Antimicrobial compounds produced by *Bacillus* spp. and applications in food

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The *Bacillus* genus is a heterogeneous group of Gram-positive, facultative anaerobic, endospore-forming bacteria spread into the environment, even though soil is generally accepted as its natural reservoir. The ability to produce endospores allows *Bacillus* to withstand extreme environmental conditions as those occurring in food processing. *Bacillus* spp., particularly *B. subtilis*, are usually found in foods such as dry cured sausages, cheeses, traditional fermented milks, sourdough, etc. in which they cooperate with other microorganisms during fermentation, releasing amylases, lipases and proteases.

One of the main characteristics shared among *Bacillus* strains is the ability to produce a wide range of antimicrobial compounds active against bacteria and fungi. Even though microbial control exerted by these metabolites was demonstrated in plant environments, few reports focused their attention on how these compounds can interact with food microbiota.

One single *Bacillus* strain is often able to produce several types of molecules stable over a wide range of pH and temperature and partially resistant to enzyme treatments. These substances are usually protein- and peptide-based compounds such as enzymes, bacteriocins and lipopeptides. Thanks to their chemical characteristics and inhibitory spectrum, *Bacillus* producer strains could be applied during food processing as innovative tools for the control of food pathogens and spoilage microorganisms.

In this paper, current and potential applications of *Bacillus* strains in food environments are discussed, focussing on antimicrobial compounds contributing to improve food safety and quality.

Keywords; *Bacillus* antimicrobials; foodborne pathogens; food processing.

1. Introduction

Bacillus genus is made up of Gram-positive aerobic or facultative endospore-forming rod-shaped bacteria that includes both mesophiles and extremophiles. These microorganisms are metabolically chemoorganotrophs being dependent on organic compounds as sources of carbon and energy. In addition, their ability to form highly resistant endospores is the key for their successful colonisation of a wide variety of environments. Due to their wide ubiquity in nature and genetic and metabolic diversity leading the production of several antibiotics and enzymes, these bacteria have become increasingly interesting for different biotechnological applications ranging from the production of fermented foods to engineered industrial enzymes used in food and detergent industries [1].

Genome sequence of the laboratory strain *B. subtilis* 168, a tryptophan auxotrophic mutant, carried out by Kunst et al. [2], has opened new scenarios to explore the molecular biology and ecology of *Bacillus*.

Recently, the finding of compounds produced by *Bacillus* strains isolated from foods and active against pathogens led to suggest a possible role of this microorganism in the control of pathogens along food chain.

In this paper, we report on the most known antimicrobial compounds secreted by these microorganisms, their inhibition activities against food pathogens and their potential applications in food process technology.

2. *Bacillus* at a glance (taxonomy and metabolism)

Bacillus, established by Chon in 1872 [3], has undergone considerable taxonomic changes. In the 2nd edition of the Taxonomic Outline of Bergey's Manual of Systematic Bacteriology [4] phylogenetic classification schemes, accomplished mainly by the analysis of 16S rDNA sequence similarities, included in the family of *Bacillaceae* the genus *Bacillus* made up by 94 species. *Bacillus* species are historically clustered into six large groups based on numerous physiological, biochemical and morphological characters [1]. Group I, including *B. polymyxa* as a reference organism, comprises facultative anaerobic species that ferment a variety of sugars and have reasonably fastidious growth requirements in the form of vitamins and amino acids. These species secrete numerous extracellular carbohydrases such as amylases, glucanases including cellulases, pectinases and pullulanases. *B. subtilis* and its relatives, *B. amyloliquefaciens*, *B. licheniformis* and *B. pumilus*, belong to group II. These species differentiate into oval endospores that do not distend the mother cell. Most of these bacteria are regarded as strict aerobes but many strains have a limited ability to ferment sugars and grow well anaerobically in the presence of glucose and nitrate as a terminal

electron acceptor. Some species such as *B. anthracis*, *B. cereus*, *B. licheniformi*s and *B. thuringiensis,* true facultative anaerobes, secrete numerous extracellular enzymes including many commercially important amylases, *ß*-glucanases and proteases. Group III species are perhaps taxonomically the least defined and are rather physiologically heterogeneous. This group is based on *Brevibacillus brevis* which is a strict aerobe that does not produce appreciable acid from sugars and differentiates into an oval endospore that distends the sporangium. Other species in this group might include *B. badius* and "*B. freudenreichii*". Bacilli which differentiate into spherical endospores are allocated to group IV. This is a phylogenetically homogeneous group of species including *B. sphaericus*, the psychrophiles *B. insolitus*, *B. psychrophilus* and some other species. These bacteria are all strict aerobes distinguished from all other bacilli by the replacement of meso-diaminopimelic acid in the peptidoglycan of their cell walls with lysine or ornithine. In particular, *B. sphaericus* does not use sugars for growth, metabolizing acetate, arginine, glutamate and histidine as carbon and energy sources. Finally, most of classification studies have recovered the thermophilic bacilli, represented by *B. stearothermophilus*, as a separate group (group V). This includes a physiologically and morphologically heterogeneous collection of species with various metabolism pathways ranging from strict aerobes to microaerophilic types. The acidophilic thermophiles (group VI) have recently been allocated to the new genus *Alicyclobacillus* in which thermophily appears to have independently evolved in many lineages. Recent researches, based on a "pan-genomic" approach, support the division of *Bacillus* into further new genera and revealed unexpected groupings [5, 6] suggesting that the final picture of *Bacillus* taxonomy is still far to be drawn.

Bacillus species are an important source of fine biochemicals, antibiotics and insecticides. Moreover, the ability of *B. subtilis* and close relatives to secrete grams per litre of proteins directly into the growth medium and their well-proven safety have also made them prime candidates for the production of heterologous proteins. In fact, about two-thirds of the enzyme market (proteases, amylases, rennet substitutes, endonucleases, glucose-dehydrogenase and pullulanase) for industrial applications are produced by fermentation from *Bacillus* species. *B. subtilis* has been used for the production of nucleotides, sold as food flavour enhancers, amino acids (such as tryptophan, histidine and phenylalanine) and vitamins such as biotin, folic acid and riboflavin [7]. Although δ-endotoxins from *B. thuringensis* are the most known and used proteinaceous metabolites derived from *Bacillus*, recently, a large variety of antimicrobial peptides have been discovered in these bacteria. Some of these peptides can play a role in competence and in the de-repression of various stationary-phase genes involved in sporulation [8].

3. Antimicrobial compounds

Among bio-preservatives, more than 500 antimicrobial compounds have been described so far. *Bacillus* genus has been reported to produce more than 45 antimicrobial molecules; some of these compounds are of clinical value, others are assayed *in vitro* to control food microbes and the remaining ones control plant diseases [9, 10]. According to their biosynthetic pathway, these metabolites can be grouped into two different classes: the first class comprises ribosomally synthesized peptides, including bacteriocins whereas the second class comprises small microbial peptides synthesized enzymatically by non-ribosomal pathways.

3.1 Bacteriocins and bacteriocin-like inhibitory substances (BLIS)

Bacteriocins usually display a high degree of target specificity against related bacteria, although some of them have a wide spectrum of activity [11].

Based on the classification of Klaenhammer [12], several antimicrobial substances produced by *Bacillus* were grouped into bacteriocin Class I, including lantibiotic, gene-encoded peptides (<5 kDa) that contain lanthionine and/or methyllanthionine residues employed to form a ring through intramolecular post-translational modifications [13]. Within this class, lantibiotics are also classified into sub-groups A and B for their general structure, molecular weight and biological activity. Type A lantibiotic (2100-3500 Da; 21–38 amino acid residues) exhibits a more linear secondary structure and kills Gram-positive target cells forming voltage-dependent pores into the cytoplasmic membrane while type B includes globular and uncharged lantibiotics [14]. Type A includes subtilin, a 32-aminoacid pentacyclic lantibiotic (3320 Da; Figure 1A) produced by *B. subtilis* ATCC 6633, structurally similar to nisin, a bacteriocin approved for use as a food preservative (E234) in over 50 countries [15, 16]. Subtilin is stable to acid and heat treatment up to 121 °C for 30-60 min and inhibits a broad range of Gram-positive bacteria including other species of *Bacillus*. Recently, Parisot et al. [17] reported that subtilin shows a more complex mechanism of action, involving the binding to a specific target or "docking molecules", the membrane-anchored cell wall precursor lipid II also targeted by vancomycin, a glycopeptide antibiotic. The interaction with the lipid II "stabilizes" the formation of pores leading to antimicrobial effects at very low concentrations of bacteriocins.

The biosynthesis of subtilin by *B. subtilis* is dependent on the products of at least 10 genes, *spa*BTCSIFEGRK [18]. During the last decade, activation, self protection, export from the cytoplasmic membrane into the extracellular space and regulation of subtilin have been also elucidated [9].

Food applications of most of these peptides is limited by their sensitivity to proteases that can be prevented, as demonstrated by Bierbaum et al. [19] for Pep5, by a further ring structure.

Ericin S (3442 Da) and ericin A (2986 Da) are two lantibiotics produced by *B. subtilis* A1/3 with strong similarities to subtilin. Purified ericins (mainly ericin S) are active against a variety of bacteria, specially against *Clavibacter michiganensis*, the causal agent of tomato bacterial canker [20].

Unlike type A lantibiotic, the type B mersacidin (1825 Da) lantibiotic, produced by *Bacillus* sp. strain HILY-85,54728, exhibits a more globular structure due to the formation of four intermolecular thioether bridges [21]. The presence of intertwined rings without elongated linear stretches increases protease resistance in comparison with other *Bacillus* lantibiotics. Mersacidin exerts its antibacterial activity by the inhibition of cell wall biosynthesis; this compound forms a complex with the peptidoglycan precursor lipid II as demonstrated for subtilin [17]. Several works showed that this peptide successfully inhibited *in vitro* and *in vivo* the growth of Gram-positive bacteria including methicillin-resistant *Staphylococcus aureus* strains (MRSA) [22] as well as enterococci expressing the VanA vancomycin resistance phenotype. Mesarcidin like subtilin is produced in stationary phase under the regulation of the sporulation transcription factor SigH [23]. The structural gene (*mrs*A) and the genes for precursor, modification enzymes (MrsM and MrsD), transport protein (ABC transporter MrsT) and regulator proteins are organized in a 12.3 kb biosynthetic gene cluster on the chromosome of the producer strain [24]. Recently, sequence homology-based studies of proteins involved in the production of lantibiotics have allowed to detect two mesarcidin-like peptide lichecidins (3020 Da and 3250 Da) in the cell-free culture supernatant of *B. licheniformis* strains (ATCC 14580 and DSM 13, respectively) which exhibit antimicrobial activity against all *L. monocytogenes*, methicillin-resistant *S. aureus* and vancomycin-resistant *Enterococcus* strains [25].

Due to the structural features, two additional *Bacillus* lantibiotics, subtilosin A and sublancin 168 were classified into a separate group of lantibiotic [9]. Subtilosin A (3399.7 Da; Figure 1A) is an anionic macrocyclic peptide, without lanthionine and methyllanthionine residues, produced by *B. subtilis* ATCC 6633 and other *Bacillus* spp. strains as well as *B. amyloliquefaciens* [26, 27] retaining strong bactericidal activity against *L. monocytogenes* at high temperatures and within 2-10 pH values. Sublancin 168 (3877.78 Da), produced by *B. subtilis* 168, contains a single lanthionine linkage and two unusual disulfide bridges; this compound exhibits bactericidal activity mainly against Gram-positive bacteria including important pathogens such as *B. cereus*, *Streptococcus pyogenes* and *S. aureus* [28].

Class II of bacteriocins consists of heat-stable, membrane-active peptides without modified residues. This group includes pediocin-like bacteriocins such as coagulin (4612 Da), a protease-sensitive peptide produced by *B. coagulans* I4 [29]. Coagulin proved bactericidal activity against pathogens and food spoilage bacteria as *Leuconostoc*, *Oenococcus*, *Listeria*, *Pediococcus* and *Enterococcus*.

Not well characterized antimicrobial proteins are known as bacteriocin-like inhibitory substances (BLIS). BLIS from *B. cereus* as cerein 7A, 7B, MXRI and 8A have attracted special attention particularly due to their possible application as natural food biopreservatives [30-32].

B. thuringiensis, phylogenetically similar to *B. cereus,* is also able to produce BLIS such as tochicin (10.5 kDa) and the large family of thuricins [33] with a broad inhibitory spectrum. The well characterised thuricin S is heat stable, with a molecular mass less than 10 kDa that inhibits the growth of *L. monocytogenes, Salmonella enterica* and *Pseudomonas aeruginosa*; thuricin 17 [34] is inhibitory against other *Bacillus* spp. including *B. cereus* strains; thuricin H [35] is active against a wide range of Gram-positive bacteria such as *Bacillus* spp., *Geobacillus stearothermophilus, Listeria* spp*., S. aureus* and *Carnobacterium maltaromaticum.*

B. licheniformis 26L-10/3RA, isolated from water buffalo rumen, produced lichenin, a chromosomally encoded hydrophobic BLIS, under anaerobic conditions. This peptide, sensitive to atmospheric oxygen, heat stable, active over a wide pH range, retained its biological activity mainly against rumen microorganisms [36].

3.2 Non-ribosomal biosynthesized peptides

Bacillus spp. strains produce several non-ribosomal compounds through a multistep mechanism including the selection and condensation of amino acid residues such as cyclic lipopeptidides (iturin group) and macrolactones (surfactins, fengycins and plipastatins), as previously reported [9, 10]. Some examples of these molecules are shown in Figure 1B.

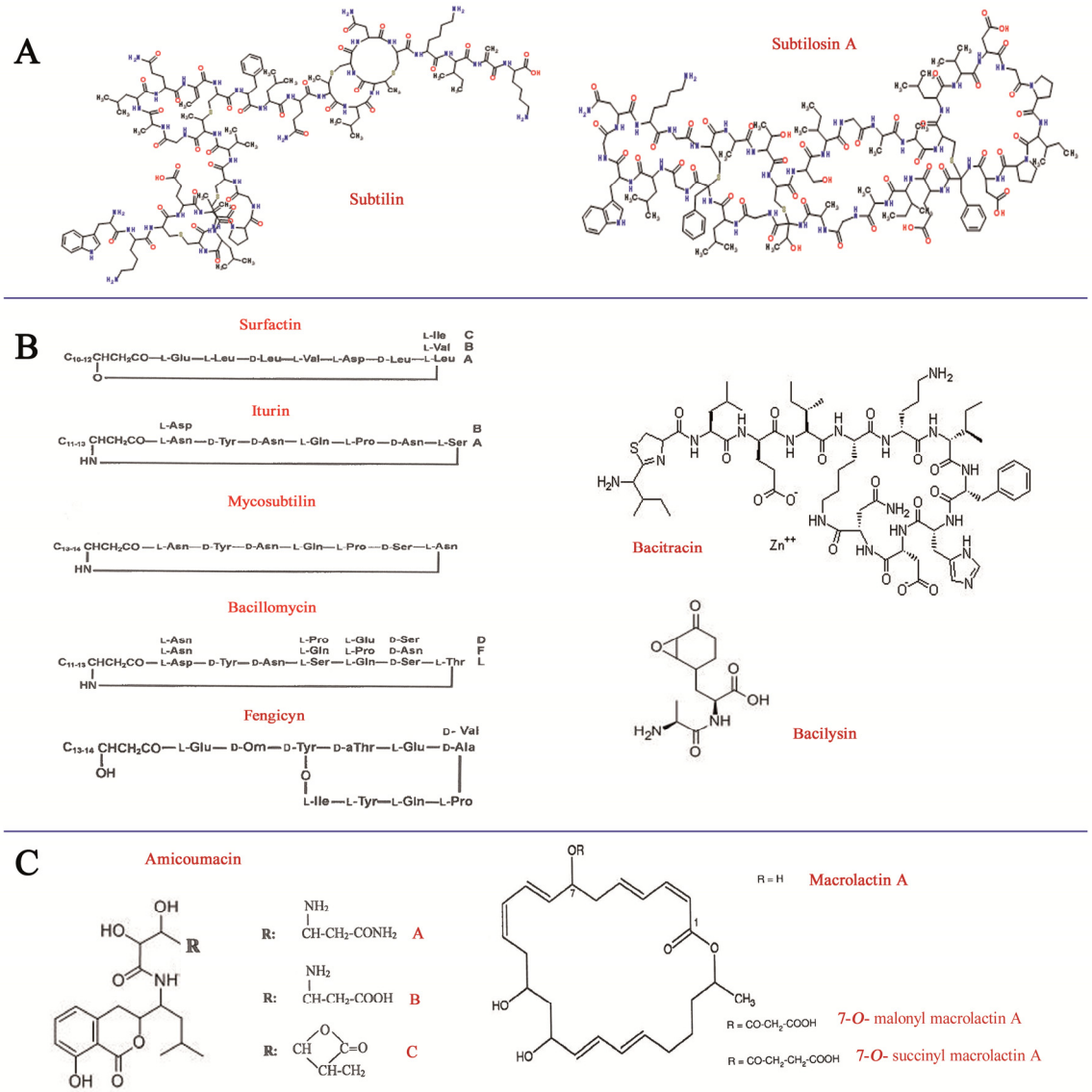

Fig. 1 Chemical structure of some *Bacillus* antimicrobial compounds: A) bacteriocins; B) non-ribosomal biosynthesized peptides; C) antibiotics.

Iturin group includes A, C, D and E isoforms [37], bacillomycin D, F and L [38] and mycosubtilin [39]. All these compounds contain a cyclic heptapeptide acylated with ß-amino fatty acids (chain length C14-C16). Iturin A, bacillomycins and mycosubtilin form channels in bacterial cell membrane [40]. Mycosubtilin alters the permeability of the plasma membrane, releasing nucleotides, proteins and lipids [39].

Fengycin class, including the closest plipastatin, consists of a ß-hydroxy fatty acid connected to the N-terminus of a decapeptide. Fengycin isoforms, containing unusual amino acids such as ornithine and allo-threonine, are classified into types A and B based on their amino acid sequences [41].

Iturins and fengycins exhibit strong biocontrol of plant diseases, inhibiting the growth of a wide range of plant fungal pathogens (*Fusarium graminearum, Rhizoctonia solani* and *Aspergillus flavus*) or post-harvest pathogens as *Botritis cinerea* and *Penicillium expansum* [42, 43].

Lipopeptides of the surfactin family (surfactin, lichenysins and pumilacidins) are produced by several *B. subtilis, B. licheniformis, B. natto* and *B. pumilus* strains and contain a cyclic heptapeptide that forms a lactone bridge with ß-hydroxy fatty acids. The length of the carbon chain of ß-hydroxy fatty acid ranges from C_{13} to C_{18} with amino acid sequence completely different from iturins [44].

Surfactins are the most powerful biosurfactants known and are well characterized for their exceptional emulsifying, foaming, anti-viral and anti-mycoplasma activities [45]; even though these peptides are not fungitoxic, they display some synergistic effects on the antifungal activity of iturin A.

Among small peptides secreted by *B. subtilis*, bacilysin contains an N-terminal alanine residue and L-anticapsin; the release of L-anticapsin irreversibly inhibits glucosamine synthase [46], involved in the synthesis of nucleotides, amino

acids and coenzymes and resulting in the lysis of microbial cells such as *S. aureus* and *Candida albicans* [47]. Some strains of *B. subtilis* also produce chlorotetaine, a chlorinated derivative of bacilysin with similar antibacterial activity [48].

The non-ribosomal dodecapeptide bacitracin (1486 Da), released by some *B. licheniformis* and *B. subtilis* strains, proved to be an inhibitor of cell wall biosynthesis of Gram positive bacteria [49] and therefore employed, together with polimyxin B and neomycin, in some pharmaceutical preparations to medicate eyes and skin infections. Some cyclic, positively charged peptide antibiotics, isolated since the 1940s, are today manufactured using cultures of some *Paenibacillus* (*Bacillus*) *polymyxa* strains. Due to their high affinity for the lipid moiety of lipopolysaccharide and disruptive effect on membrane integrity, polimixins are used against Gram-negative bacteria infections [50].

3.3 Non-peptide-based antibiotics

Bacillus strains also produce a variety of non-peptide antibiotics with different chemical structures (Figure 1C). During a screening of *Bacillus* spp. harvested from 1970 to 1998, Pinchuk et al. [51] found several strains that produced amicoumacins, a class of compounds responsible for antagonistic activity against *S. aureus* and *Helicobacter pylori* [52]. Amicoumacins are a family of low molecular weight dihydroisocoumarin derivatives, subdivided into isoforms A (424 Da), B (425 Da) and C (407 Da) showing inflammatory, antiulcer and gastroprotective effects in addition to antibacterial activities [53].

Other antibiotics are represented by macrolactins and their derivates succinyl or glycosylated macrolactin, containing three separate diene structure elements in a 24-membered lactone ring. Until now, about 18 macrolactins from *Bacillus* spp. have been chemically described, including seven compounds with a molecular mass of 402 Da [54]; they are considered to be potent antiviral and cytotoxic agents that also have antibacterial activity against *S. aureus* [55].

Difficidin and oxydifficidin, isolated from fermentation of *B. subtilis* ATCC 39320, represent a class of antibiotics characterized by highly unsaturated 22-membered macrolide phosphates and exhibit a good antibacterial activity against both aerobic and anaerobic organisms [56].

B. subtilis also produces rhizocticins, phosphonate oligopeptide antibiotics containing the C-terminal non-proteinogenic amino acid (*Z*)-l-2-amino-5-phosphono-3-pentenoic acid (APPA) displaying antifungal activity [57].

4. *Bacillus* strains from foods producing antimicrobial compounds

Bacilli are widespread in the environment being found in dust, soil, water, air and vegetable matter. Contamination of milk or carcasses with spores through fodder and silage can be considered the primary source of entrance of endospore forming bacteria into food chain as illustrated in Figure 2 [58].

A complex *Bacillus* population can occur in processed foods as result of the relationships between processing and natural contamination of raw ingredients.

In particular, cheeses can harbour many *Bacillus* species such as *B. cereus*, *B. circulans*, *B. coagulans*, *B. licheniformis* and *B. pumilus* [59, 60], whereas artisanal and industrial cured sausages were shown to carry mainly *B. pumilus* and *B. subtilis* strains [61]. Unfermented foods such as surimi and zucchini puree can be also carriers of different *Bacillus* species such as *B. amyloliquefaciens*, *B. cereus*, *B. circulans*, *B. licheniformis*, *B. simplex* and *B. subtilis* [62, 63].

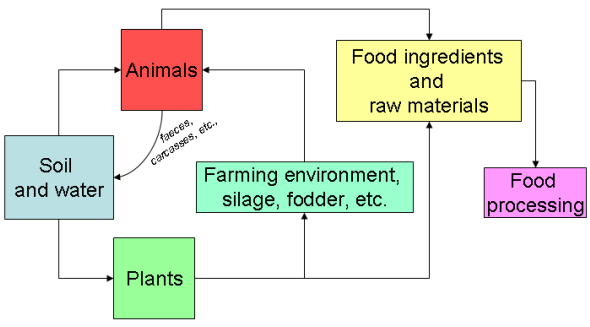

Fig. 2 Diagram of contamination of food by spore-forming bacteria. Modified from Carlin [58]

Among fermented foods, Natto, a traditional Japanese food made from soybeans, considered safe for healthy humans [64] and inoculated only with *B. subtilis* subsp. *natto* strains producing surfactin [65].

However, most outbreaks of *Bacillus* food poisoning are associated with the consumption of cooked foods cooled too slowly and/or incorrectly stored, providing optimal growth conditions for species belonging to *B. cereus* group which increased up to significant numbers (usually $>10^5$ cfu/g).

Within these complex *Bacillus* populations, a number of isolates were found able to produce antimicrobial compounds, that, as demonstrated for bacteriocins, are generally recognized as a safe strategy for food bio-preservation [66]. In fact, the ability to produce some antimicrobial substances is exploited by the formulation of commercial *Bacillus* probiotic products [10].

The *Bacillus* sp. strain CS93 isolated from Pozol, a lactic acid bacteria fermented maize dough manufactured in south-east Mexico, consumed for centuries by the indigenous Mayan peoples, was found to produce several antimicrobial substances [48]. Further studies demonstrated that antibacterial activity of *Bacillus* sp. CS93 against *Escherichia coli* and *Staphylococcus aureus* was supported by a cluster of 12 surfactins showing changes in some amino acids and different lengths in the *β*-hydroxy fatty acid chain [67]. The finding of all these antimicrobial metabolites was considered responsible for the antibacterial activity of Pozol as reported by Herrera and Ulloa [68], even though their production by *Bacillus* sp. strain CS93 was never demonstrated in this food.

A preliminary study [69] showed the ability of *B. subtilis* 20B to produce molecules similar to the surfactin. This strain, when co-inoculated on agar plates, showed inhibitory activity against several fungi such as *Chrysosporium indicum*, *Alternaria burnsii*, *Fusarium oxysporium*, *F. udum*, *Trichoderma herzanium* and *Rhizoctonia bataticola*.

In an extensive screening program for new antibiotic compounds, Pinchuk et al. [51] isolated 51 *Bacillus* strains in Tajikistan and Ukraine that were tested for the production of isocoumarin in a liquid starchy medium; only 11 strains were found able to produce amicoumacins after 72 h (mean sporulation cycle) of growth. Four of them, isolated from flour, resulted to belong to *B. subtilis* species. These Authors did not further investigate whether the production of amicoumacin released in the starchy medium could be associated to their isolation from flours.

Subtilosin produced by a *B. amyloliquefaciens* strain, isolated from a contaminated fermented dairy beverage [27] and *B. subtilis* 22, isolated from Chinese fermented soybean [70], was found to be active in the growth reduction of pathogens such as *Listeria monocytogenes, Salmonella typhimurium, S. aureus* and *Pseudomonas aeruginosa*. Even though the release of subtilosin in foods has never been demonstrated, it has been suggested that the ability of these strains to inhibit food pathogens could be an attractive option useful for the food industry [27] .

Bacillus strains producing bacteriocins were also isolated from Kimchi, a Korean traditional fermented vegetable. In particular, Hyung et al. [71] reported that *Brevibacillus brevis* 430 produced a bacteriocin very active against *Shighella dysenteriae* and formulated the hypothesis to use this strain in a traditional Korean soybean paste mainly fermented by *Bacillus* strains.

Martirani et al. [72] suggested that bacillocin 490, a bacteriocin of approximately 2 kDa produced by the dairy *B. licheniformis* 490/5, could be suitable for milk-based foods on the basis of the inhibition of growth of the *B. smithii* PRO/S in water-buffalo milk.

In addition to the action of one single antimicrobial compound, Caputo et al. [73] and Quintieri et al. [74] supposed that the inhibitory activity of the meat-borne *B. subtilis* TR50 against ten food-borne pathogens was supported by the presence of a mixture of metabolites (Figure 3). Baruzzi et al. [75] demonstrated that *B. subtilis* TR50, enumerated in minced meat at about 4.7 log cfu/g, survived after 25 days of ripening when it accounted for more than 80% of total *Bacillus* viable cells.

Pinchuk et al. [52] also reported that at least two antibiotics, produced by *B. subtilis* 3 in a starch based medium, were responsible for the anti-*Helicobacter pylori* activity. Thanks to its ability to inhibit other human pathogens such as *Campylobacter* spp. [76], *B. subtilis* 3 is one of the basic components of the commercial probiotic Biosporin for human use, even though it was originally isolated from animal fodder.

Food application of antimicrobial compounds produced by *Bacillus* strains was rarely evaluated [72, 76, 78] differently from the application of lactic acid bacteria (LAB) bacteriocins against food pathogens and/or spoilage bacteria. This fact is due to the GRAS status of most of LAB and to their involvement in food fermentation and processing.

In past, it was demonstrated that *Clostridium botulinum* was sensitive to the polypeptide antibiotic subtilin and for this reason this compound was investigated for its potential use (at levels of 5-20 ppm) in the preservation of canned foods [79]. Peptide antibiotics such as mersacidin and cerein 8A were found to inhibit food-spoilage and several pathogens including methicillin resistant *S. aureu*s [21, 80]. Interestingly, the addition of cerein 8A during the manufacture of Minas-type cheese caused a delay in the development of *L. monocytogenes* in comparison with cheese samples without the bacteriocin [78].

To the best of our knowledge, no data are available on the production of antimicrobial compounds by *Bacillus* spp. in foods.

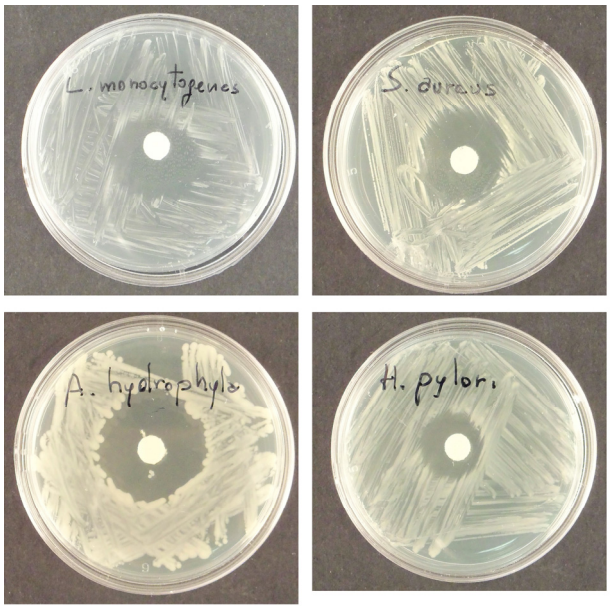

Fig. 3 Inhibition halos from disk diffusion test of *B. subtilis* TR50 against four food pathogens [73]

5. Future perspective and conclusions

The surge during the past 20 years of the spread of antibiotic-resistant bacteria in foods and the consumer demand for foods without or with a reduced use of chemical preservatives has stimulated research for natural antimicrobial agents such as plant extracts, bacteriophages, enzymes interfering with microbial life cell cycle, essential oils and antimicrobial peptides. In particular, bacteriocins are considered ideal candidates for food preservation thanks to their potential absence of harmful effects on humans and their antagonistic activity against specific pathogens.

Recently, campylobacteriosis, salmonellosis, listeriosis and Shiga toxin-producing *E. coli* represent the most common food-borne diseases in the developed Countries.

The state of the art of scientific literature, as just described in the previous sections, the GRAS status of most of *Bacillus* species and the ability of many *Bacillus* strains from foods to produce inhibitory compounds, mainly active against food-borne pathogens, lead to suppose that these metabolites could be applied in food productions to reduce the level of pathogens. As *Bacillus* spp. generally survive along food chain, further researches should be addressed to ascertain the release of antimicrobial compounds during food fermentation and processing in order to carry out a continuous microbial control in each phase of food processing and storage.

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