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BACILLUS SPECIES: AN EXCELLENT FERMENTER OF LEGUMES AND OIL SEEDS

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ABSTRACT

Bacillus species is generally active in the fermentation of legumes and oil seeds due to its highly efficient protein system and adaptable metabolism; this makes it relevant for use as probiotics or feed additives where their heat-stability can ensure survival in food matrix or enable long term storage at ambient temperature. In this review, the metabolism of *Bacillus* species in the fermentation of legumes and oil seeds is being observed alongside with its bio-characterization and phenotypic identification. Oil seeds and legumes are very high in protein component, hence, the fermentation of oil seeds and legumes into flavoring condiments by solid state fermentation results from extensive hydrolysis of protein and carbohydrate components as well as lipids, which are mostly used up by these fermenting microorganisms for energy. These proteins are broken down into its main components (amino acids and peptides) after fermentation has taken place likewise carbohydrates (passing through sugar metabolism later converting to organic acids, alcohols and carbonyl compounds) which are present in the resulting metabolite as a result of the microbial activities. Certain metabolites are produced by *Bacillus* fermenters in the cause of the fermentation; some of which contain certain antibiotics which have been demonstrated to exert antimicrobial activities towards various pathogens, antioxidants which have the ability to prevent risk of infections by protecting cells against harmful oxygen radicals thereby stabilizing cellular membrane, enzymes that possess antithrombolytic activities that can be supplemented with food to be consumed or taken as medication to improve or prevent cardiovascular conditions in humans and other humanly beneficial properties which can be applied in various sectors of life ranging from individual use to industrial use.

KEYWORDS: *Bacillus*, probiotics, hydrolysis, condiments, metabolites.

INTRODUCTION

The microbial interaction during the traditional fermentation of African condiments is determined by the microbiological status of the raw materials, utensils, handlers and production environment which can vary from one community to another and from one processor to another. Various microorganisms grow and enrich the fermentation system of vegetable proteins. Hence, these complex metabolic activities result in production of flavor compounds, metabolites as well as some other technologically useful compounds.

The genus *Bacillus* is a large, heterogenous collection of Gram-positive or Gram-variable spore forming, aerobic or facultative anaerobic bacteria. They can either be obligate aerobes or facultative anaerobes. Cultured species test positive for enzyme catalase if oxygen has been used or present (Favaro *et al.*, 2016). They are ubiquitous in nature and sporulate, therefore, resistant to heat, cold, radiation, desiccation and disinfection. Hence, they are difficult to eliminate from materials and are a frequent source of contamination (Christie and Setlow, 2020). *Bacillus* have a long history of consumption (Mohammed *et al.*, 2014) and safe use as probiotics (El Shagabee *et al.*, 2017), neutraceuticals (such as vitamins) and carotenoids and have been used for the synthesis of several health supplements. *Bacillus* species are typically found in non-dairy products and are important in diets of lactose intolerant individuals. *Bacillus* offer a potential for industrial applications, being commonly found in the Asian and West African *Bacillus* fermented foods (BFFs), based on soybeans and locust beans(Gopikrishna *et al.*, 2021).

The major fermenting microorganisms involved in fermentation of most vegetable proteins have been identified as proteolytic *Bacillus* species. They include: *Bacillus subtilis, B. megaterium, B. circulans, B. amyloliquefaciens, B. licheniformis, B. pumilus, B. sphaericus, B. coagulans, B. clausii, B. badius, and B.*

fusiformis (Isu and Ofuya, 2000; Sanni *et al.*, 2002). However, *B. subtilis* appears to be predominant of all other members followed by *B. coagulans*, *B. pumilus*, *B. licheniformis* and *B. clausii* (Jezewska-Frackowiak *et al.*, 2018). Probably, the spores of all *Bacillus* species can germinate in the gastro-intestinal tract and undergo limited rounds of proliferation before being shed in feaces (Cartman *et al.*, 2008; Tam *et al.*, 2006). They share sporulation ability, forming one oval endospore per cell. *Bacillus* probiotics have been shown to temporarily reside as symbiotic organisms within the host (Dong *et al.*, 2009). The rationale for their beneficial use is based largely on heat-stability of the spore ensuring that the product can be incorporated into foodstuff and animal feeds as well as in liquid formulations (Pepin *et al.*, 2004).

Seeds of legumes may account for up to 80% of dietary protein. Their cooked forms are eaten as meals and commonly used in fermented forms as condiments to enhance flavors of foods (Achi, 2005b). Legumes and oil seeds have constituted significant proportion of the diet of many people. The major components of legumes and oil seeds responsible for condiment production through fermentation are carbohydrates, proteins and fats. As a result, the organisms in charge of fermentation must be able to utilize and hydrolyze these components by degradation, fat oxidation, glycolysis and other reactions to produce primary metabolites such as amino acids, short chain peptides, fatty acids, nucleotides *etc*. These primary metabolites can be utilized by microorganisms to develop and generate secondary metabolites which can then be broken down further to produce volatile compounds.

Fermentation of oil seeds and legumes into food condiments

"Iru" and "ogiri" are two most popular indigenous fermented condiments produced from oil seeds and legumes in Nigeria (Omafuvbe *et al.*, 2004); with different names among the different ethnic groups and different varieties of substrates (Ibeabuchi, 2014). Legumes and oil seeds are fermented by allowing the microorganisms to act on them through enzymatic activity to yield condiments in extensive hydrolysis of carbohydrates and protein components (Ibeabuchi *et al.*, 2014). The fermentation is usually carried out in a moist solid state, involving contact with appropriate inoculum of assorted microorganisms and is accomplished by the natural temperatures of the tropics.

The oil seeds and legumes (such as African locust bean, castor seeds, fluted pumpkin seeds, melon seeds *etc*) serving as the raw materials will be sorted to remove bad seeds and unwanted materials and then boiled for a long period of time (about 8-12 h) depending on the raw material used to soften it. The seeds with firmly attached seed coats will be dehulled by slightly pounding with a mortar and pestle, so the seed coats will be washed out of the seeds and the seeds cooked again for about 16 h and the hot water removed. Then, the seeds or cotyledons will be spread into a calabash tray, covered with wooden tray and wrapped in sacks or banana leaves as the case may be; this is in order to keep the system warm at ambient temperature for 4 days in order to ferment. The end product of this fermentation is our food condiment. This may follow further treatment for preservation depending on the physiological requirements of the substrate and how it is wanted by the consumer.

Effects of fermentation on the major components of legumes and oil seeds

The main compositions of legumes and oil seeds which are subjected to degradation during fermentation include:

Carbohydrates- Certain carbohydrate degradation enzymes such as fructofuranosidase, galactanase, and glucosidase are produced by *Bacillus* species (Kiers *et al.*, 2000) and play active role in the hydrolysis of oligosaccharides and indigestible carbohydrates such as raffinose, starchyose and arabinogalactan which are abundant in most legumes (Omafuvbe and Kolawole, 2004) to produce compounds that are readily digestible by human. The nutritional significance of hydrolysis of oligosaccharides is evident in the drastic reduction of the level of indigestible carbohydrates (Nurudeen and Princewill, 2019). Microorganisms produce acids, alcohols and other flavor substances through polysaccharide decomposition (Kashyap *et al.*, 2001). Microbial polysaccharide metabolism involves amylosis, cellulose decomposition and pectinolysis into molecules ready for absorption and utilization (Xu *et al.*, 2020). Microbial amylases hydrolyze carbohydrates into sugars that are digestible by humans; galactanases soften the texture of the seeds and liberate sugars for digestion, sucrases also reduces the total flatus factor of legumes and oil seeds (Nurudeen and Princewill, 2019), starch decomposition is catalyzed by amylase, pectinase also play its role by decomposing pectin (which is widely found in plants, bacteria, molds and yeasts) while the fermented product contains an increased amount of resistant starch (Asencio-Granu *et al.*, 2020). All these enzymes are secreted by *Bacillus* species while degrading carbohydrate components during fermentation of legumes and oil seeds. Polysaccharide decomposition produces alcohols, acids, and other flavor substances (Kashyap *et al.*, 2001). Monosaccharides are converted into pyruvate through sugar metabolism and are further converted into some organic acids, alcohols and carbonyl compounds and other flavors including ethanol, acetaldehyde, acetic acid and diacetyl (Bartowsky and Pretorius, 2009).

Proteins- Proteolytic activity has been found to steadily increase with increase in fermentation period (Ogueke and Aririatu, 2004) and is a major biochemical activity that takes place in fermentation of legumes and oil seeds due to origin of the plant (Ghosh *et al.*, 2013). Metabolic and hydrolytic activities of the *Bacillus* species serve to breakdown the protein into amino acids (Ghosh *et al.*,

2013) with increasing population of *Bacillus* species from the beginning of fermentation process. Due to high level of hydrolytic enzyme production by *Bacillus* species have been reported to have one or more enzymatic hydrolytic properties during fermentation (Oguntoyinbo *et al.,* 2007). These enzymes interact with endogenous enzymes of the food itself to degrade proteins into free amino acids and small molecular peptides through ammonia conversion, decarboxylation and dehydrogenation processes, these amino acids are converted into certain aromatic compounds such as aldehydes, ketones, alcohols, acids, indoles, phenols *etc* (Olasupo and Okorie, 2018). *Bacillus* strains obtained from fermenting African oil bean seeds and locust beans have been found to produce glutamic acid and extracellular proteinases, which play active role in the fermentation process of these seeds (Nurudeen and Princewill, 2019). Amino acids produced because of protein metabolism are responsible for the gradual pH increase and leveling off towards 7.5- 8.0 (Achi, 2005b).

Lipids- The degradation of lipids mainly involves endogenous enzymes produced by the substrates and lipases produced by microorganisms which catalyze autoxidation and enzymatic oxidation. The significant lipolysis of legumes yields predominantly palmitic acids, stearic acids, oleic acids and linoleic acids (Ouoba *et al.,* 2003), octadecenoic acid and other fatty acids are also produced; all of which are further oxidized and degraded to produce linear aldehydes. When lipid is hydrolyzed by lipase, small molecules can further generate flavor compounds including fatty aldehydes, ketones, alcohols and more. Reduction in fat content is attributed to metabolism of *Bacillus* species in the processed product; the breakdown of lipids by lipase and their use as energy source for bacteria (Onwurafor *et al.,* 2014). Many reports confirm that vitamin levels are higher in fermented protein foods than in the raw materials, especially for riboflavin, thiamine, niacin, vitamin C and folic acid due to the metabolic activities of *Bacillus* species (Leejeerajumnean *et al.,* 2001). Food condiments made from vegetable proteins may be a good source of B-vitamins, but they are found to be deficient in ascorbate and some fat-soluble vitamins, which are lost during fermentation (Ogbonna *et al.,* 2001).

BIOTECHNOLOGICAL SIGNIFICANCES OF ACTIVITIES OF *BACILLUS* SPECIES

Antimicrobial Compounds Produced by *Bacillus* Species

In recent years, *Bacillus* species have been demonstrated to exhibit broad-spectrum activity against microbes (Chen *et al.,* 2019). *Bacillus* group has been reported to produce more than 45 antimicrobial molecules; some of these compounds are of clinical values, others are assayed *in vitro* to control food microbes and the remaining ones control plant diseases (Stein, 2005). These metabolites can be grouped majorly into two according to their biosynthetic pathways namely; the peptide antibiotics (also called antimicrobial peptides-AMPs) and the non-peptide based antibiotics (also known as the polyketides) (Puan *et al.,* 2023).

A. The peptide antibiotics- These are ribosomally synthesized and non-ribosomally synthesized peptides from the metabolites produced by the species of *Bacillus.*

i. Ribosomally synthesized peptides- They are widely distributed in nature and contain between 12 and 50 amino acid residues. They are synthesized by ribosomes and are typically cationic and exhibit great structural diversity (Marx *et al.,* 2001). They exist as either post-translationally modified or unmodified peptides (Wu *et al.,* 2023). AMPs synthesized by ribosomes include subtilin, bacthuricin and cerein, commonly referred to as bacteriocins as they display a high degree of target specificity against closely related bacteria (Chopra *et al.,* 2015). Because of their wide diversity, bacteriocins display different modes of action such as protoplasm vesicularization, pore formation or cell disintegration (Sumi *et al.,* 2015). They are generally bactericidal with some exceptions that exhibit bacteriostatic activities (Gautam and Sharma, 2009; Oladipo *et al.,* 2015). However, those compounds display diverse metabolic activities such as quorum sensing (QS) mediation, cell lysis or induction of genetic competence (Shafi *et al.,* 2017) and so on. Bacteriocin anti-microbial spectra of *Bacillus* species are broad expanding the search of bioactive molecules to other bacteriocin-producing genera (Pederson *et al.,* 2002; Oladipo *et al.,* 2014; Sumi *et al.,* 2015).

ii. Non-ribosomally synthesized peptides- *Bacillus* species strains produce several non-ribosomal compounds through a multistep mechanism including the selection and condensation of amino acid residues such as cyclic lipopeptide (iturin group) and macrolactones (surfactin, fengycins and plipastatins (Stein, 2005) by multienzyme thiotemplates. Large multi subunit enzymes play a key role in the synthesis of these peptides. The non-ribosomally synthesized peptides are assembled from among more than 300 different precursors. These peptides can be linear or cyclic and also contain cyclic branched structures containing hydroxyl group, L-amino acids nor D-amino acids. These peptides can be further modified by N-methylation, acylation, glucosylation or heterocyclic ring formation (Riccardo *et al.,* 2022).

In general, these AMPs are cationic and hydrophobic or amphiphilic, and the cellular membrane of bacteria, in most cases, is the main target for AMPs to exert antimicrobial activity (Zhang *et al.,* 2021). In addition to antibacterial and antifungal properties, *Bacillus*-derived AMPs have also shown antiviral, antitumor and immunomodulatory activities, making them another attractive alternative in recent years (Caulier *et al.,* 2019; Basi-Chipalu *et al.,* 2022).

Mechanisms of Action of antimicrobial peptides: Multiple models have been proposed with exact mechanism probably on the specific peptide, concentration and bacterium. Bacteria have also been shown to respond to antimicrobial peptides and even to evolve resistance to their toxic effects (Scott *et al.,* 2008). Different species of *Bacillus* produce bacteriocins and bacteriocin-like substances with different modes of action. Tochicin, lichenin (Pattnaik *et al.*, 2001), thuricin 439(Ahern *et al.*, 2003) and thuricin S (Chehimi *et al.*, 2010) have all been established to exert a bactericidal effect. In general, bacteriocins are cationic peptides that exhibit hydrophobic or amphiphilic properties, and in most cases, their primary target is the bacterial cell membrane. (Sirtori *et al.,* 2006). Several models have proposed that the mechanism of action of cationic peptides involves the formation of channels through which ions can pass, as well as the disruption of bacterial cytoplasmic membranes (Huang *et al.*, 2009; Palffy *et al.,* 2009). Killing of bacteria via formation of pores in the bacterial membrane requires 3 principal steps: (a) Binding to the bacterial membrane (b) Aggregation within the membrane and (c) Formation of channels. Channel formation leads to breakage of internal cell contents and consequently, cell death.

In addition, antimicrobial peptides must cross the negatively charged outer wall of Gram-negative bacteria, which contain lipopolysaccharides (LPS), outer cell wall of Gram-negative bacteria, which contains acidic polysaccharides. In many cases, specific metabolism of the target microbes provides critical conditions or pore formation (Palffy *et al.,* 2009).

Lipopolysaccharides readily bind to the bacterial surface layer and the local lipid organizational linkages on negatively charged fatty acids, ultimately restructuring the lipid bilayer and thus preventing cellular processes. The fatty acid moiety of lipopeptides contain β-hydroxy fatty acids with 14 carbon chain and inhibit different species of fungi but have only narrow antibacterial activity (Baindara *et al.*, 2013). Gaofu *et al*. (2010) reported that the antifungal mechanism of a new member of the surfactin family, produced by *Bacillus amyloliquefaciens* WH1 and named WH1-fungin, exhibits two types of antifungal actions. At high concentrations, it forms pores in the fungal cell membrane, while at low concentrations, it induces apoptosis. Additionally, it inhibits glucan synthase, leading to reduced callose synthesis in the fungal cell wall. WH1-fungin has also been found to bind to an ATPase on the mitochondrial membrane, thereby decreasing its activity in fungal cells. Iturins typically exert their antifungal effects through fungal membrane permeabilization. However, bacillomycin L, produced by *B. amyloliquefaciens* K103 strain, interacts with other intracellular targets (such as DNA) after disrupting the cell membrane (Zhang *et al.,* 2013). Furthermore, subtilisin produced by *B. subtilis* has been reported to kill target cells by depleting the transmembrane pH gradient component of the proton motive force and causing an efflux of intracellular ATP (Noll *et al.,* 2011).

B. Non peptide-based antibiotics: Non peptide-based antibiotics are also called the polyketides. *Bacillus* species secrete three antimicrobial polyketide groups and their variants (bacillaene, difficidin and macrolactin) and they exhibit antimicrobial activities by selectively inhibiting protein synthesis and may also have synergistic effects against pathogens (Chen *et al.,* 2019). Pinchuk *et al.* (2001) also reported that at least two antibiotics, one of which is amicomycin, produced by *B. subtilis* in a starch-based medium were responsible for anti- *Helicobacter pylori* activity. About 18 macrolactins from *Bacillus* species have been chemically described and some of them are considered to be potent antiviral and cytotoxic agents that also have antibacterial activity against *Staphylococcus aureus* (Romco-Tabarez *et al.*, 2006). Difficidin and oxydifficidin are also produced during fermentation of *B. subtilis* and this represent a class of antibiotics that are active against aerobic and anaerobic organisms (Baruzzi *et al.,* 2011). Hence, bioactive peptides are formed during fermentation by proteolytic microorganisms (De mejia and Dia, 2010).

Anti-oxidant Activities of *Bacillus* species

Bacillus fermented foods and condiments have been proven to possess various antioxidants activities. Antioxidants are substances (produced by microorganisms) that can prevent or slow down chemical reactions that may damage cells caused by free radicals such as nitric oxide, nitrous oxide and peroxynitrite (Ozabor *et al.*, 2020). The antioxidant activity of a biochemical compound corresponds to its capacity to delay or prevent the oxidation of a substrate, resulting from an imbalance between reactive oxygen species (ROS) production and their degradation by antioxidants. Several studies have reported the potentials of *Bacillus* species as biosurfactant producers such as lipopeptide type surfactants (Joshi *et al.*, 2013) which are amphiphilic cyclic peptides that are linked to a fatty acid hydrocarbon chain and belong to surfactin, iturin and fengycin families; synthesized by non-ribosomal peptide synthases without involving messenger RNA (Leclere *et al.*, 2005) and are found to possess specific biological activities such as antioxidant activities (Tabenne *et al.*, 2012; Ben Ayed *et al.*, 2015).

Carotenoids are antioxidants associated with a range of health benefits, including a reduced risk of cardiovascular diseases, (Fraser and Bramley, 2004; Rao and Rao, 2007) and carotenoid producing bacilli have also been found to be able to reduce symptoms of metabolic syndromes in rats (Cresenzo *et al.,* 2017). Species such as *Bacillus indicus* produces C30 carotenoids (Khaneja *et al.*, 2010; Perez-Fonz *et al.*, 2011) that have been bioavailable at all levels that are possibly superior to other common carotenoids such as astaxancin and lycopene (Sy *et al.,* 2013a). The stability and bioavailability of these *Bacillus* carotenoids have led to the introduction of a number of products worldwide (Kotowicz *et al.*, 2019).

Anti-thrombolytic Activities of *Bacillus* species

Bacillus species secrete various hydrolytic enzymes into their surroundings, and amylases and proteases are the most important enzymes widely utilized for various industrial applications (Schallmey *et al.*, 2004). All fermented foods made from legumes and oil seeds depend on the strong enzyme activities of *Bacillus* species. *Bacillus subtilis*, the primary fermenting agent of several fermented oil seeds and legumes, has been extensively studied for its ability to produce extracellular proteases that effectively prevent thrombosis. Among these, certain proteases exhibit strong fibrinolytic activity. Notably, nattokinase and bacillopeptidase-F are two enzymes with significant fibrinolytic effects, currently utilized as nutritional supplements and considered promising alternatives to conventional drugs for the treatment and prevention of fibrin-related thrombosis. Oral administration of nattokinase has been shown to enhance plasma fibrinolysis *in-vivo*. In Korea, *Bacillus* producing these enzymes were isolated from soy products such as *doenjang* (fermented soy paste), *ganjang* (soy sauce) and *cheongukjang* (fermented and boiled soy) (Yao *et al.*, 2020). Indonesian fermented "*terasi"* fermentation explores fibrinolytic capability of several microorganisms such as *Bacillus* species as naturally occurring bacteria in foods; increasing the knowledge regarding the health benefits of its consumption (Pinonthan *et al.*, 2024). Bacteria strains of *Bacillus subtilis* A26 (Agrebi *et al.*, 2009), *Bacillus amyloliquefaciens* (Wei *et al.*, 2011) and *Bacillus* spp. (Anh *et al.*, 2015) were recently adopted in the production of fibrinolytic enzymes. In addition, research of fibrinolytic enzymes from fermenting *Bacillus* in fermented legumes such as Chinese soy paste (Wei *et al.*, 2011), Indonesian red *oncom* and *Gembus Tempeh* (Afifah *et al.*, 2014), soybean paste (Lee *et al.*, 2001), Japan *tofuyo* (Syahbanu *et al.*, 2019) and several other traditional fermented foods from legumes and oil seeds are of interest in developing functional foods beneficial to public health (Yoon *et al.*, 2002).

Other significances include

1. **Enhancement of organoleptic properties**- *Bacillus* species contribute to the development of unique flavor and texture of foods during fermentation while producing peptides and amino acids from proteins and functional materials (Yao *et al.*, 2020). Proteases, for instance, released by the fermenting *Bacillus* species, produce peptides and amino acids from the protein components of the substrate (such as the low molecular weight peptides and amino acids), improve taste and flavor of the food condiment (Ogbonna *et al.*, 2001). The organoleptic properties of the fermented foods make them more important since it has a wider acceptance than the unfermented foods. Hence, they become more palatable as there will be improvement on the organoleptic properties, texture, aroma and flavor (Osungbaro, 2009).
2. **Provision of nutritional quality-** Improvement in the nutritional value and digestibility of foods has been associated with the actions of *Bacillus* bacteria in vegetable fermentation (Nout, 2009). The enzymes like amylase, proteases, lipases and phytates modify the primary food products through hydrolysis of polysaccharides, phytates, proteins and lipids (Adeyemi and Muhammed, 2008). The quantity of proteins and the content of the water-soluble vitamins increase, while the antinutrient factors (ANFs) in the foods decline during fermentation (Santos *et al*., 2008). This leads to increased bioavailability of minerals such as zinc, calcium, phosphorous iron and amino acids (Murwan and Ali, 2011).
3. **Food preservatives-** The increasing trend of limiting the use of chemical preservatives has generated interest in the use of natural alternatives. *Bacillus* has emerged as a promising bio-preservative due to its ability to grow efficiently on a large scale at low cost and to produce pH-tolerant bacteriocins with broad-spectrum activity, thereby extending the shelf life of foods (Hoang *et al.*, 2022). Recently, antimicrobial lipopeptide microcapsules prepared from the spray drying of the *B. amyloliquefaciens* ES-2 strain were tested as food additives (Wang *et al.*, 2014c). Subtilosin is an attractive food preservative alternative to nisin because of its efficacy against *L. monocytogenes* and other foodborne pathogens (Jung *et al*., 2008; Sutyak *et al.*, 2008). *Bacillus* species have been reported to inhibit and control these microorganisms. These antimicrobials can also be purified and applied in food matrix as natural-based preservatives to extend shelf-life of food products as they are considered safe for human consumption due to their antimicrobial and antioxidant properties (Cattelan *et al.*, 2013).
4. **Detoxification-** Enzymes produced by *Bacillus* species during fermentation of vegetable proteins hydrolyses macromolecules i.e. complex carbohydrates, proteins and fats, thereby enhancing bioavailabilty and digestibility of the fermented product compared to the unfermented substrate (Kiers *et al.*, 2000). Also, enzymatic degradation during fermentation reduces naturally occurring toxic components, allergens and antinutritional components in the raw substrate, thereby, transforming otherwise inedible, difficult to digest or potentially raw materials into palatable and culturally desirable food products that deliver essential nutrients, thus, contributing to a complex rich traditional dietary diversity, with important food security and sustainability implications (Parkouda *et al.*, 2009).
5. **Improvement of health-** Many of the *Bacillus* strains predominant in alkaline fermented foods are also used as probiotics. Some of these strains

include *B. cereus, B. clausii, B. coagulans, B. licheniformis, B. polyfermenticus, B. pumilus* and *B. subtilis*- all of which exhibit probiotic activities in both spores and vegetative forms. Fermented plant-based foods could be used as carriers for delivery of *Bacillus* probiotics into the mammalian systems (Hoa *et al.*, 2000). The influence of probiotics on human gut microbiome is well reported (Thursby and Juge, 2017; Kawai *et al.*, 2018) including that of *Bacillus* species. *Bacillus* strains stimulate antimicrobial, anticancer, antihypertensive, antioxidant, fibrinolytic and immunomodulatory activities *in vitro* and *in vivo*. These biological properties can be stimulated by metabolic processes of probiotics in alkaline fermented foods.

CONCLUSION

Bacillus species have unique properties, and they produce a number of metabolites: enzymes, antibiotics, vitamins and amino acids. Alkaline fermentation of plant-based foods such as legumes and oil seeds offer several sensorial, nutritional and health benefits. For example, guided selection of probiotic strains could be used to stimulate the *in situ* biofortification of plant-based foods through alkaline fermentation as a strategy to prevent deficiencies in nutrients such as vitamins, minerals and proteins.

Many *Bacillus* species can produce copious number of enzymes which are used in various industries such as in the production of alpha amylase used in starch hydrolysis and protease subtilisin used in detergents. Some others can synthesize and secrete lipopeptides (Nigris *et al.*, 2018; Paul *et al.*, 2021) and several other anti-microbial peptides (Rahman *et al.*, 2020).

Studies on *Bacillus*-derived antimicrobials have already yielded promising results in the field of human health. Therefore, *Bacillus* species can be used as a source of ideal therapeutic tools because of their broad specificity and specific and rapid killing activity against various pathogens. So, effective use of *Bacillus*-derived antimicrobials can be established in medical settings and various industries based on their modes of action, toxicities, and immunogenicities in humans.

Some species of *Bacillus* are newly discovered to be rich in antioxidants; producing riboflavin and C30 carotenoids. Besides providing a source of vitamin B2 and gastric stable carotenoids, increase in spore levels might confer long-term health benefits. Antioxidants ensure probable migration of bioactive compounds derived from foods, offering a healthier alternative while maintaining oxidative stability and sensory acceptance as well as ingestion of healthy foods containing functional ingredients. *Bacillus subtilis* is exploited in industry for some B-vitamin production in large scale fermentations, producing reasonable amounts for industrial purposes. For example, *B. subtilis* produces riboflavin extracellularly, therefore referred to as ideal cell factories for riboflavin production as they enhance riboflavin level enrichment in fermented foods.

The high protein contents in vegetable proteins including legumes and oil seeds have the potential of being utilized as a media for growing proteolytic and fibrinolytic microorganisms such as the members of the genus *Bacillus* to identify their strong activities and indicate their beneficial health importance. Therefore, proteolytic activities of *Bacillus* are prerequisite of their fibrinolytic activities. Studies about fibrinolytic protease enzymes of bacteria from fermentation products offer novelty and discovery of new proteolytic enzymes potential to be used in cardiovascular diseases treatment. Hence, the bacteria producing fibrinolytic protease enzymes applied in cardiovascular disease treatment are from *Bacillus* group (Hayatun *et al.*, 2020).

Finally, fermented legumes and oil seeds can help in management of lactose intolerance, lowering serum cholesterol level, improving uptake of nutrients, support health management and reducing need for antibiotics.

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