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Review Article

Comprehensive overview on *Bacillus subtilis* antibacterial metabolites production

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Abstract:

Over the last 70 years, Food processors and the plant protection sector have both benefited from *Bacillus subtilis*. Their capacity to manufacture endospores for survival, as well as a multitude of antimicrobial substances has piqued industrial interest in areas such as food preservation, medicinal agents, and biopesticides. In light of the growing trend of food healing and the protection of bacterial plants, this review suggests a holistic approach to visualizing the antimicrobial screen described in Group B. This review aims to make easy and updated classification of antimicrobial metabolites in group *B. subtilis*, its complex phylogeny that tends to perpetuate development.

Key words: *B. subtilis,* antimicrobial compounds, biopesticides, therapeutic agents and metabolites

Introduction:

Bacillus genus comprise of three hundred seventy seven species (last updated in January 2019).They are G+ and rod shaped bacteria [1]. Their assortment in living conditions, their ability to make endospores and to produce large amounts of synthetic antimicrobial compounds (AMCs) are endemic to the soil, aquatic environment, arthropods and mammals and gut microbiota [2], A total of four unique species of the group (*B. subtilis, B. amylolicifaciens, B. bumilus* and *B. licheniformis*) were discovered 40 years ago [1]. Many unique genres and subspecies have been re-examined and interpreted since the discovery of their molecular and chemical structures [3]. The desert has a unique flower and the Bacillus species is one of the most prominent. Members of this Gram-positive, aerobic, and spore-forming bacterium. Various types of Bacillus have been isolated in various places including soil, water, food and stone [4]. The natural environment remains an important final repository for microorganisms, which are able to produce energy for the metabolites antimicrobials metabolites. Such germs are able to form ants to cope with critical conditions such as high salt, pH and temperature and make them survive and produce different enzymes or proteins [5]. Bacillus strains form many secondary metabolites, including antiretic and antifungals compounds. These viruses are well known for the production of a large number of unrelated genes, including lipo-peptides such as iturin, survilin, fengycins and bacteriocin as a barrier material. An antibacterial compound from the genus Bacillus has shown activity against many drug-resistant pathogens in the clinic [6]. Recently in Pakistan, Bacillus species isolated from saline soils and are known to produce antibiotic peptides. Previous studies have been conducted to distinguish novel bacteria from the desert soil of Cholistan, Pakistan [7].

Production of Bacteriocin:

It is projected that 99% of archaea and bacteria can yield minimum one bacteriocin. Factually, lactobacillus (LB) has been deliberate as major producers of bacteriocin, largely because they are being used in food for longer time and considered safe [8]. *L. lactis* subsp Lactis produce Nisin. Since its introduction as a dietary supplement in the 1968- 1962 period, it has been

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used in over fifty nations for antibacterial action against G+ bacteria such as *Clostridium* spp. and *Bacillus* spp [9]. However, in the late 1990s, the search for novel bioactive compounds expanded to include additional bacteriocin producing variations, with specific focus on the antimicrobial spectra of GRAS (more commonly known as Bacillus species), their bacteriocin, in the late 1990s [10]. The common biomarker alleyway of Bacillus Species bacteriocin involves numerous alterations, including proteinolytic division of the lead peptide at the N-terminal end [11]. Modification of the active peptides may vary depending on the type of bacteriocin responsible for its bacteriocin protection and infection.

Although many years apart, the logical way to deal with Bacillus' diversity is to replace it with their biomarker method as earlier described with *Streptococcus* spp. also *Enterococcus* spp. Bacteriocins [12]. In line with this, the three main classes are divided into several different classes. They can be divided into subtilis groups as, the class I assembles modified peptides after lantibiotic conversion, and the unchanged peptides are isolated from class II; The third category includes bacteriocins superior than 10 kDa [12]. We summarize the different RPs produced by subtilis group brains and their comparative reporting activity.

Phase I comprises minor amps (19-39 AA) with many alterations. Normal I-1, I-2 and I-3 remain in their lantibiotic assembly, showing the fractions of thioester residues of residue formation in modified AA residues. Lantibiotics including 2,3-dihydrohydroalanine (Tha) and (Z) Intramolecular incorporation of Ta or TB into cysteine rings leads to formation of suitable lanthionine bridges and methylthionine [13]. It is the most deliberate bacteriocin in the subtilis group. Its assembly dividends many similarities with the Nisin A lantibiotic [12]. The peptides isolated by subclass I.4 constitute other types of alterations. For example, subtilosin A is a tail to tail peptide with an unlinked surface between residues [14].

Amp Enzymes

In the B. subtilis group, 2 main kinds of enzymes exhibited antitumor deeds lytic enzymes and individuals complicated in quorum secretion. Many types of subtilis group have truly been recognized as being able to yield powerful biocontrol enzymes [9]. They are called cellulases, glucanases, proteins, and chitinases and commonly in cell wall enzymes (CWDE) [15]. Chitin and glucan are important components in their cell wall because they are deposited on different glycoproteins [16].

B. Subtilis Polyketides

Among the included microorganisms, polyketides (PKs) are the most popular from the human health sector for their various activities, including anti-bacterial, immune suppressants, antitumor and many other anti-immune agents. The most common PKS species in the subtilis group. They were developed from Azol CoA preursors malonate and methyl malonate. Their biosynthesis is dependent on modfunctional polyketide synthases (PKSs). Their assembly stayed first extracted from fatty acid synthases (FASs), which share similarities with elastic rocks, precursors and general construction [17]. PKS is made up of a series of expansion modules, surrounded by the opening and disconnecting modules [18]. The activation block is collected of 2 domains: the acyltransferase (AD) domain, which beginners and promotes the binding of a monomer substrate to the domain of acyl carrier's protein (ACP). ACP then functions as the second instructional domains found in the next extension block. This domain, β-ketosyl synthase (KS), triggers a chain elongation feedback caused by a decarboxylative classene dioster capacitor. These subtypes of ketoerection (KR), cardiac output (DH), or enolase deficiency (ER), appear before the stress response. This mutation greatly enhances the formation and diversity of mature PKs [18]. Finally, a cleavage block containing an additional domain of the deregulation (TE) enhances PK release [17].

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Thiotemplate Nrps – Lipopeptides:

Lipopeptides are often synthesized by the incorporation of AA residues into the NRPS, either in active or inactive form. Like BKS, the NRPS has a functional structure that works by opening, expanding, and configuring modules. Each block is divided into intermediate domains, and its network domains and carriers are slightly different from those of PKS. This biomarker, begins with a dispute domain that binds and AA monomer is phosphorylated to the aminoacyl adenylate precursor [19]. The intermediary is then connected to the protein peptide carriers or the deooxygenation dominion (T domain or PCP) via a diastereomer bond. PCP deeds as a channel and confirms the interaction between aminoacyl and the formation of acyl peptide through the C-N bonding generator domain (C domain). The elimination block contains a diastereose (TE) domain, which promotes the proclamation of the concluding peptide acyl chain [20]. Expansion modules can be provided by the downstream domain such as the cyclic domain (cy), the epimerization domain (e) and the methyl purview. Those domains can mimic the peptide growing chain, leading to the formation of various mature molecules [21]. Because LP biosynthesis pathways are highly variable, the LPs produced are quite different. There are four main families: kurstakins, survivants, iaturins, and fungicins [22]. All intimate dividends similar basic topographies depending on the type and composition of the fatty acid tail or peptide tail of the three families of LP [23].

Volatile Inorganic Compounds (VICS):

Mixed components of the various elements made up of microorganisms are made from basic dietary items mainly CO, CO_2, H2, H_2S, HCN, NH_3, NO and N_2. Compounds containing N_2 are mostly produced by bacteria from the upper layers of agitated sediments. Nitric oxide is generated in large amount in this process by NO reductase or NO synthase [24]. Compounds containing VIC found in Group *B. subtilis* have a wide spectrum of antimicrobial properties e.g NO can cause systemic acquired resistance (SAR) in plants against viruses like *R. solanacearum* [25]. From the catabolism of the amino acid L-aspartate , ammonia is well known to be highly potent against Oomycetes such as *Pythium* spp [26,27]. By blocking metal containing cytochrome c oxidase- activating enzymes in the respiratory chain, HCN, generated from glycine catabolism has direct anticancer action against aerobic microorganisms.

Different VIC_s such as H_2S or H_2 are produced germs under low humidity deep in the soil. These molecules can act as antimicrobial metabolites and electron acceptors. *B. subtilis* can generate hydrogen sulphide as a by-product of L-cysteine and L-methionine catabolism by direct removal of L-methionine or transamination followed by constipation reduction [28]. It has antifungal action against phytopathogen such as *Aspergillus niger* or *Penicillium italicum*or as well as food-borne pathogens or human pathogens. Surprisingly, it is also thought to function as a defence mechanism against viruses [24]. Amonia, it turns out, boost both G^+ and G^- resistance to other bacteria [12].

Volatile Organic Compounds (VOCs)

High vapour pressure, low molecular mass (100-500 Da), lipophilic moiety, low boiling point are all and having fewer than 20 carbon atoms are all characteristics of volatile organic molecules. These characteristics allow simple evaporation and long distance dispersion, both of which are advantageous in a complex matrix such as soil [24]. The availability of nutrients, oxygen, soil moisture, temperature, texture, the physiological condition of microorganisms, pH, and architecture are all important variables in their production by soil- borne bacteria and diffusion [25]. The bulk of VOC_s are produced as a result of glucose oxidation, which includes glycolysis and following cycles like the tricarboxylic acid cycle (TCA). However, they can also be produced by a variety of different processes, including fermentations, terpene synthesis, aerobic heterotrophic carbon

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metabolism, Sulphur reduction and AA degradation [26]. Five types of VOC_s may be differentiated based on prior evaluations provided in [27]: terpenoids, fatty acids and derivatives, metalloid or halogenated containing VOC, Sulphur containing VOC_s and nitrogen containing VOC_s. In the VOC 2.0 database almost 2,000 compounds generated by nearly 1,000 species of microbes have been recorded. According to this database, fatty acid derivatives (alkanes, aldehydes, alcohols, alkenes, acids and ketones) account for about 70% of bacillus VOC_s, followed by Sulphur and nitrogen containing compounds.

The Embden-meyerhof (glycolysis), Entner-Doudoroff, heterolactic and homolactic fermentation pathways provide the majority of the precursors for volatile fatty acids and their derivatives. Under anaerobic circumstances [28], bacteria like *B. subtilis* ferment pyruvate to make ketone molecules like 2, 3-butanedione or acetoin (3-hydroxy-2-butanone). Microbes also employ other intermediates from fatty acid biosynthesis or oxidation as precursors, which are then decarboxylated or reduced to produce VOC_s [28]. They not only supply necessary hydrocarbons, but also fatty acid derivatives. Several amino acids can be oxidatively deaminated to produce aldehyde, ketone or alcohol volatiles. The breakdown of L-tyrosine or L-phenylalanine, for example can be the first step in the production of aromatic volatile chemicals like benzene or its carbohydrate derivatives. Finally, bacteria can produce benzenoid via the shikimate pathway, which results in the production of a natural precursor of aromatic aminoacids known as chorismate [29]. Benzenoid volatiles can also be produced by the degradation of intermediates from aromatic amino acids or shikimate pathway [30].

The most frequent kind of VOC produced by bacteria is volatile fatty acids and their derivatives, which account for up to 87% of known antimicrobial VOCs produced by *B. subtilis*. They are classified as either carbohydrates (alcohols, acids, esters, aldehydes, ketones, furans, benzenoids and lactones) or hydrocarbons (alkenes, alkanes and alkynes). The most common sub- category is benzenoids, which is followed by alcohols, aldehydes, ketones, alkanes and acids. Even though benenoids are a separate category, they are also fatty acid derivatives since the vast majority of antimicrobial benzenoids volatiles generated by B. subtilis have a benzene core connected to fatty acid derivatives.

Benzenoids come in a wide variety of forms, some of which are connected to carbohydrates chains containing nitrogen, Sulphur or both. The majority of these antimicrobial volatiles exhibit fungicidal properties, although several have also been identified as antibacterial or nematicidal. Their mechanism of operation is rarely described in detail. For example, following exposure to *B. subtilis* VOC [31], morphological defects on fungal and bacterial cells have been observed. Volatiles like 2, 3- butanediol and 1, 3-butadiene have also been shown to alter the expression of genes associated to *Pactobacterium carotovorum* and *R. solanacearum* pathogencity [32]. Fatty acids volatile have a variety of biological roles in addition to their direct antibacterial properties.2- butanone and Acetoin, for example, have the capacity to activate produce stress tolerance or plant defenses in plants, both of which enhance plant development. *B. amyloliquefaciens*, *B. velezensis* and *B. subtilis* strains are mostly responsible for their production [12].

Terpenes and their derivatives (sometimes called terenoids or isoptrenoids) are among the most common secondary metabolites discovered in biological systems [33]. Isopentenyl pyrophosphate (IPP) and its allylic isomer, dimethylallyl pyrophosphate are the two major precursors (DMAPP). The deoxy-xylulose phosphate pathway (DOXP), which starts with pyruvate and glyceraldehyde-3-phosphate derived from glucose metabolism, also produces IPP and DMAPP. Isoprene molecules can be used

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to make terpenoids [34]. Isoprene is not produced via the MVA or DOXP routes in B. subtilis, but it might be a result of the methylerythritol phosphate (MEP) pathway, as it is in plant systems [35].

Conclusions

B. subtilis produces a large spectrum of resistant compounds with a variety of biological activities. Because of their wide variety of anti-food or phytopathogenic plants, as well as their history of safe food usage, *B. subtilis* has piqued industrial and ecological interest. The most recent information on AMC's well known group B. Based on their metabolic routes (e.g. RPs, PKs, NRPs, volatiles) and the chemical environment, B. subtilis offers an unstable framework for differentiation

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