Bacteriocins

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Keywords: Bacteriocins, Membrane-active compound

Abstract

Bacteriocins are antibiotic peptides produced by eubacterial bacteria. They form pores in the cells which disrupt membrane potential and leads to cell death. They are similar to antibiotics produce by fungi like penicillin. Bacteriocins have a wide possible application as a food preservative. We will discuss about the classification, general characteristics and mode of action on membrane.

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Introduction

Bacteriocins were first discovered by A. Gratia in 1925 [3]. Bacteriocins are proteinaceous compounds lethal to bacteria other than the producing strain. As a group, bacteriocins are heterogeneous. Some bacteriocins are peptides consisting of only 19 to 37 amino acids, whereas others are large peptides with molecular weights of up to 90,000. Some small bacteriocins contain unusual amino acids originating from modifications of conventional amino acids after translation. The activity spectrum of bacteriocins can be narrow and confined to inhibition of closely related species, or it can be relatively broad and include many different bacterial species In contrast to the currently used antibiotics, bacteriocins are often considered more natural because they are thought to have been present in many of the foods eaten since ancient times. The bacteriocin nisin actually has GRAS (generally recognized as safe) status. Nisin and other bacteriocins produced by lactic acid bacteria have received a great deal of attention because they are produced by bacteria largely considered beneficial to human health and to food production.

Classification

Different authors classify bacteriocin in different ways like method of killing (pore forming, dnase, nuclease, murein production inhibition, etc.), genetics (large plasmids, small plasmids, chromosomal), molecular weight and chemistry (large protein, polypeptide, with/without sugar moiety, containing atypical amino acids like lanthionine) and method of production (ribosomal, post ribosomal modifications, non-ribosomal).

Klaenhammer classified bacteriocin into four groups on the basis of their molecular mass, thermosability, enzymatic sensitivity, presence of post translationally modified amino acids, and mode of action [4]. On a sound scientific basis three defined classes of bacteriocins are:

- Class I, the lantibiotics;
- Class II, the small heat stable non lantibiotics
- Class III, large heat labile bacteriocins.
- Class IV, undefined mixture proteins, lipids and carbohydrates.

Class I bacteriocins

This group comprises lantibiotics. Lantibiotics are produced by a large number of Gram-positive bacteria such as Streptococcus and Streptomyces to attack other Gram-positive bacteria. Lantibiotics are a class of peptide antibiotics that contain the characteristic polycyclic thioether amino acids lanthionine or methyllanthionine, as well as the unsaturated amino acids dehydroalanine and 2-aminoisobutyric acid [5, 6].They are produced by Streptococcus and Streptomyces. Lantibiotics can be further divided into two subgroups on the basis of structure and charge of the compound. Group Ia, which consists of screw-shaped, amphipathic, small cationic peptides. Group Ib, which consists of anionic or neutral peptides having globular shape. Example of Group Ia bacteriocin are nisin, subtilin, epidermin. Some Group Ib bacteriocin are mersacidin [8], actagardin [9], and cinnamycin [10].

Class II bacteriocins

Class II bacteriocins includes heat-stable peptides with no modified amino acids and smaller molecular mass (less than 10kDa). This class is further divided into four groups. Group IIa consist bactericides that disrupt the integrity of the cytoplasmic membrane, producing ionic imbalance and leakage of the organic phosphate to exert their killing actions. Bacteriocins like pediocin AcH/PA1, mesentericin Y 105, Sakacin A, Sakasin P and carnobacteriocin B2 are included in it. Group IIb consists of pore-forming complexes requiring two peptides for their activity. These may act alone or as synergist (enterocins L50A and L50B) [11]. Group IIc includes two types of bacteriocins *i.e.* with one or two cysteine residues (thiolbiotics and cystibiotics, respectively) and without cysteine (lactococcin A and acidocin B).

Received Manuscript 01-04-2013 Accepted Manuscript- 15-06-2013

Table 1 Classification of Class IIcbacteriocins by Klaenhammer [4]

Bacteriocin	Producer Strain	Molecular mass (Da)	Amino acids	p*I*	Number of cysteine residues
Cerein 7/8	*Bacillus cereus Bc7*	4893	56	8.38	2
Enterocin B	*Enterococcus faecium* T136	5465	53	9.70	2
Carnobacteriocin A	*Carnobacterium piscicola* LV17A	5053	53	9.02	2
Lactococcin A	*Lactococcus lactis* LMG2130	5778	54	9.21	0
Lactococcin B	*Lactococcus cremoris* 9B4	5328	47	9.25	1
Divergicin A	*Carnobacterium divergens* LV13	4225	46	9.96	2
Acidocin B	*Lactobacillus acidophilus* M46	5754	59	7.38	0

Class III bacteriocins

Class III bacteriocins are peptidic antibiotics that are heat liable proteins with a molecular mass larger than 30 kDa. Major producers of these bacteriocins are genus Lactobacillus. For eg. Helveticin J [12] by L. helvecticus 481, and lacticin B [13], produced by L. acidophilus.

Class IV bacteriocins

This group consists of their glycoproteins (lactocin) [27, 14] or lipoproteins (lacstrepcins) [15] that require non-protein moieties for their activity.

Characteristics of bacteriocins

Bacteriocins must have two requirements to become lethal, to be cationic and highly hydrophobic. Most of the class I and II bacteriocins are cationic at pH 7.0. Their high isoelectric point [7] allows them to interact at physiological pH values with the anionic surface of the bacterial membrane. This leads to insertion of the hydrophobic moiety into the bacterial membrane. This build up the transmembrane pore which cause gradient dissipation and cellular death.

Heat-stability is another property of bacteriocins (low molecular weight). Complex pattern of monosulfide and disulfide intramolecular bonds helps in the stabilization [16] of secondary structures by reducing the number of possible unfolded structures (entropic effect). Cintas et al. [17] observed that most of the supernatants of bacteriocins producing strains are resistant to autoclaving conditions and heat treatment (100 and 121^{0}C). However, some bacteriocins produced by *Lactobacillus* strains (helveticin J [12]) were inactivated by 10 to 15min treatments of 60-100^{0}C.

Nisin produced by *Lactococcuslactis* [18] strains, was discovered in 1928 and is most studied bacteriocins. It consists of 34 amino acids and has five lanthionine bonds. It has high solubility, antimicrobial activity, and thermostability at pH 2.0. It is inactivated at pH 7.0. Sensitivity of nisin to digestive enzymes made it a product of choice as food preservative [19].

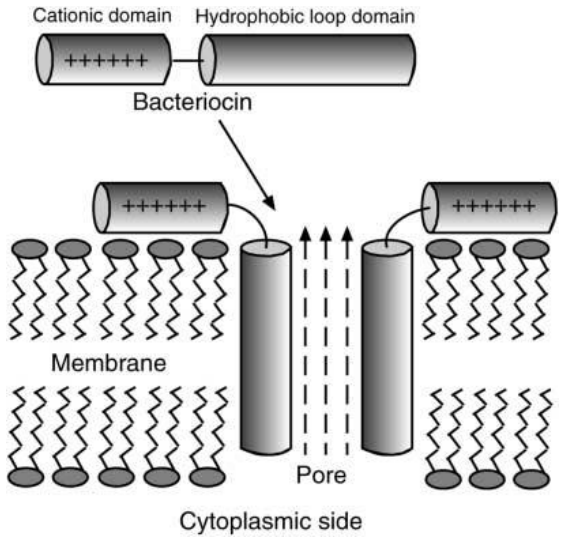
General Mode of Action

Bacteriocin act of target by forming membrane pores that disturb the energy potential of sensitive cells. Different bacteria produce different bacteriocins which have their own mode of action.

Mode of action of nisin is best studied. This bacteriocins associates electrostatically with phospholipilds, which causes interaction of bacteriocins's hydrophobic residues with the cytoplasmic membrane of target cell [20]. Electrostatic interaction is caused by Lysine (cationic amino acid). The interaction between the hydrophobic part of nisin and membrane generates ionic channels. It is favoured by high transmembrane potentials, presence of anionic and absence of cationic lipids [21]. It is hindered by divalent cations because they neutralize the negative charges of the phospholipids, reducing the fluidity of the membrane. Pores produced by nisin create passive efflux of K+ and Mg2+ , amino acids, ATP and proton-motive-force dissipation and cell death.

Class I bacteriocins show a pronounced anti-listerial specificity due to the presence of sequence YGNGV in their N-terminal region [22]. The current mechanism to explain mode of action includes electrostatic binding of antibiotic to the target membrane mediated by a putative membrane-bound receptor molecule, although the necessity of this specific receptor is still controversial. The hypothetical receptor would be responsible for the recognition of the YGNGV anti-listerial motif present in these peptides.

Fig. 1 Killing action of Bacteriocins



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