7. A method according to any one of claims 1 to 6, wherein the first nucleic acid sequence is operably linked to an inducible promoter.

8. A method according to any one of claims 1 to 7, wherein the first nucleic acid sequence comprises a sequence coding for an immunity gene whose expression confers to its host a resistance to the presence of a specific bacteriocin in the medium.

9. A method according to claim 8, wherein the sequence encoded by the first nucleic acid sequence confers to its host a resistance to the presence of at least two distinct bacteriocins in the medium.

10. A method according to claim 9, wherein the bacteriocin is B17, C7 or ColV and the immunity conferring resistance to a B17 is McbG, to C7 is either MccE or C-terminal MccE and to a ColV is Cvi.

11. A method according to any one of claims 1 to 10, wherein the auto-replicative extra-chromosomal nucleic acid molecule is a plasmid.

12. An auto-replicative extra-chromosomal nucleic acid molecule comprising a first nucleic acid sequence whose genetic activity confers an advantage to a microbial host wherein the genetic activity of said first nucleic acid sequence is controlled,
and optionally comprising a second nucleic acid sequence that is directly or indirectly involved in the production of a product of interest.

13. An auto-replicative extra-chromosomal nucleic acid molecule according to claim 12, wherein the first nucleic acid sequence is operably linked to an inducible promoter.

14. An auto-replicative extra-chromosomal nucleic acid molecule according to claim 12 or 13 which is a plasmid.

15. An auto-replicative extra-chromosomal nucleic acid molecule according to any one of claim 12 to 14, wherein the first nucleic acid sequence comprises a sequence coding for an immunity gene whose expression confers to its host a resistance to the presence of a specific bacteriocin in the medium.

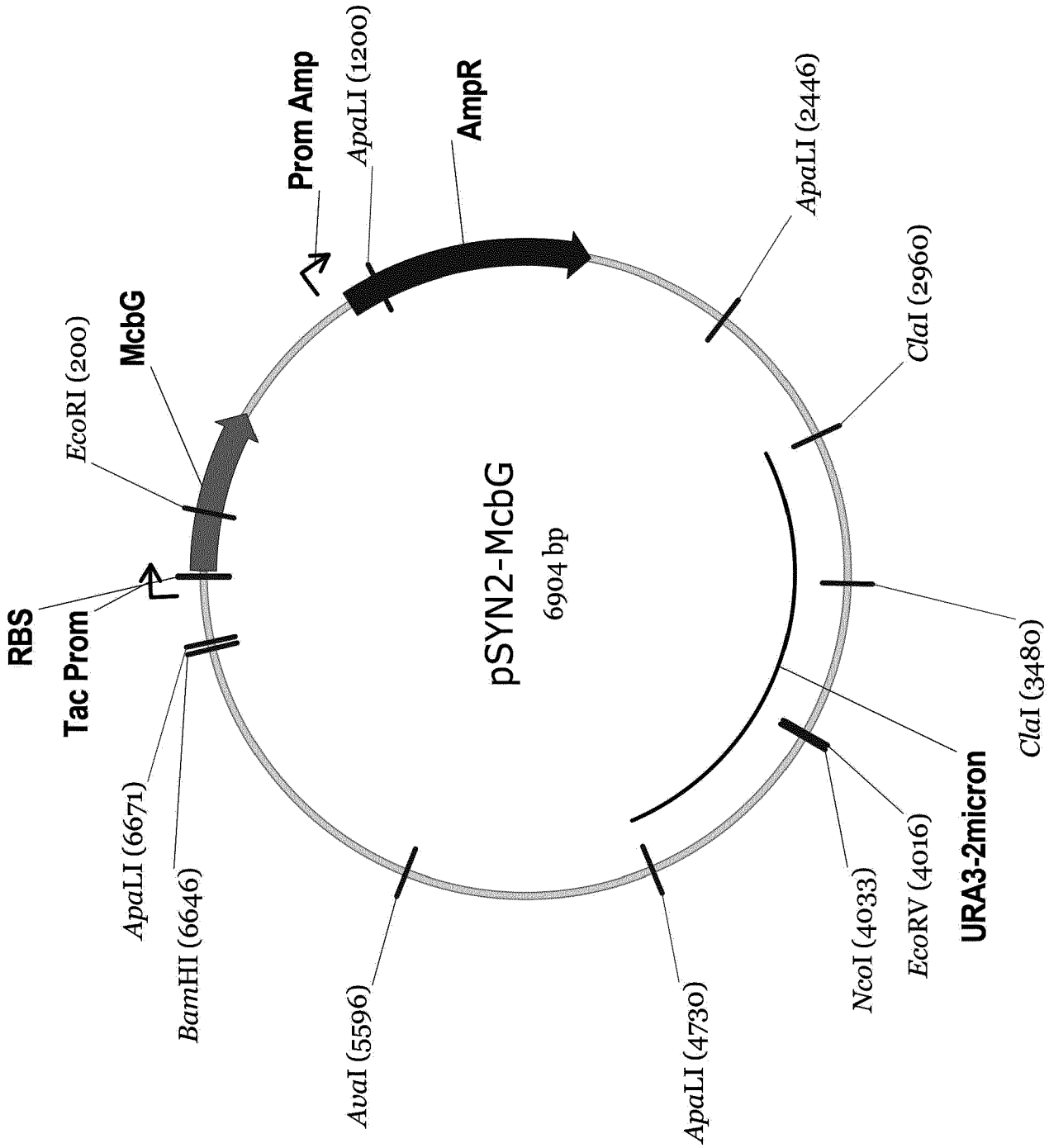
16. An auto-replicative extra-chromosomal nucleic acid molecule according to any one of claim 15, wherein the sequence encoded by the first nucleic acid sequence confers to its host a resistance to the presence of at least two distinct bacteriocins in the medium.

17. An auto-replicative extra-chromosomal nucleic acid molecule according to claim 16, wherein the bacteriocin is B17, C7 or ColV and the immunity modulator conferring resistance to a B17 is McbG, to C7 is either MccE or C-terminal MccE) and to ColV is Cvi.

18. A microbial host comprising the auto-replicative extra-chromosomal nucleic acid molecule of any one of claims 11 to 16, optionally wherein the microbial cell is a bacterium, yeast, filamentous fungus or an algae,


pSYN2-McbE/F
7807 bp
RBS
Tac Prom
McbE
McbF
Prom Amp
AmpR
URA3-2micron
ClaI (413)
ApaLI (2103)
ApaLI (3349)
ClaI (3863)
ClaI (4383)
NcoI (4936)
ApaLI (5633)
AvaI (6499)
BamHI (7549)
ApaLI (7574)

Fig. 1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AmpR
McbG
RBS
Tac Prom
URA3-2micron
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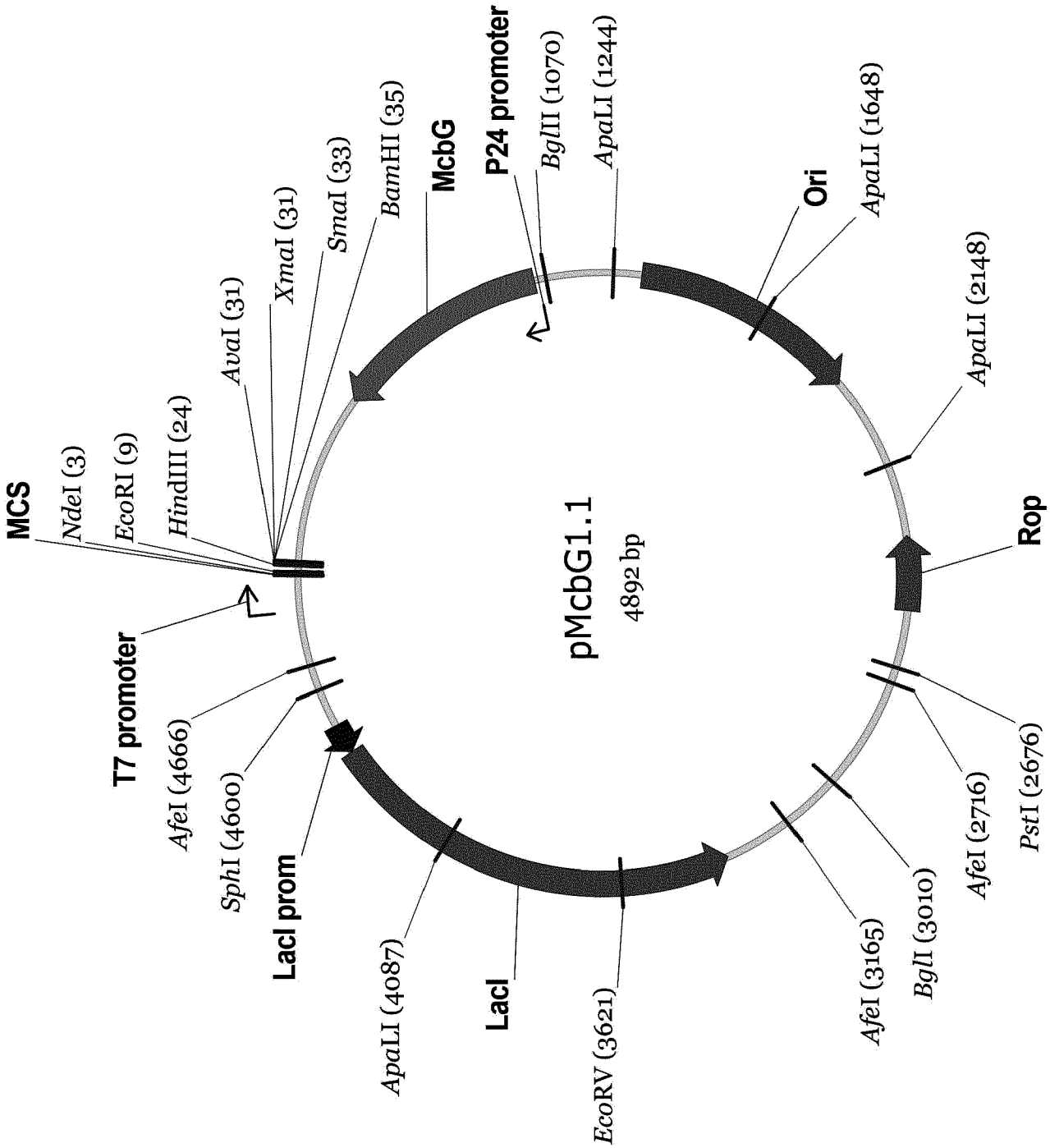
EcoRI (200)
ApaLI (2446)
ClaI (2960)
ClaI (3480)
ApaLI (6671)
BamHI (6646)
AvaI (5596)
ApaLI (4730)
NcoI (4033)
EcoRV (4016)

Fig. 2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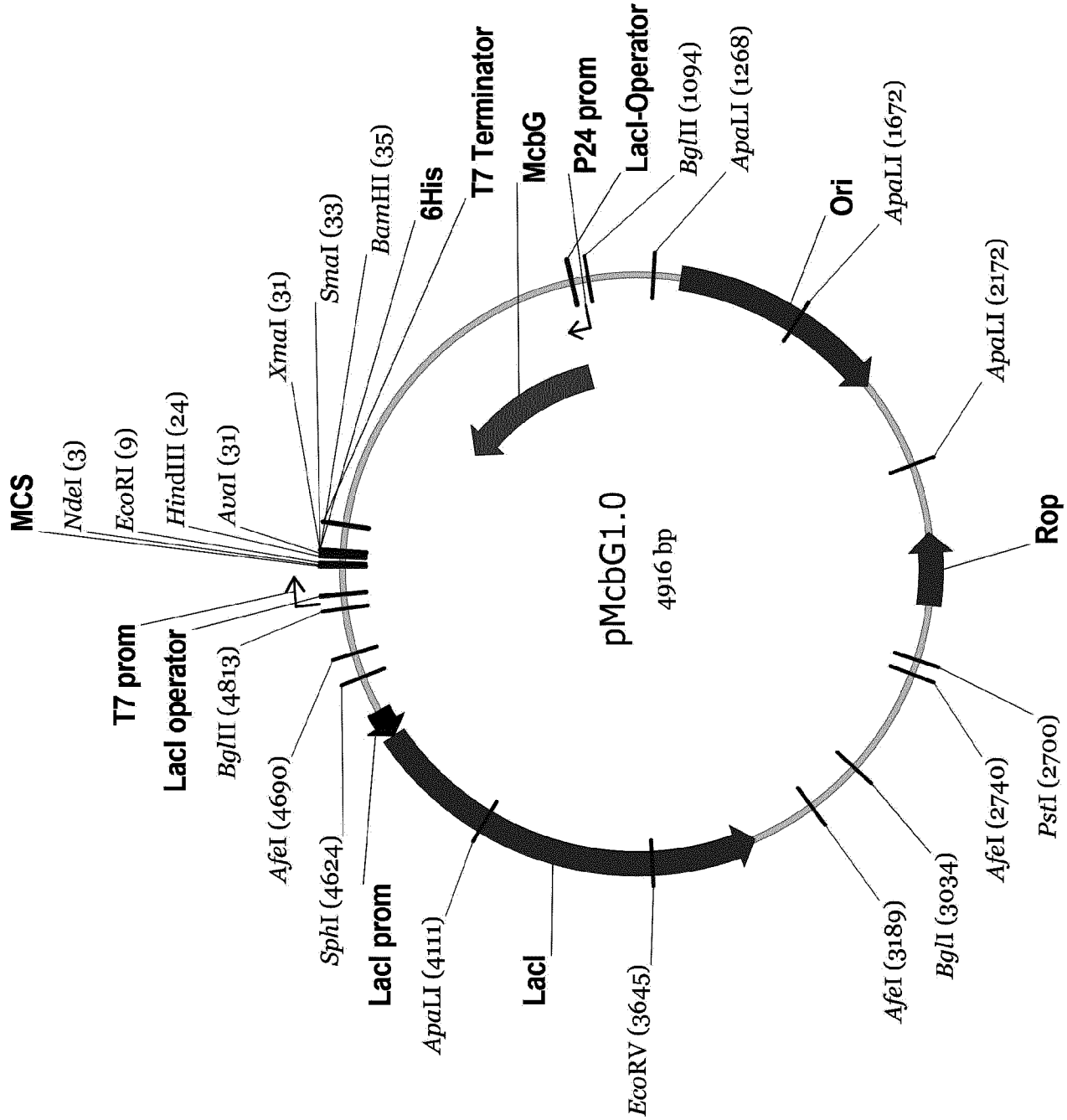
EcoRI (9)
HindIII (24)
AvaI (31)
XmaI (31)
SmaI (33)
BamHI (35)
McbG
P24 promoter
BglII (1070)
ApaLI (1244)
Ori
ApaLI (1648)
ApaLI (2148)
Rop
PstI (2676)
AfeI (2716)
BglI (3010)
AfeI (3165)
EcoRV (3621)
ApaLI (4087)
LacI
LacI prom
SphI (4600)
AfeI (4666)
T7 promoter

Fig. 3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EcoRI (9)
HindIII (24)
AvaI (31)
XmaI (31)
SmaI (33)
BamHI (35)
6His
T7 Terminator
McbG
P24 prom
LacI-Operator
BglII (1094)
ApaLI (1268)
Ori
ApaLI (1672)
ApaLI (2172)
Rop
PstI (2700)
AfeI (2740)
BglI (3034)
AfeI (3189)
EcoRV (3645)
LacI
ApaLI (4111)
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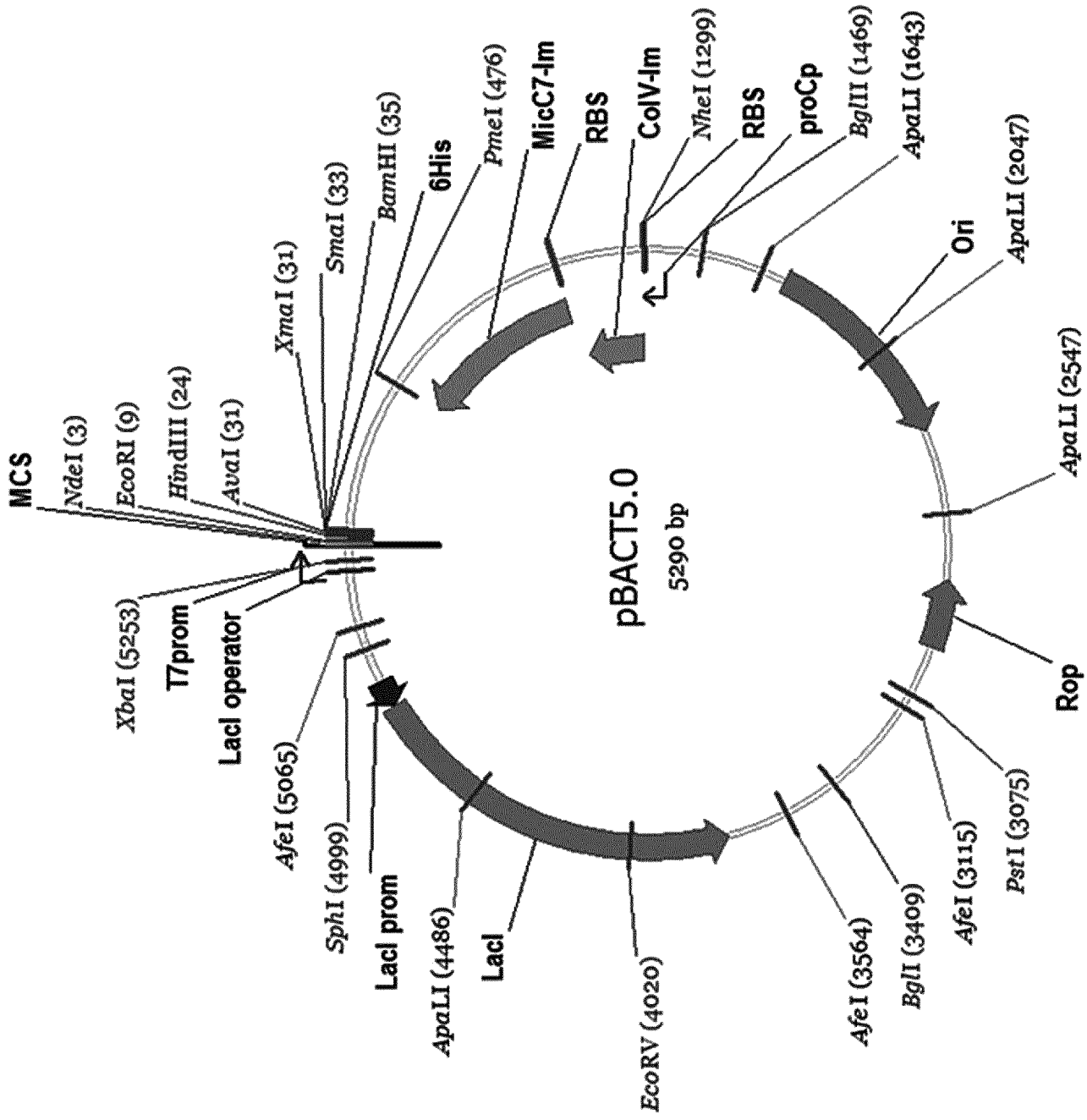
SphI (4624)
AfeI (4690)
BglII (4813)
T7 operator
LacI operator
T7 prom

Fig. 4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HindIII (24)
AvaI (31)
XmaI (31)
SmaI (33)
BamHI (35)
6His
PmeI (476)
MicC7-Im
RBS
ColV-Im
NheI (1299)
RBS
proCp
BglII (1469)
ApaLI (1643)
Ori
ApaLI (2047)
ApaLI (2547)
Rop
PstI (3075)
AfeI (3115)
BglI (3409)
AfeI (3564)
EcoRV (4020)
LacI
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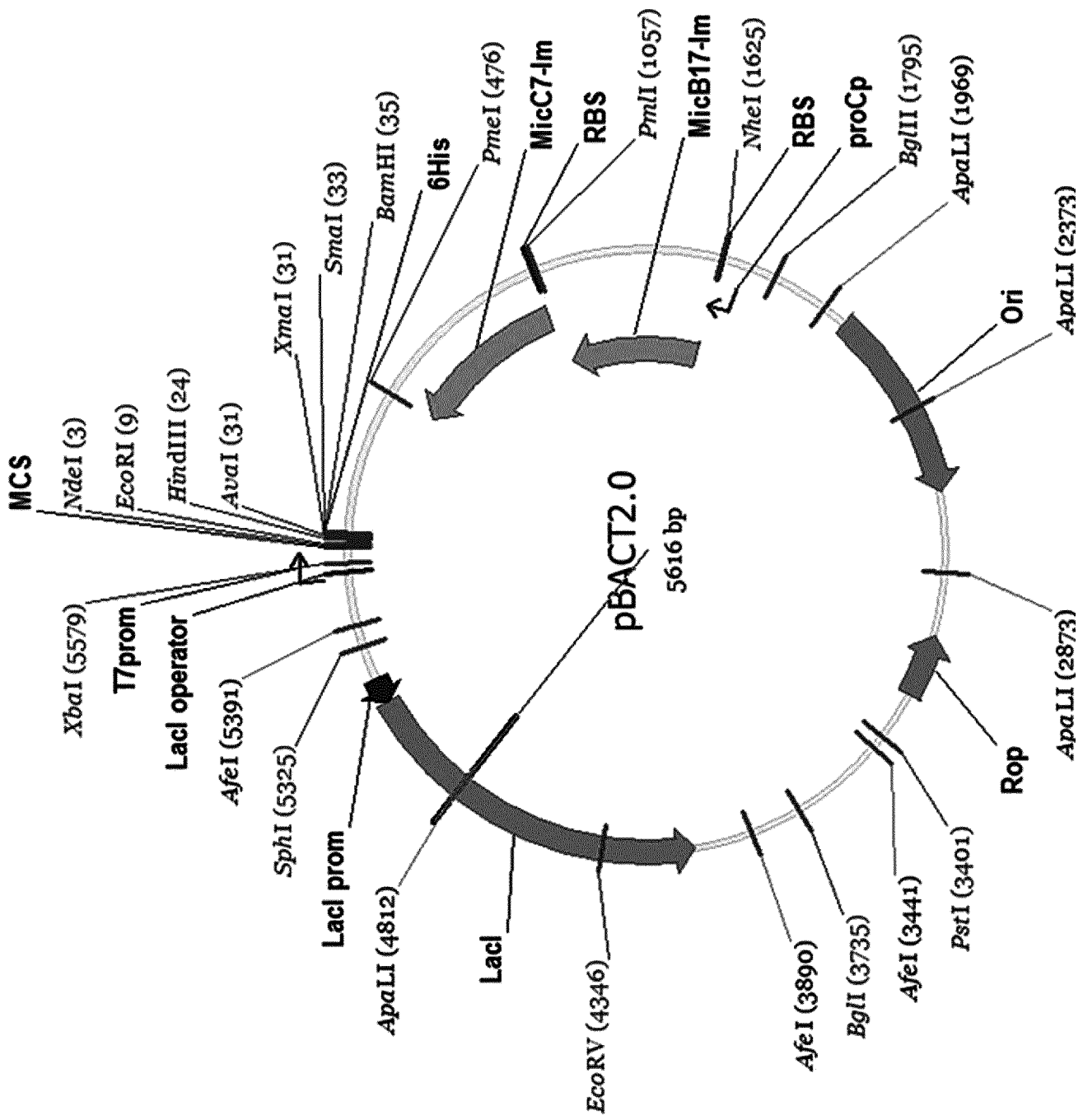
LacI prom
SphI (4999)
AfeI (5065)
LacI operator
T7prom
XbaI (5253)

Fig. 5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XmaI (31)
SmaI (33)
BamHI (35)
6His
PmeI (476)
MicC7-Im
RBS
PmlI (1057)
MicB17-Im
NheI (1625)
RBS
proCp
BglII (1795)
ApaLI (1969)
Ori
ApaLI (2373)
ApaLI (2873)
Rop
PstI (3401)
AfeI (3441)
BglI (3735)
AfeI (3890)
EcoRV (4346)
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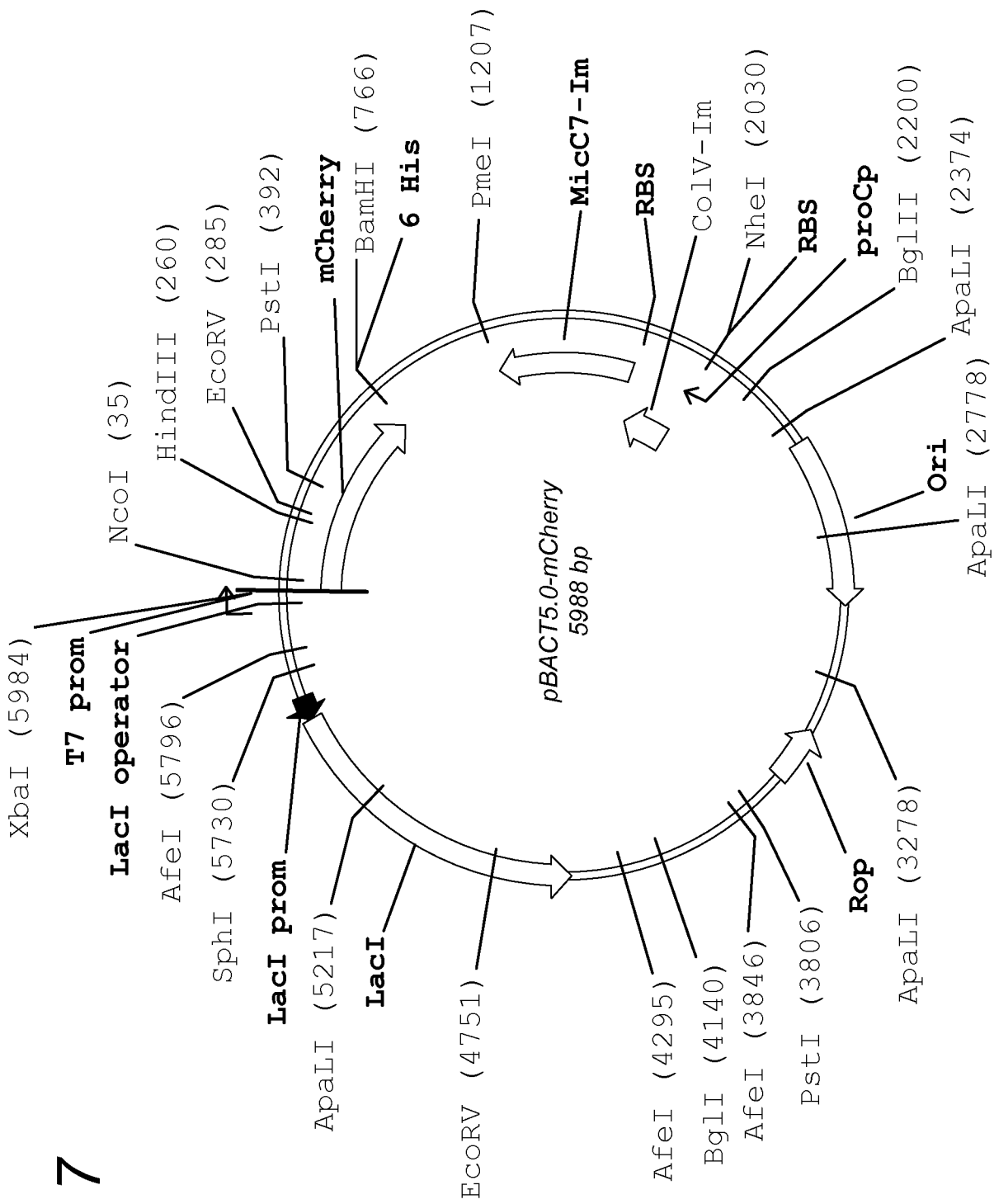
ApaLI (4812)
LacI prom
SphI (5325)
AfeI (5391)
LacI operator
T7prom
XbaI (5579)

Fig. 6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T7 prom
LacI operator
NcoI (35)
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EcoRV (285)
PstI (392)
mCherry
BamHI (766)
6 His
PmeI (1207)
MicC7-Im
RBS
ColV-Im
NheI (2030)
RBS
proCp
BglII (2200)
ApaLI (2374)
Ori
ApaLI (2778)
ApaLI (3278)
Rop
PstI (3806)
AfeI (3846)
BglI (4140)
AfeI (4295)
EcoRV (4751)
LacI
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LacI prom
SphI (5730)
AfeI (5796)

Fig. 7

Fig. 8

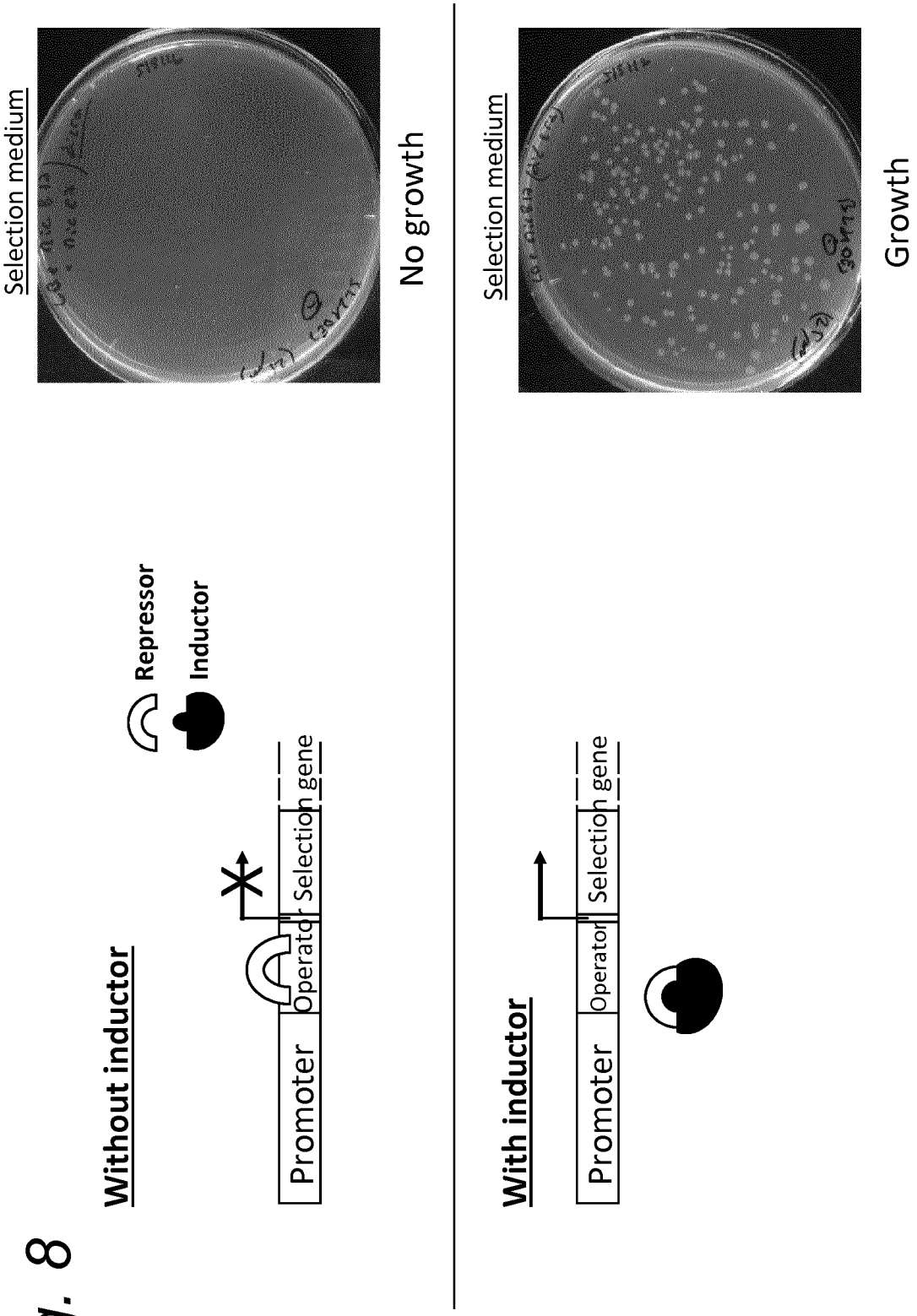
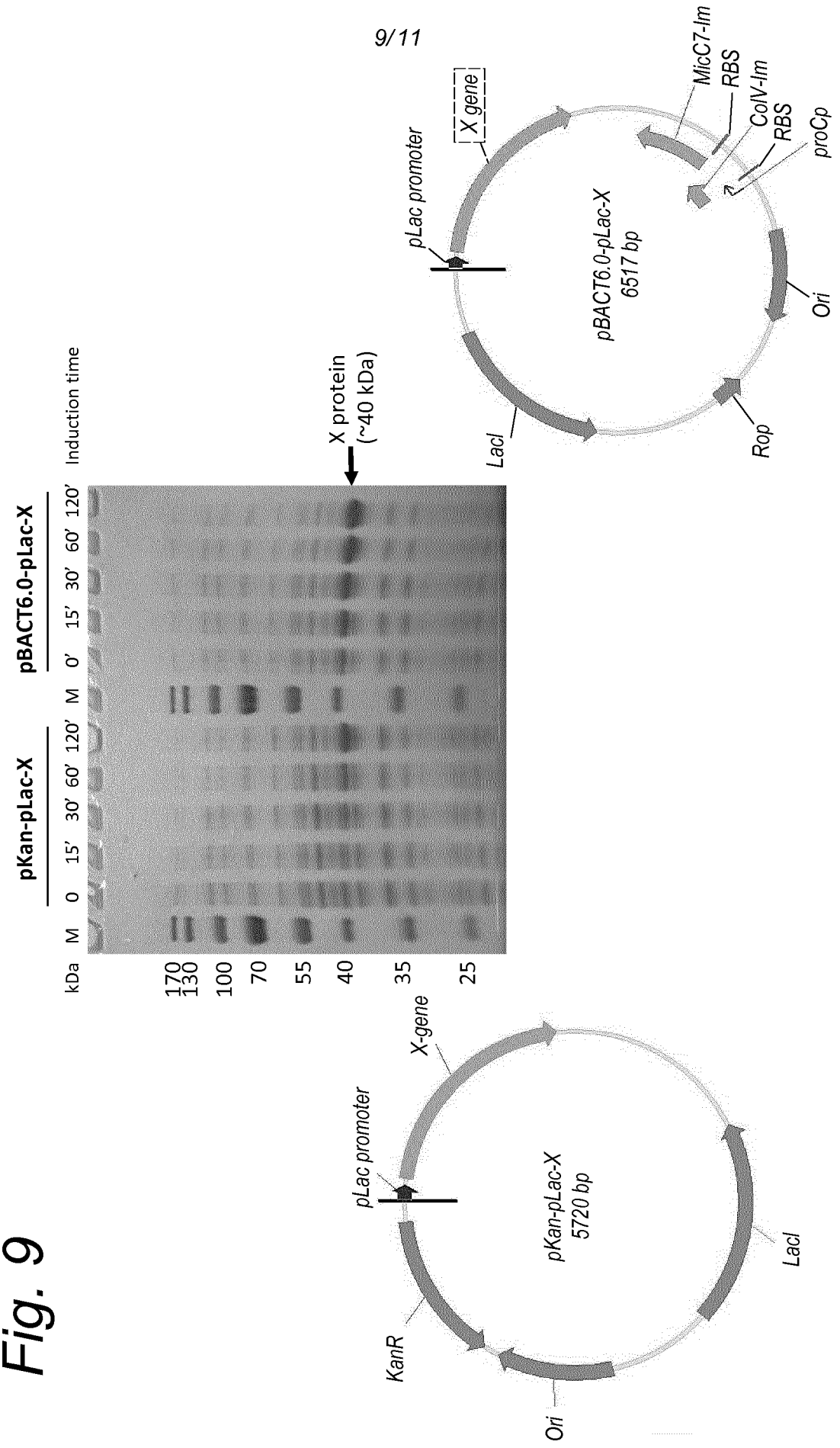

Without inductor
Repressor
Inductor
Promoter
Operator
Selection gene
Selection medium
No growth
With inductor
Promoter
Operator
Selection gene
Selection medium
Growth

Fig. 9


pKan-pLac-X
pBACT6.0-pLac-X
Induction time
kDa
M
0
15'
30'
60'
120'
170
130
100
70
55
40
35
25
X protein
(~40 kDa)
pLac promoter
X-gene
pKan-pLac-X
5720 bp
KanR
Ori
LacI
pLac promoter
X gene
pBACT6.0-pLac-X
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MicC7-Im
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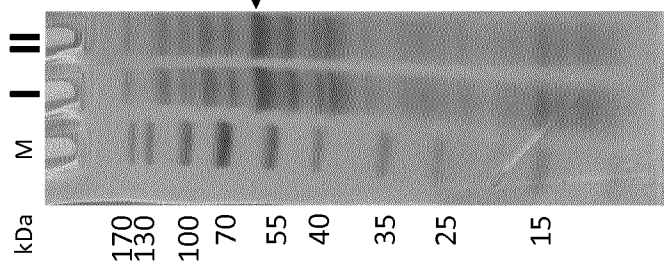
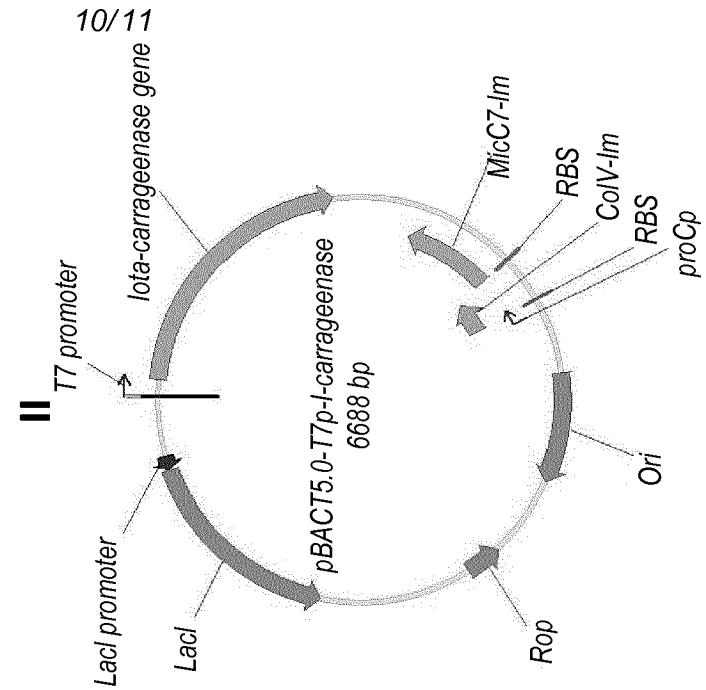
ColV-Im
RBS
proCp
Ori
Rop
LacI

Fig. 10


kDa
M
I
II
170
130
100
70
55
40
35
25
15
Iota-carrageenase protein (~56 kDa)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Iota-carrageenase gene
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RBS
ColV-Im
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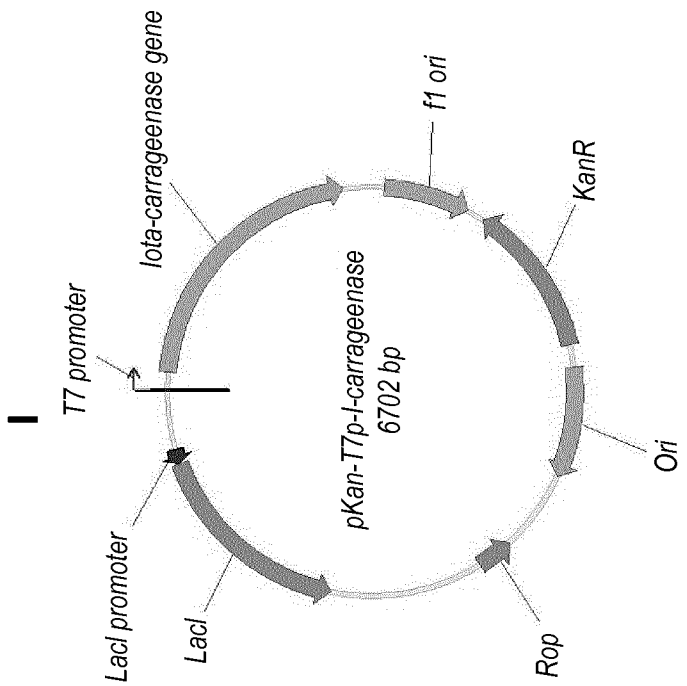
6688 bp
Ori
LacI promoter
LacI
Rop


I
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Iota-carrageenase gene
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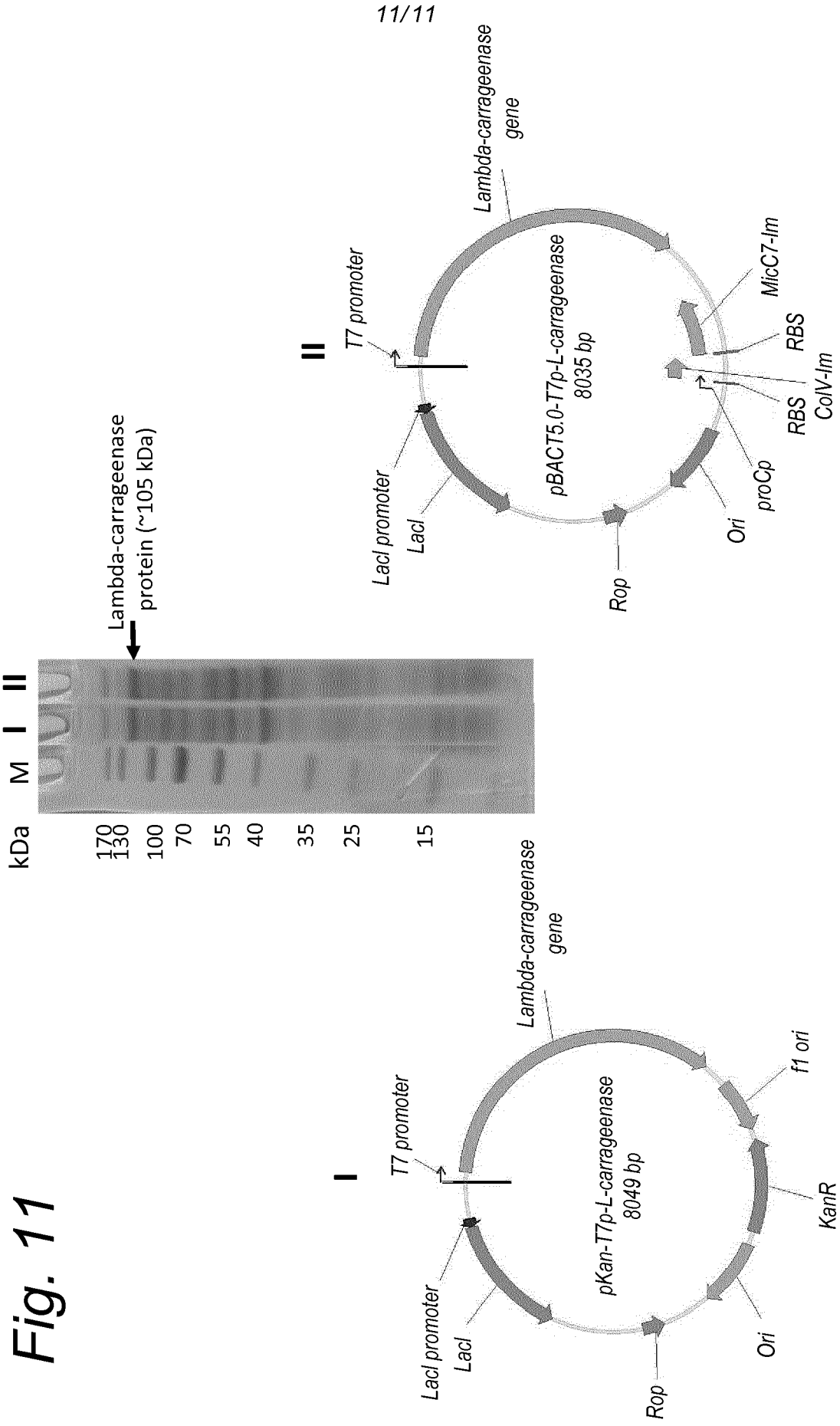
KanR
pKan-T7p-I-carrageenase
6702 bp
Ori
LacI promoter
LacI
Rop

Fig. 11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Lambda-carrageenase gene
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KanR
Ori
Rop
LacI
LacI promoter
pKan-T7p-L-carrageenase
8049 bp
II
T7 promoter
Lambda-carrageenase gene
MicC7-Im
RBS
ColV-Im
RBS
proCp
Ori
Rop
LacI
LacI promoter
pBACT5.0-T7p-L-carrageenase
8035 bp
kDa
M
I
II
170
130
100
70
55
40
35
25
15
Lambda-carrageenase protein (~105 kDa)

INTERNATIONAL SEARCH REPORT

International application No
PCT/EP2018/085941

A. CLASSIFICATION OF SUBJECT MATTER
INV. C07K14/245 C12N15/70
ADD.

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
C07K C12N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
EPO-Internal

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	G E Allison ET AL: "Functional analysis of the gene encoding immunity to lactacin F, lafI, and its use as a Lactobacillus-specific, food-grade genetic marker", Applied and Environmental Microbiology, 1 December 1996 (1996-12-01), pages 4450-4460, XP055467010, UNITED STATES Retrieved from the Internet: URL:http://aem.asm.org/content/62/12/4450.full.pdf#page=1&view=FitH abstract page 4456, left-hand column, paragraph 2 - right-hand column, paragraph 1 page 4459, left-hand column, paragraph 1 figure 3 ----- -/--	1-18

[X] Further documents are listed in the continuation of Box C.

[X] See patent family annex.

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"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
1 March 2019	11/03/2019

Name and mailing address of the ISA/	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Fax: (+31-70) 340-3016	Sonnerat, Isabelle

1

Form PCT/ISA/210 (second sheet) (April 2005)

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	D. A. BREDE ET AL: "Identification of the Propionicin F Bacteriocin Immunity Gene (pcfI) and Development of a Food-Grade Cloning System for Propionibacterium freudenreichii", APPLIED AND ENVIRONMENTAL MICROBIOLOGY, vol. 73, no. 23, 1 December 2007 (2007-12-01), pages 7542-7547, XP055467000, US ISSN: 0099-2240, DOI: 10.1128/AEM.01023-07 abstract page 7544, left-hand column, paragraphs 3,4 page 7545, right-hand column page 7546, left-hand column, paragraph 2 -----	1-18
X	WO 2009/011940 A2 (UNIV MARYLAND [US]; GALEN JAMES E [US]; FANG CHEE-MUN [US]) 22 January 2009 (2009-01-22) abstract paragraphs [0080] - [0083] example 6 -----	1-18
X	C.-M. FANG ET AL: "Use of mchI Encoding Immunity to the Antimicrobial Peptide Microcin H47 as a Plasmid Selection Marker in Attenuated Bacterial Live Vectors", INFECTION AND IMMUNITY, vol. 76, no. 10, 1 October 2008 (2008-10-01), pages 4422-4430, XP055467029, ISSN: 0019-9567, DOI: 10.1128/IAI.00487-08 the whole document -----	1-18
X	TAKALA T. ET AL: "A food-grade cloning vector for lactic acid bacteria based on the nisin immunity gene nisI", APPLIED MICROBIOLOGY AND BIOTECHNOLOGY, vol. 59, no. 4-5, 1 January 2002 (2002-01-01), pages 467-471, XP055018982, ISSN: 0175-7598, DOI: 10.1007/s00253-002-1034-4 the whole document -----	1-18
A	SOPHIE DUQUESNE ET AL: "Microcins, gene-encoded antibacterial peptides from enterobacteria", NATURAL PRODUCT REPORTS, vol. 24, no. 4, 1 January 2007 (2007-01-01), page 708, XP055467441, GB ISSN: 0265-0568, DOI: 10.1039/b516237h the whole document -----	1-18

1

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No
PCT/EP2018/085941

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 2009011940 A2	22-01-2009	US 2010112674 A1	06-05-2010
		WO 2009011940 A2	22-01-2009