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Review Paper

Applications of Lipopeptide(s) from a *Bacillus* sp: An Overview

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Abstract

In the recent years, continuous and excess use of chemical pesticides in the field has created an adverse ecological balance that affected the whole environment. Most of the part of crops is destroyed by the phytopathogens such as fungi, bacteria and yeast causes to economic losses to the farmers. Extensive use of the chemicals for controlling the plant diseases in the field has unbalanced the delicate environmental harmony of the fertile land, causes soil water contamination, evolution of new races of microorganisms and risk for humans health. The constant and continue demand for new bio-therapeutic agents with an effective mode of action has activated the intensive/deep research in the field of diverse natural bioactive molecules having antimicrobial activity. Among these active molecules, lipopeptide(s) are a unique class of bio-active secondary metabolites with increasing scientific, therapeutic and biotechnological interest. Bacillus subtilis produced mainly a four different types of lipopeptides with a potential for biotechnological and biopharmaceutical applications. Among the all Lipopeptide(s) classes, Iturin and Surfactin shows attractive antibiotic properties. The main family of the microbial lipopeptide(s) is surfactin, which mainly originated from the B. subtilis. These properties of the surfactin make a momemtous drug (surfactin) for the resolution of a number of global issues in medicine. Cancer and phytopathogens are the major problem in the today's scenario. The bacterial lipopeptide(s) have lower toxicity for plants and animals, high biodegradability, low irritancy and compatibility with human skin.

Keywords: Lipopeptides, *Bacillus* sp, Iturin, Surfactin, Anticancer, Phytopathogens.

Introduction

Continuous exercise of the hazardous pesticides in nimiety for management of the diseases (plants) caused by the pathogens, has interrupted whole environmental equity of the land causes evolution of the rebellious new strains of the microbial pathogens causes' soil water contamination as well as fitness exposure to humans[1]. The greatest environmentally-safe challenge to be showed to microbiological as well as pathological scientists of plants in the coming time is of the invention of environmental substitutes to replace the currently using hazardous pesticides by the biopesticides[2]. These llipopeptide(s) are reckoned as potent alternatives to the resistance problems of generally used antibiotics, life threatening diseases and fungal infections. Demand of the new bio therapeutic compounds with unique or new mode of action is increasing continuously that has promoted the research in the field of lipopeptide like active compounds that shows antimicrobial activity. Need of the lipopeptide(s) is also surging by leaps and bound, in the present time, due to their usefulness for the human welfare with respect to wide applications like bio-control of phyto-pathogens causing diseases in the plants. There are many types of lipopeptide(s) amongst these Iturin, Surfactin (Fig.1) and Fengycin are the key type of lipopeptide(s) which were discovered first and studied for the anti-microbial activity against the broad compass of phytopathogens. The bacterial lipopeptide(s) antibiotics are getting more attention for their various applications such as antifungal activity, lower toxicity and high biodegradability[3]. Continuous increasing resistance of the drugs to the bacterial strains has prompted the urgent need of the development of new antibiotics have applications in the field of medical, food and dairy products[4]. These lipopeptides have crucial properties in the field of biomedical and biotechnology. These molecules have proposed as the solution of increasing drug resistance to the conventional antibiotics and fungal diseases.

Types of Microbial Lipopeptide(s)

There are many types of lipopeptide(s) which are classified on the basis of their amino- acid sequences and lipid moiety length. Broadly lipopeptide(s) can be categorized mainly in the three types namely Iturin, Surfactin, and Fengycin and Kurstakins that are produced in the medium by the various bacterial species.

Iturin lipopeptide: Out of these four types of microbial lipopeptide(s), Iturin is an antibiotic of puny molecular mass (M_r) comprising of ~ 1.04 kDa. Iturin consist of two hefty parts including lipid and small peptide chain of seven D and L amino-acids (Figure-1). Structure of Iturin lipopeptide clearly indicates towards the amphiphilic character of the molecule, thus membrane of the microbes is the main susceptible to attack for the site of the action[5]. Iturin lipopeptide contains cyclic ring consisting of seven D/L amino acids residues connected with a

chain of fatty acid (β-amino) which can deviate from the C_{14}-C_{17} carbon molecules. These molecules of Iturin are of very interesting due to the chemical, biological along with physico-chemical properties, which may be used or exploited in the field of oil, food along with therapeutics. About all the strains of *B. subtilis* able to evolve this Iturin like of lipopeptide(s). Operon of Iturin, made up of ~ 38 to 40 kb in size have four open reading frame (ORF) like Itu A, Itu B, Itu C, and Itu D[6].

Surfactin lipopeptide: Surfactin also an amphipathic cyclic lipopeptide (CLP) of molecular mass ~1.36 kDa contains ^{L}Glu-^{L}Leu-^{D}Leu-^{L}Val-^{L}Asp-^{D}Leu-^{L}Leu seven amino acids that play a momentous role in the surfactant properties. These D and L, seven amino acids in the cyclic ring connected with a fatty acid (β-hydroxy) chain of C_{12}-C_{16} carbon chain which forms a close ring (lactone) (Figure-2) structure[7]. Types of Surfactins (Surfactin A, B, C and D) deviate on the basis of order of the amino acids as well as the size/chain of lipid moiety/ portion[8]. Leu and Val amino acids of the Surfactin are situated at 2, 3, 4, 6 and 7th position while the acidic Asp and Glu residues of the surfactin are placed at the positions of 1st and 5th, respectively. Generally, isomers of the Surfactin cohabit into the bacterial cells as a mash of various peptide chains with a various fatty acid chain length[9]. In the Surfactin, bacterial strain and its culture conditions, both affect the amino-acids pattern and fatty acids (β-hydroxy) length[7]. β-turn in the protein, are formed by the hydrogen bond (Intramolecular) whereas the β-sheet of the peptide depends on the intermolecular hydrogen bonds[10].

Fengycin: This is the third family of lipopeptide(s) also called plipastatin. This bio-active lipopeptide molecule contains 10 amino acid and a fatty acid chain, which may saturated/unsaturated. Fengycin molecules do not have haemolytic activity at more extent compared to Iturin and Surfactin that have surfactant activities involved in the hemolytic activity. Iturin and Surfactin show strong anti-fungal activity against selected filamentous fungi[11-12]. The mode of action of Fengycins is not well known as compared to Iturin and Surfactin but up to some extent have the capability to disintegrate the membrane by the pore formation ultimately change the structure of the lipid membrane. It does not have any surfactant activity and show little antimicrobial activity. In the bacterial membrane, these extracellular lipopeptides are synthesized by an enzyme system consisting of 1000 amino acids encoded by an operon of five open reading frame (ORF; fenA-E). Due to the antimicrobial activity, these lipopeptide(s) are considered as biocontrol agents.

Kurstakins: This is a newly discovered type of the lipopeptides from *Bacillus* sp.[13]. These type of lipopeptides shows antifungal activity against the selected fungal strains such as *Stachybotrys charatum.* These lipopeptides are absorbed on spore surfaces of the fungal sp. and attack at the cell wall causes pore formation leads to kill/growth inhibition.

Applications of lipopeptide(s)

Amongst the all lipopeptide(s), Surfactin has acknowledged for its enormous applications (Figure-2). Lipopeptide(s) have surfactant properties that make the strong interest of these biosurfactants for different applications[14] in foods[15], environmental[16], biomedical[17] and cosmetics[18]. From the last history, Surfactin which was examined first, for its potent bio therapeutic applications such as anti-bacterial, anti-tumor and hypo cholesterol activities. Anti-mycoplasma and anti-viral properties of the Surfactin were discovered in the end of 1990s. These properties lead to the proposal of its use to ensure the bio-safety of pharmaceutical and biotechnological products[19]. Presence of mycosubtilin like lipopeptide(s) in the fermented food products or secretion of these active molecules by microbial sp[20] allows considering for their potent application(s) in the field of food processing field.

Figure-1
Cyclic structure of lipopeptides Iturin and surfactin
[A] Iturin contains seven amino acid residues attached to a 14-carbon chain indicates its amphiphilic nature. The amino acids are three D-amino acids (Tyr, Asn, and Asn) and the four L-amino acids (Pro, Ser, Asn, and Gln). [B] Heptapeptide cyclic structure of Surfactin, containing both hydrophobic and hydrophilic amino acids. The structure containing amino acids: two D-amino acids (Leu, Leu) and five L-amino acids (Val, Asp, Leu, Glu, and Leu), indicates its amphipathic nature

Lipopeptide(s) as inducer of plant resistance: Fengycin and surfactant type lipopeptide(s) are capable to interact with the plant cells, where these lipopeptides interact with the bacteria and induce the immune response for the detection of bacterial sp. associated with the plant. In tomato and bean, surfactin and fengycin play a key important role in the plant defence mechanism for the bacterial strains. In a conclusive way, an important tender effect was observed when the plant are treated with the different concentrations of the LPs derivatives obtained from the *Bacillus subtilis* 168, which is not capable to synthesize these lipopeptide(s) type active compounds and also not active on the surface of plants.

***Bacillus* lipopeptide(s) as biocontrol agents:** *Bacillus* sp. is a commercialize bio-control agent. The market of the lipopeptides globally increasing day by day for phytosanitary products that were used in all over the world to increased the crop production yield up to the $26.7 billion in the year of 2005[21]. *Bacillus* lipopeptide(s) have potent antimicrobial activities to control the phytopathogens such as bacteria, fungi and yeasts. Iturin is a good antifungal compound while Surfactin knows for its antibacterial activity against the pathogenic microorganisms.

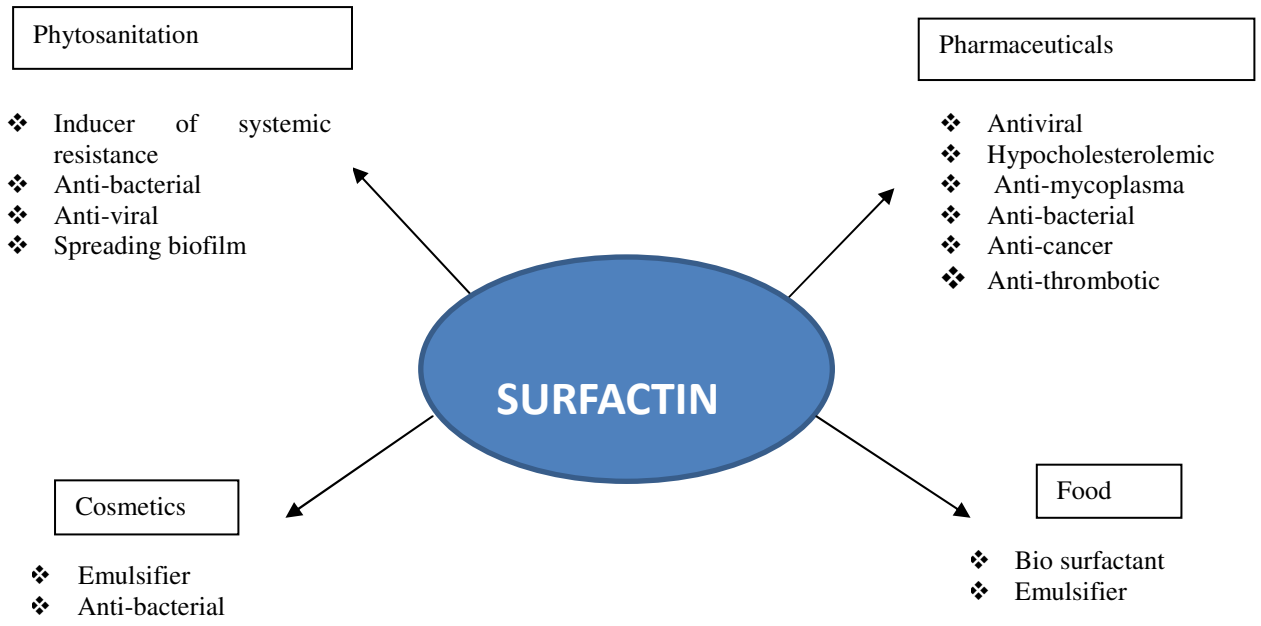

Figure-2
Broader applications of Surfactin in food and pharmaceutical industries, Applications are shown in different areas such as phytosanitation, pharmaceuticals, food, and cosmetics

Table-1
Lipopeptides from *Bacillus* sp. as biocontrol agents

Plant disease	Phytopathogen	Lipopeptide producing microorganism	Lipopeptide(s) inhibited phytopathogens	Reference(s)
Damping off of bean	*Pythium ultimum*	*B. subtilis* M4	Iturin	22
Root infection in *Arabidopsis*	*Pseudomonas syringae*	*B. subtilis* 6051	Surfactin	23
Powdery mildew disease of cucurbits	*Podosphaera fusca*	*B. subtilis*	Iturin	24
Foliar/root disease of soybeans	*Xanthomonas axonopodis*	*B. amyloliquefaciens* KPS46	Surfactin	25
White mould disease	*Sclerotinia sclerotiorum*	*B. amyloliquefaciens*	Surfactin	26

Lipopeptide(s) in the nanotechnology: According to Plaza *et al*., lipopeptide(s) which have property of biosurfactants have capability to synthesize the nanoparticles (NPs)[27]. According to Reddy *et al*., Surfactin is able to synthesize both silver and gold nanoparticles in which this lipopeptide (Surfactin) used as a template or stabilizing agent play a key role in the stabilization of the nanoparticles[28]. Gold NPs quality assured by their stability. To synthesize the stable gold nanoparticles, $AuCl_4$ is reduced in the aqueous solution of sodium borohydrate in the presence of Surfactin lipopeptide from the *B.subtilis*. Reddy *et al*., used Surfactin lipopeptide as a stabilizer where it play key role in the nanostructured shape of the zinc oxide nanoparticles by the precipitation method. Surfactin act as templating agents which help to provide nano shape[29]. Singh *et al*., used the same lipopeptide Surfactin from the *B. amyloliquefaciens* to synthesize the stable and sound cadmium sulfide- nanoparticles (CdS-NPs)[30]. These nanoparticles of CdS synthesized from the crude Surfactin and covered nanoparticles with the Surfactin were found stable upto six months without any change in the structure[30].

Lipopeptides as immunomodulators: According to Infante *et al*., the lipopeptides developed from the *B. burgdorferi*, able to stimulate the expression of IL-17 (a Cytokine produced by T_C-cells) in T_h cells of both murine and human[31]. They induce the expression of IL-17 unitedly with Tumor Necrosis factor (TNF-α), so these are co-expressed with other pro-inflammatory cytokines. Das *et al*., observed the antimicrobial activity of the lipopeptide biosurfactant (Surfactin) produced from a marine bacterium *B. circulans*[32]. The produced biosurfactant from *B. circulans* have the potential for the potent anti-microbial activity against the gram positive and gram negative pathogenic microbial strains. Moreover, this biosurfactant was found to be non-haemolytic.

Conclusion

Microbial lipopeptide(s) has emerged as a new tool to overcome the future problems of the human being. These lipopeptides are safe to use without any adverse effects on the humans. These are various types on the day of today, but some types like surfactin and Iturin are the much important because of their wide applications. According to various earlier reports, Surfactin is an anticancer agent without any effect on the normal cells.

It also has antiviral, antimalarial, antifungal and antibacterial activities. On the day of today, most urgent need is the replacement of the hazardous chemical pesticides with the safe and biodegradable lipopeptide like biopesticides, which are molecules act as biocontrol agents against the phytopathogens. Lipopeptides are made a special class of bioactive secondary metabolites have potential applications in the field of medical, agriculture, phytosanitation. Due to the various applications of the lipopeptides, these molecules of low molecular mass are considered as versatile weapons.

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