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Mini Review

Bacteriocin-like protein produced Brevibacillus laterosporus that can inhibit the growth of drug resistant bacteria

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Abstract

This minireview contains a compendium of bacteriocin or bacteriocin-like proteins produced from *Brevibacillus laterosporus* that can inhibit the growth of drug resistant bacteria. A good number of bacterial secondary metabolites/ bacteriocin/ bacteriocin-like proteins are reported to have anti-drug resistant bacteria activity or anti-cancer activity comparable to the existing chemical synthesis antimicrobial drugs or sometimes even better. Information regarding the mode of action of bacteriocin leads to insight into their activity relationship and potency. A further well defined strategy is required to exploit these active molecules used as anti-drug resistant bacteria drugs.

Introduction

Currently, resistance of pathogenic organisms to approved antibiotics has become a worldwide problem with serious consequences on the treatment of infectious diseases [1-2]. The increased use/misuse of antibiotics in a treatment of infectious diseases is mainly causing to the phenomenon and/or pathogenic bacteria develop mechanism of antibiotic resistance [2]. There is an alarming increase of antibiotic resistance of bacteria that cause either community infections and/or hospital-acquired infections. Of particular interest are the multidrug resistant pathogens such as methicillin-resistant *Staphylococcus aureus* (MRSA), vancomycin-resistant MRSA, penicillin-resistant *Streptococcus pneumoniae* (PRSP) and vancomycin-resistant *Enterococcus* (VRE) [3]. Including, ESBLs (extended-spectrum beta-lactamases)-producing strains such as *Klebsiella pneumoniae*, *Pseudomonas aeruginosa* and *E. coli* etc., which can be transferred antibiotic-resistant gene to the other species by conjugation [4]. In many multidrug resistant pathogens, MRSA and ESBLs-producing strains are the most serious pathogens because they are a major cause of nosocomial infections and non-hospital acquired infections that associated morbidity and mortality. In many years past, the emergence of MRSA strains resistant to all β-lactam antibiotics. Since the discovery, development and drug administration approval of vancomycin in the 1950s, this antibiotic is a mainstay for the treatment of infections caused by MRSA. However, because of the development of new anti-staphylococcal antibiotics, several researches reported vancomycin failure [5-6]. The MRSA, ESBLs-producing strains and some multidrug resistant pathogen has multi-mechanism resistance such as 1) modification of the bio-molecular target of antibiotics 2) enzymatic inactivation of antibiotic, *e.g.* β-lactamases which hydrolyze β-lactams 3) reduction of the intracellular antibiotic concentration in bacteria, by its efflux outside from the cell through bacterial trans-membrane efflux pumps. As a result, the development of novel antibiotics increases [2-3,7].

The several researches reported antibacterial/antimicrobial peptides or bacteriocins were applied as model for the production of novel antibiotics [8-9] because of the mode of action of antibacterial/antimicrobial peptides or bacteriocins are inhibit cell wall synthesis of MRSA strains with efficiency equal to that reported for vancomycin, which leaves the option

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of using the two substances in combination [8]. According to the research report in 2008, the partially bioactive compounds, which expected as bacteriocin, produced by *Brev. laterosporus* strain SA14 inhibit the growth of clinical strain of MRSA [10]. Whereas, the non-specific on targets of the bioactive compounds and/or partially bioactive compounds is a major disadvantage. The several researches reported the bioactive compounds and/or partially bioactive compounds produced by many strains of *Brev. laterosporus*, which are major proteins, were used to control *Musca domestica*, *Aedes aegypti*, Coleoptera, parasitic nematodes ova/larvae and mollusks [11-12].

Brevibacillus laterosporus

Brev. laterosporus, previously classified as *Bacillus laterosporus* (*B. laterosporus*) [13], is a Gram-positive bacilli and an aerobic spore-forming bacterium characterized by the production of a typical canoe-shaped parasporal body (CSPB) which remains firmly attached to one side of the spore after lysis of the sporangium. As a pathogen against invertebrates, its toxic activities against parasitic nematodes ova/larvae [11]. It has been found as a secondary invader during European foulbrood, which is a serious infectious disease of honey bees [14]. The *Brev. laterosporus* can produce different virulence factors: parasporal crystalline, extracellular protease [15] and lipopeptide antibiotics [16]. Including, the secretion of short-sequence peptides (bacteriocin) with broad antibiotic spectra, such as laterosporulin [17], loloatin A [18].

Antagonistic compounds produced by bacteria from the genus *Brevibacillus* have also been studied [19]. The strains of *Brevibacillus laterosporus* are well known produced antibacterial and antifungal agents [10, 20–22]. The recently characterized *Brev. laterosporus* OSY-I1 produces brevibacillin, a 1583 Da antimicrobial lipopeptide with a linear structure containing 13 amino acids and a C6 fatty acid at the N-terminus [23]. Brevibacillin shows strong antimicrobial activity against some pathogenic and food-spoilage Gram-positive bacteria, particularly MRSA, *Listeria monocytogenes* and *Bacillus cereus*. In addition, some strains of *Brev. laterosporus* also produced the medically important substance such as spergualin, which is a new antitumor antibiotic [13], and bacithrocins A, B and C [14].

Bacteriocin and Bacteriocin produced by Brevibacillus sp.

Bacteriocins, antibacterial peptides, which are produced by bacteria as a defense mechanism in complex environments [17]. It is short-sequence peptides that categorized into different classes based on structural and functional characteristics as follows 1) the class I bacteriocin (molecular weight <5 kDa) called as lantibiotics, are well studied with wide applications in both therapeutic and preservation of food products at industrial scale [24-25] 2) the class II bacteriocin (molecular weight <10 kDa) are further divided into different sub-classes, including antilisterial one-peptide pediocin like bacteriocin as sub-class IIa [26-29], the two-peptide bacteriocin as sub-class IIb [24,27–30], the sec-dependent bacteriocin as sub-class IIc 3) the class III bacteriocin (molecular weight >30 kDa) containing cyclic and heat-labile protein bacteriocin 4) the class IV bacteriocin (large protein) composed of one-peptide, lipid(s) and carbohydrate(s) in bacteriocin molecule [25, 28, 30-31]. The mode of action of bacteriocins is most likely based on its amphiphilic nature and the ability of the cationic amino acids to interact with the negatively charged phospholipids of the cell membrane, causing the disruption and depolarization of the membrane [23]. A similar mechanism was observed in the case of paenibacterin, a broad-spectrum antimicrobial lipopeptide produced by *Paenibacillus thiaminolyticus* [32]. In addition, it was found that the marine bacterial isolate *Brevibacillus laterosporus* PNG-276 showed broad-spectrum antibiotic activity (producing polyketides the basiliskamides A and B and non-ribosomal peptides: loloatins A-D and bogorols A-E) against the human pathogens MRSA, VRE, *Mycobacterium tuberculosis*, *Candida albicans*, and *Escherichia coli* [33].

The laterosporulin, a novel bacteriocin produced by *Brevibacillus laterosporus* strain Gl-9 [34]., which are class IId bacteriocin because of molecular weight of peptide to be of 5.6 kDa. The open reading frame (ORF) encoding laterosporulin were identified a 4 kb region from the draft genome sequence of Gl-9. The 4 kb region contained the putative structural gene encoding laterosporulin and its flanking genes (transcriptional regulator, hypothetical protein, ABC transporter and alkyl hydroperoxide reductase). The ORF of 153 nucleotides followed the putative Shine-Dalgarno sequence most likely codes for the bacteriocins [17]. Laterosporulin is active against both Gram-positive and Gram-negative bacteria and was found to be resistant to a range of proteolytic enzymes. Structural studies revealed that the peptide consists of twisted Antimicrobials from new *Brevibacillus laterosporus* strains [https://doi.org/10.1371/journal.pone.0216773; 35]. β-sheet and includes three disulfide bonds [36]. Laterosporulin is relatively rich in cysteine and polar amino acids, which is atypical for bacteriocins in general, whereas its structure showed similarities with mammalian defensins. More recently, laterosporulin 10, produced by the strain *Brevibacillus* sp. SKDU10 was characterized [37] and, while considered similar, shows only 57.6% identity with laterosporulin. In addition, this novel bacteriocin has a different antimicrobial spectrum to laterosporulin as activity is limited to Gram-positive bacteria. However, laterosporulin 10 has also proven to be a promising new anti-cancer molecule that exhibits a cytotoxic effect on cancer cells [38].

The strains of *Brev. laterosporus* have been sequenced and and their genomes deposited in Genbank. These include LMG 15441 (under accession number AFRV00000000, [39]), GI-9 (under accession numbers CAGD01000001 to CAGD01000061, [40]), B9 (under accession numbers CP011074-CP011076, [41]), Lak 1210 (under accession number NDIP00000000, [42]), OSY-I1 (under accession number NOLX00000000, [43]), and SA14 (accession number KF718856.1, [https://www.ncbi.nlm.nih.gov/nuccore/KF718856.1]).

Unfortunately, the manufacturing of important substances (bioactive compound, anticancer/antimicrobial-peptides and/or bacteriocins) used the high cost and time-consuming. Nevertheless, it can be produced very less amounts which cannot industrial use. However, the advancement of genetic engineering was used to solve the above problems. The novel

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bacteriocin-encoding genes will be inserted into appropriate shuttle / expression plasmid vector. The recombinant plasmids containing bacteriocin gene will be transferred to host cell-free protein expression system for introduce the novel bacteriocin production. With the hope, the constructed shuttle plasmid vector may be high segregational stability which can be introduce the novel bacteriocin production in large scale for industrial use.

Conclusion

It will be difficult to treat the drug resistant bacteria without increased funding for drug discovery. Bacteriocin producing bacteria are applicable used for treatment of infectious disease caused from drug resistant microorganisms. Though anti-drug resistant microorganisms MIC of bacteriocin is higher but they have resistance modifying properties. Therefore, bacteriocin derived drugs can help in fighting the drug resistance. Unfortunately, there is no bacteriocin derived molecule either in market or under trial for treatment of drug resistant infections. Majority of studies focused on identification of bacteriocin structures or extracts with anti-drug resistant microorganisms properties and has not been extended to identification of bioactive-compound metabolites. Therefore, an integrated approach of identification of secondary metabolites/bacteriocin with anti-drug resistant microorganisms activity followed by identification of bioactive molecules will speed up the research and development of bacteriocin derived drug molecules for drug resistant microorganisms infections.

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