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Bacillus sp.: Potential Biotechnological Applications.

Isabel Cristina Rodríguez Luna[1], Alejandro Sánchez Varela[2]*
[1]*Laboratorio de Biotecnología Genómica, Centro de Biotecnología Genómica, Instituto Politécnico Nacional, Boulevard del Maestro S/N, Esq. Elías Piña, Colonia Narciso Mendoza, CP 88710, Reynosa, Tamaulipas, México.*
[2]*Laboratorio de Biotecnología Genómica, Centro de Biotecnología Genómica, Instituto Politécnico Nacional, Boulevard del Maestro S/N, Esq. Elías Piña, Colonia Narciso Mendoza, CP 88710, Reynosa, Tamaulipas, México.*

ABSTRACT: Bacillus sp. comprises a group of Gram-positive bacteria containing meso diaminomelic acid and peptidoglycans in their cell walls. They are rod-shaped and generally form endospores. This genus is widely distributed in the environment and is a major food contaminant. Human pathogens such as Staphylococcus aureus exist, which, while part of the human body's microbiota, can also cause infections, ranging from mild to severe, including sepsis and pneumonia. Furthermore, a wide range of biotechnological and pharmaceutical applications have emerged from the genus Bacillus, including human and animal probiotics (Hong et al., 2005), biological control products and applications for plant protection, enzyme production and synthesis, antimicrobials, food preservatives that improve food quality and safety, pharmaceuticals, bacteriocin production, and agents for bioremediation and microbial biodegradation. Bacillus safensis is a source of industrially important enzymes such as amylase, lipase, protease, cellulase, chitinase, inulanase, β-galactosidase and keratinase, as well as various other applications.

Keywords - Bacillus, biotechnological, enzymes, bioremediation, biodegradation.

I. INTRODUCTION.

Bacillus sp. comprises a group of Gram-positive bacteria containing mesodiaminomelic acid and peptidoglycans in their cell walls. They are rod-shaped and generally form endospores. This genus is widely distributed in the environment and can be isolated from food, such as milk, through the presence of Bacillus spores in pasteurized milk, leading to recontamination, as is the case with Bacillus thermolactis, among other species. Some human pathogens include Staphylococcus aureus, a Gram-positive bacterium that is part of the human microbiota. However, it is one of the most important pathogenic bacteria, acting in a wide range of infections, including osteoarticular infections, endocarditis, sepsis, and pneumonia [1] [2]. On the other hand, a wide range of biotechnological and pharmaceutical applications have also emerged that have been attributed to the genus Bacillus, including human and animal probiotics, products and applications for biological control of plant protection products, production and synthesis of enzymes with biotechnological applications in food and pharmaceuticals, as well as agents for bioremediation and microbial biodegradation [3].

Bacillus safensis is a source of industrially important enzymes such as amylase, lipase, protease, cellulase, chitinase, inulanase, β-galactosidase, and keratinase. Bacillus phytases are enzymes belonging to the phosphatase subfamily. This group of enzymes is classified into alkaline phosphatases, high and low molecular weight acid phosphatases, and protein phosphatases. They are differentiated by their optimal pH for catalyzing the hydrolysis of ester bonds, their affinity for metal cations, their substrate specificity, and possibly their reaction mechanisms [4].

Currently, food biopreservation is a practice considered among the sustainable solutions for extending shelf life and improving food safety. It is important to consider using safe, natural, or controlled microbial cultures or their antimicrobial compounds. They prevent the growth of pathogens and contaminating microbes, and suppress the formation of unwanted metabolites that affect food quality [5] [6].
In food preservation, bacteriocins are considered notable candidates for shaping the food microbiome. These peptides, synthesized by ribosomes, can prevent the colonization of specific microbes, typically those closely related to the bacteriocin producer [7].

In addition to bacteriocins, Bacillus species produce other secondary metabolites such as polypeptides, terpenes, siderophores, and other synthesized ribosomal and non-ribosomal peptides. The genus Bacillus is known for the production of biosurfactants, including cyclic lipopeptides such as lichenisin, bacillomycin, fengycin, and surfactin [8]. Minerals and heavy metals, such as cyanide, are present in soil and water. Mining, mineral processing, electroplating, and the plastics industry contribute significantly to cyanide pollution in the environment. Bacillus sp. can degrade cyanide into less toxic products, as these microorganisms can use cyanide as a nitrogen source, producing byproducts such as ammonia and carbon dioxide [3].

II. FUNCTIONAL ENZYMES

Bacillus Nitrogenases. The function of these enzymes is to reduce the N2 molecule, and they have two components: Component I, or dinitrogenase, possesses a cofactor that associates iron and molybdenum (FeMo), called a heterotetramer, which forms part of the active site. The Fe-M protein, where the metal (M) can be molybdenum, vanadium, or iron, is an α2 β2 tetramer composed of 30 iron atoms and two of the corresponding metal in two forms: cubic or P-type packing (8 Fe, 7S2), and the Fe-M cofactor. This protein is inactivated by oxygen [10]. Component II, or dinitrogenase reductase, transfers electrons to Component I with the consumption of ATP, and Component I subsequently donates the electrons to the substrate. The enzyme includes a cofactor, which is where the reaction with the substrate takes place. It can be permanently associated with the enzyme, in which case it is called a prosthetic group, or transiently associated as a co-substrate. As a result of their simultaneous activity, nitrogenases reduce molecular nitrogen and carry out nitrogen fixation processes, converting it into ammonia that is easily assimilated by plants [10]. Bacillus phytases are enzymes belonging to the phosphatase subfamily. They comprise a group of enzymes classified as alkaline phosphatases, high and low molecular weight acid phosphatases, and protein phosphatases. These differ in their optimal pH for catalyzing the hydrolysis of ester bonds, their affinity for metal cations, their substrate specificity, and possibly their reaction mechanisms [4].

Bacillus safensis strains are a source of a wide variety of enzymes with broad applications. Bacillus safensis is a source of industrially important enzymes such as amylase, lipase, protease, cellulase, chitinase, inulanase, β-galactosidase, and keratinase. A Bacillus safensis strain with good production of bacterial lipases has been reported [11]. Keratin consists of fibrous proteins found in hair, feathers, wool, nails, horns, and other epithelial coverings. These proteins are rich in beta-helices linked by cysteine bridges. Keratin substrates are considered a major contributor to contamination due to their recalcitrant nature, resulting from their stable structure through hydrogen bonds, disulfide bonds, cysteine bridges, and hydrophobic interactions. The development of enzymes through microbiological methods, specifically the hydrolysis of keratin-containing substrates into soluble proteins and amino acids, could be a highly attractive, economical, and straightforward method for producing valuable products. Keratinases (EC 3.4.21/24/99.11) are a class of proteases capable of breaking various bonds in keratin-containing substrates. There are primarily serine or metalloproteases that possess the ability to degrade structures containing keratin proteins. Keratinolytic activity has been reported in various microorganisms, including fungal species and actinomycetes, with enzymes produced in solid-state and submerged fermentations. There are also reports of activity in bacterial strains, such as Bacillus safensis, through mutagenesis upon exposure to ultraviolet light. Keratinolytic enzymes have applications in the detergent, medical, cosmetic, textile, and skin care industries, in pesticide degradation, and in the biodegradation of biofilms, adhesives, etc. [12]. Strains of B. safensis (MS11 and JUCHE1) were isolated from Mongolian desert soil and whey, respectively [13] [14]. A thermostable hydrolase (β-galactosidase) was isolated and characterized from B. safensis JUCHE 1, and its production process was tested via fermentation using different carbon sources [15] [16]. B. safensis DVL-43, isolated from a soil sample from Haryana (India), produces a new hydrolase (lipase) that is stable in organic solvents and is readily applicable for the synthesis of methyl laurate from lauric acid [17]. Other strains show potential for lipase production [11] [18].

Oxidoreductases are the largest class of enzymes applied in biotechnology. They are responsible for the oxidation-reduction reactions used in the interconversion of functional groups [19] [20]. This biocatalysis is clean and a greener alternative to traditional methodologies, reducing the use of solvents and toxic reagents [21]. This occurs naturally or can be engineered using microorganisms that can be used in free, immobilized, or whole-cell form. Each approach has advantages and limitations; processes using free enzymes are usually regio-enantio-selective, but these generally require the addition of cofactors [22].

II.1 BIOTECHNOLOGICAL APPLICATIONS OF BACILLUS SPECIES

Food biopreservation is currently considered a sustainable solution for extending shelf life and improving food safety. It is important to manage microbial cultures that are safe, natural, or controlled, and to use antimicrobial compounds [5] [6]. They prevent the growth of pathogens and contaminating microbes and suppress the formation of unwanted metabolites that affect food quality.

In preservation, bacteriocins are considered notable candidates for shaping the food microbiome [7]. These peptides, synthesized by ribosomes, can prevent the colonization of specific microbes, typically those closely related to the bacteriocin producer. There are bacteriocins that are commercially used for food safety applications such as: Nisin (Nisaplin; Danisco, Dupont), NisinA/Z and NisinA/Z P (Handary), Chrisin (Chr. Hansen), Pediocin PA-1 (ALTA 2351/2341; Kerry Group), Fargo 23 (Quest International) and Canocyclin A (Micocin, Griffith Foods) [23] [24] [25]. Bacillus sp. are Gram-positive, rod-shaped bacteria that form endospores and are widely distributed in the environment, including soil, water, food, air, and the intestinal tract of arthropods and mammals. Many Bacillus strains are generally recognized as safe by the FDA. Some Bacillus species can also produce various antimicrobial substances, bacteriocins, and antibiotic lipopeptides.

Based on post-translational modifications, bacteriocins can be divided into two classes. Class I bacteriocins, or lantibiotics, are small peptides (≥ 5 kDa) containing serine, threonine, and cysteine amino acid residues that have undergone post-translational modifications. Class II bacteriocins are small linear peptides (≥ 10 kDa) without post-translational modifications. Circular bacteriocins have recently been reclassified to class I of bacteriocins, due to a post-translational circulation created by covalent bonds between the functional group between the N and C terminal amino acids [25]. Circular bacteriocins are subdivided into two groups based on their biochemical characteristics. Group I primarily consists of cationic peptide residues and has high isoelectric points (pI > 9), while Group II peptides exhibit increased hydrophobicity, acidic residues, and lower isoelectric points than Group I (pI < 7). This mode of action involves direct interaction of circular bacteriocins with the target bacterial cell membrane. This leads to increased cell permeability, causing ion leakage. As a result, the membrane potential is altered, leading to cell death [26]. In addition to bacteriocins, Bacillus species produce other secondary metabolites such as polypeptides, terpenes, siderophores, and other synthesized ribosomal and non-ribosomal peptides. The genus Bacillus is known for the production of biosurfactants, including cyclic lipopeptides, such as lichenisin, bacillomycin, fengycin, and surfactin [27].

Bacteriocins produced by Bacillus species have the potential to be used in various biotechnological applications, including medical, veterinary, and food preservation applications. For example, the bacteriocins CAMT2, bacthuricin F103, and bacicyclicin XIN-1, produced by B. amyloliquefaciens ZJHD3, B. thuringiensis, and Bacillus sp. Xn1, respectively, have demonstrated anti-Listeria monocytogenes activity in pork [28], ground beef [29], and skim milk [30]. Additionally, the bacteriocin BpSl14, produced by Bacillus safensis, stimulates apoptosis in human A549 lung carcinoma cells, preventing their proliferation [31]. Antimicrobial compounds, secondary metabolites, and a new bacteriocin called safencin E were purified and identified from Bacillus safensis APC 4099, isolated from bees [32].

II.2 BIOREMEDIATION AND MICROBIAL BIODEGRADATION

Cyanide is a compound produced by living organisms, including bacteria, fungi, algae, and plants, as a defense mechanism. However, these cyanide concentrations are negligible compared to those produced by anthropogenic activities. Mining, mineral processing, electroplating, and the plastics industry contribute significantly to cyanide pollution in the environment [33]. Many tons of sodium cyanide have been produced over decades and used in the mining industry for gold extraction. Cyanide is highly toxic to living organisms [34]. Cyanide present in wastewater not only poses a threat to aquatic organisms but also to organisms that use nearby water sources. Most wastewater ends up in wastewater treatment plants (WWTPs), where biological processes are employed for bioremediation. The presence of cyanide in wastewater will cause the organisms normally used in WWTPs to be susceptible to cyanide toxicity, rendering the biological stage of WWTPs useless [35] [36]. The treated water from wastewater treatment plants (WWTPs) contaminates drinking water resources, as it is typically discharged into rivers, ultimately causing ecological and environmental pollution. Various treatment methods have been developed to remediate cyanide-containing wastewater. However, these chemical and physical methods require considerable capital investment and generate excess sludge that requires further treatment. Biological treatment of free cyanide in industrial wastewater has proven to be a viable and robust method for treating cyanide-containing wastewater. Several bacterial species, such as Bacillus sp., can degrade cyanide into less toxic products, as these microorganisms can use cyanide as a nitrogen source, producing byproducts such as ammonia and carbon dioxide. These bacterial species secrete enzymes that catalyze the degradation of cyanide into various end products [36]. In a bacterial consortium containing several strains of Bacillus safensis isolated from a wastewater electroplating process, it showed a high tolerance to free cyanide [37].

III. CONCLUSIONS.

The genus Bacillus is widely distributed in the environment, in soil and water, and is one of the main food contaminants. Human pathogens such as Staphylococcus aureus exist, which, although part of the human body's microbiota, can also cause infections, ranging from mild to severe, such as sepsis and pneumonia. There are phytopathogenic species of the genus Bacillus, as well as others considered beneficial, from which biotechnological applications have emerged, such as enzymes, food preservatives, probiotics, and bacteriocins, and pharmaceutical applications as antimicrobials, surfactants, and biodegraders of mineral contaminants and heavy metals.

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DISCLOSURE OF CONFLICT OF INTEREST.

The authors declare that there is no conflict of interest.

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Isabel Cristina Rodríguez Luna[1], Alejandro Sánchez Varela[2]*

[1]Laboratorio de Biotecnología Genómica, Centro de Biotecnología Genómica, Instituto Politécnico Nacional, Boulevard del Maestro S/N, Esq. Elías Piña, Colonia Narciso Mendoza, CP 88710, Reynosa, Tamaulipas, México.

[2]Laboratorio de Biotecnología Genómica, Centro de Biotecnología Genómica, Instituto Politécnico Nacional, Boulevard del Maestro S/N, Esq. Elías Piña, Colonia Narciso Mendoza, CP 88710, Reynosa, Tamaulipas, México.

**Corresponding Author: asanchezv@ipn.mx*