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REVIEW ARTICLE

Properties and antimicrobial activity of the smear surface cheese coryneform bacterium *Brevibacterium linens*

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Abstract Surface microorganisms contribute to the ripening of some low-moisture cheese varieties and the composition of the surface microflora is dynamic. *Brevibacterium linens* is an important surface microorganism that is present in the smear of surface-ripened cheeses and is commonly regarded as the organism primarily responsible for the characteristic taste, aroma, and color of surface cheese. The enzymology and biochemical characteristics of *B. linens* influence the ripening and final characteristics of smear surface-ripened cheeses. Proteolytic, peptidolytic, esterolytic, and lipolytic activities are of particular importance in the ripening process. Because of its putative importance to the ripening in smear-ripened cheeses, *B. linens* is the best studied component of the microflora, although in comparision with other dairy-related microorganisms, it is poorly characterized. *B. linens* produces antimicrobial substances that inhibit the growth of many food poisoning bacteria as well as several yeast and moulds. Some inhibitory substances produced by this species were identified as bacteriocins. Bacteriocins could appear as potential agents to be applied in food conservation systems in order to provide microbiologically stable foods. This article describes the properties of *B. linens* and discusses about the potential of this species to produce bacteriocins and other antimicrobial substances, which are important for production of high quality cheese.

Keywords *Brevibacterium linens* · Cheese · Bacteriocin · Biochemical properties · Antimicrobial activity

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Introduction

The surface of smear ripened cheeses such as Limburger, Münster, Tilster, and Brick is covered by a layer of yeast and bacteria, and *Brevibacterium linens* has long been recognized as an important dairy microorganism. Apart from the influence of the physical and chemical parameters of the cheese, these cultures contribute to the appearance and aroma development, as well as to the complexity of the cheese ripening [1]. It is now recognized that, in addition to cheese, brevibacteria exist in a number of different habitats, and especially where there is a high salt concentration, dairy products, and skin.

The metabolism and physiology of the microorganism determine its growth on smear surface-ripened cheeses and the effect of such growth on the characteristics of the cheese. *B. linens* is a strictly aerobic microorganism, with a rod–coccus growth cycle. In this cycle, cells from older cultures, 3–7 days old, are composed largely or entirely of coccoid cells, 0.6–1.0 μm in diameter, or occasionally of coccal rods. On transfer to a suitable fresh medium, these forms grow out to give the irregular, slender rods characteristic of exponential phase cultures. The optimum growth conditions are at a temperature of 20–30 °C and pH 6.5–8.5 [2]. The microflora of smear surface-ripened cheese is generally complex and of a transient nature. Because of the brine salting, only halotolerant microorganisms predominate on the surface of the cheese. The growth of *B. linens* on the surface is thought to be an essential prerequisite for the development of the typical color, flavor, and aroma of the cheeses.

Cheeses are known to be sources of foodborne listeriosis. Due to the production process and ripening procedures, semi-soft and soft cheeses are considered as problematic cheese types. *Listeria monocytogenes* is a pathogenic

microorganism whose control in food is made difficult by its ubiquity in the environment, its ability to grow at refrigeration temperature, and its tolerance towards low pH (lower than pH 5.0) and sodium chloride levels upto 10% [3]. Studies on antagonistic systems that could prevent the growth of *L. monocytogenes* in cheese are of great interest since foodborne listeriosis have been associated with the consumption of cheese and dairy products. Among antagonistic systems produced, hydrogen peroxide, diacetyl, organic acids, and bacteriocins have been observed and investigated [4].

Bacteriocins are antibacterial peptides that are inhibitory to microorganisms that are usually, but not always, closely related to the producer strain. Bacteriocin production is widespread and well characterized for gram-positive bacteria [5], and lactic acid bacteria have been the subject of intensive investigation. Many attempts are being made to incorporate bacteriocins into processes and products [6, 7]. However, only nisin has been granted Generally Recognized as Safe (GRAS) status by the FDA and was shown to substantially reduce the level of *L. monocytogenes* in Camembert cheese made with artificially contaminated milk, but was unable to prevent the growth of this organism during ripening [8]. Therefore, there is a strong need for cheese surface flora exhibiting inhibitory properties against *L. monocytogenes*. Detailed information about bacteriocins or bacteriocin-like substances from smear cheese microorganisms is limited. Some studies have described the anti-listerial effect of coryneform bacteria [9–11].

Apart from their importance in the maturation of smear-ripened cheeses, these organisms may be important sources of food-grade enzymes and antimicrobial substances. This review will cover the properties of *B. linens* and discuss about the potential of this species to produce antimicrobial substances and bacteriocins, which are important for production of high quality cheese.

The genus *Brevibacterium*

Taxonomy and classification

Brevibacterium is a unique genus of the Brevibacteriacea family located in the suborder Micrococcineae, order Actinomycetales, subclass Actinobacteridae, and class Actinobacteria. The genus *Brevibacterium* was first proposed by Breed (1953), with *B. linens* as type species [12]. Later, three more species were added to this genus, namely, *B. iodinum*, *B. casei*, and *B. epidermidis* [13, 14].

Several bacterial species have been incorporated in the genus *Brevibacterium*, making it very heterogeneous. Nowadays, several reclassifications have been made and the genus has been restricted for those bacteria with a close resemblance with the type species *B. linens*, based on 16S rDNA sequence analysis and DNA–DNA hybridization experiments. Currently, the genus consists of twelve species [15].

Members of the genus *Brevibacterium* may be confused with members of the other coryneform and morphologically similar genera, which contain meso-DAP in the cell wall or which show a rod–coccus growth cycle. The presence of teichoic acids in the cell wall polysaccharide of brevibacteria distinguishes them from all other coryneform bacteria so far examined [16].

Brevibacterium linens is phenotypically similar to *Arthrobacter globiformis*, although cellular pigmentation, cell wall composition, DNA/DNA hybridization, and 5S RNA analysis show that *Brevibacterium* is markedly different [17]. PFGE analysis indicates that diversity within the species is related to polymorphisms in the 16S rRNA genes with genome sizes ranging from 3.2 to 3.9 Mbp [18].

Molecular techniques like amplified ribosomal DNA restriction enzyme analysis (ARDRA), pulsed field gel electrophoresis (PFGE), and ribotyping have been used to differentiate *Brevibacterium* species isolated from smear-ripened cheeses. *B. linens* showed to be quite a heterogenic group. The genotypes 1–3 of *B. linens* cultures can be classified by *Xmn*I restriction, but genotype 4 cannot be distinguished from *B. casei* by ARDRA [19].

Fourier-transform infrared (FT–IR) spectroscopy can be used to identify microorganisms on the basis of their chemical composition [20]. Reference databases for the identification of several groups of microorganisms, including coryneform bacteria, have been established applying FT–IR spectroscopy [21]. Two smear cheese microbial consortia were compared by FT–IR microspectroscopy [22]. This technique enables the measurement of microcolonies without producing a pure culture, allowing the identification of *B. linens* among other coryneform strains.

Phenotypical characteristics

Brevibacterium species are strictly aerobic, chemo-organotrophic bacteria, belonging to the Brevibacteriacea family. During growth on complex medium, a marked rod–coccus cycle occurs: cells are rod-shaped during the exponential growth phase but become coccoid-shaped in the stationary phase (Fig. 1). Both the rod and coccoid forms are gram-positive but older cells decolorize readily during the staining protocol.

Brevibacterium is a non-motile, non-spore formed, non-acid fast, gram-positive coryneform bacteria that tolerates high salt concentrations (8–20%), and is capable of growing in a broad pH range with an optimum at pH 7.0. They also survive carbohydrate starvation and drying for extended periods. *B. linens* is unusual as it alkalinizes the

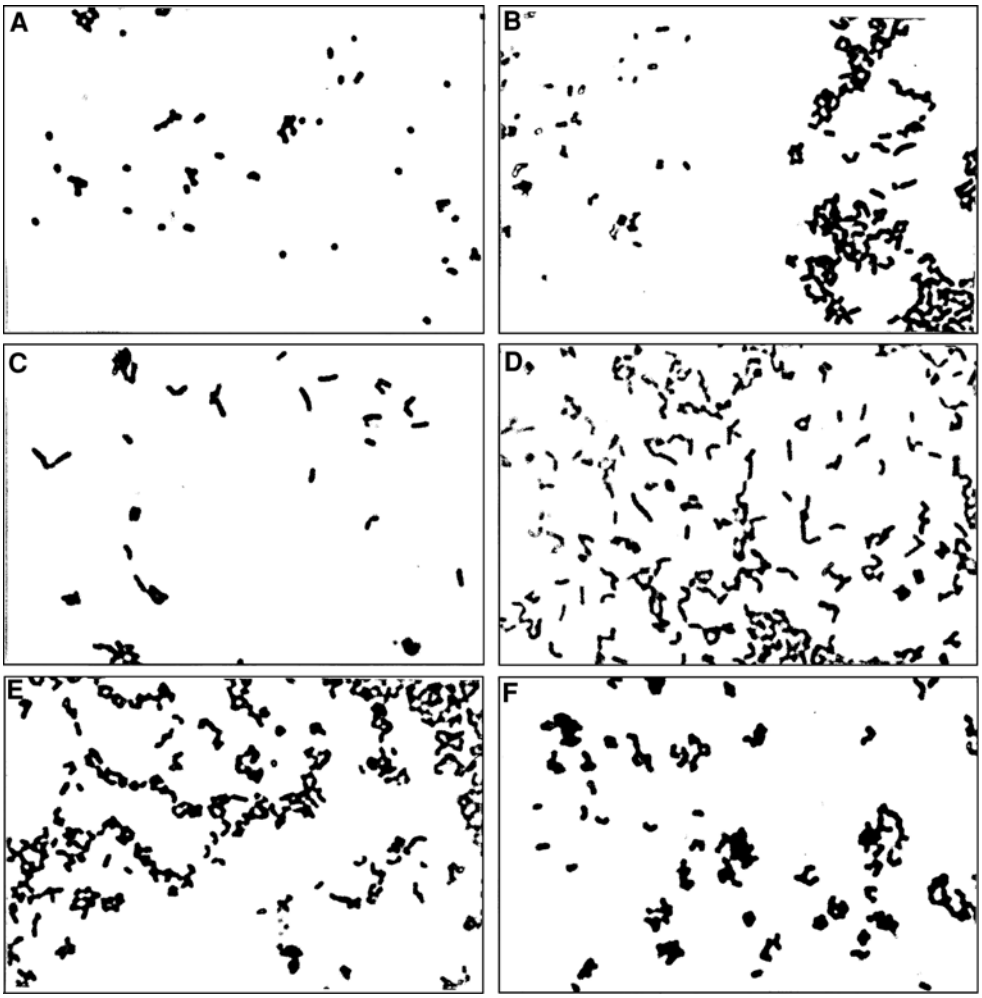

Fig. 1 Rod–coccus growth cycle of *B. linens*. The strain ATCC 9175 was cultivated in TSB medium at 25 °C with initial inoculum cocoid cells (**a**). After 6 h of growth, outgrowth of rods are seen from coccoid cells (**b**). After 12 h of cultivation (**c**), and after 48 h of cultivation (**d**). At 96 h, observed cells in rod–coccus shape and coccoid cells (**e**). After 168 h, the cells were observed in the coccoid shape (**f**)

medium during cultivation, increasing the pH to 9.5 within 24–36 h [2]. Some strains of *Brevibacterium* produce distinctive red–orange carotenoid-type pigments when exposed to light during growth. The color of the colonies varies from orange (*B. linens*), through gray-white (*B. epidermidis* and *B. casei*) to purple (*B. iodinum*). The orange pigmentation (carotenoids) of the type-strain is often light-dependent. The purple coloration of *B. iodinum* results from the production and secretion of purple crystals of a phenazine derivative, called iodinin [12].

Brevibacteria often show a good growth on peptone-yeast extract agar at near neutral pH. The optimum growth temperature is 20–30 °C or 37 °C, depending on species and strain. All studied strains tolerate or are sometimes stimulated by the addition of NaCl to the medium. The respiratory metabolism mode is used and little to no acid is produced from glucose or other carbohydrates in peptone medium. Brevibacteria are catalase positive, oxidase and nitrate reductase variable depending on the species, hydrolyzing gelatin and casein but not starch or urea. *B. linens*, *B. casei*, and *B. epidermidis* produced methanethiol from L-methionine [15].

To date, it appeared that *B. linens* and *B. iodinum* are rarely, if ever, associated with human infections, and the eventual safety concerns are rather associated with the dermal species. Some cases of human opportunistic infections caused by *B. casei*, *B. otitidis, B. epidermidis*, and *B. mcbrellneri* are reported [23]. At least one case involving *B. linens* has been reported, which was associated with a pleural empyema [24], although the species identification may have been questionable. There are few case reports of infections caused by *Brevibacterium* species, and the first case of endocarditis (caused by *B. otitidis*) was reported in a patient with prosthetic heart valves. The patient responded to 6 weeks of treatment with vancomycin and 2 weeks with gentamicin, and received long-term maintenance therapy with oral azithromycin [25]. The natural susceptibility of 20 strains of *B. casei* was tested. All species were naturally

sensitive to tetracyclines, most aminoglycosides, carbapenems, macrolides, lincosamides, glycopeptides, and rifampin. Susceptibility patterns indicate natural resistance to pipemidic acid, sulfamethoxazole, and cotrimoxazole. This database can be applied for the validation of susceptibility testing results [26].

Environmental growth conditions

The growth of *B. linens* in smear cheeses is stimulated by vitamin production by the growing yeasts. The major factors that influence the individual characteristics intrinsic to the cheeses are: pH, water activity, redox potential, composition and size, the environmental parameters (ripening temperature, relative humidity), and the technological conditions during manufacture (ripening time, degree of mechanization, and microflora of cheese equipment) [27].

Brevibacterium linens and related organisms utilize a number of substances as carbon energy sources. There were some differences between the strains of *B. linens* examined. These differences may be correlated with the genetic heterogeneity detected among strains of *B. linens*, but there is no sufficient evidence to support this theory.

In addition to cheese, brevibacteria exist in a number of different habitats and especially those where there is a high salt concentration. The usual habitat of the strains of *B. linens* is on the exterior of surface-ripened cheeses of the Limburger variety, but it also occurs on cheeses such as Brick, Camembert, Roquefort and other (Table 1). *B. linens* was believed to contribute to the surface color of Limburger and similar cheeses. That work reported the production of methanethiol from L-methionine by the seven strains of *B. linens* tested [2]. Methanethiol is an important constituent of the aroma of Cheddar cheese and those researchers suggest that the production of this compound might also be important in the aroma and flavor of surface-ripened cheeses. *B. linens* and *B. casei* have been implicated in contributing to the aroma of various cheeses by the production of methanethiol and other sulfur compounds. Furthermore, some strains of *B. linens* have been shown to produce *S*-methylthioacetate, an important aroma component of smear-coated cheeses.

The interest in *B. linens* has focused around their ability to produce the self-processing extracellular proteases [28], their ability to produce high levels of volatile sulfur compounds [29, 30], bacteriocin production [31], cell membrane-associated carotenoid pigment production, and aromatic amino acid metabolism [32].

Antibacterial and antifungal properties of *B. linens*

In recent years, the dairy industry faced severe problems when *L. monocytogenes* and *Staphylococcus aureus* were found on the brick cheese surface. Because the traditional method of old-young-smearing was considered to be the source for undesirable microorganisms, attempts were made to replace this method by using a well defined red smear culture. Unfortunately, all efforts to amend the traditional method were unsuccessful. The resulting cheeses were of low quality or showed a prolonged ripening period. Certainly, one reason for this failure is a very limited knowledge about the species composition of the red smear [33].

The knowledge about the natural species composition and their function in the ripening process should enable us to design a specific ripening culture by selecting appropriate species. Such culture should remain stable in the dairy, develop antagonistic activity on the cheeses against pathogens such as *L. monocytogenes* and *S. aureus* and especially, prevent recycling of undesirable microorganisms. It is interesting to know whether anti-listerial activity in red smear cheese microbial consortia is widespread. It has been reported that microorganisms isolated from surface flora of red smear cheese display antagonistic effects against moulds [34], *Listeria* spp., and other bacterial contaminants [35]. The culture supernatant from *B. linens* was demonstrated to inhibit the germination of spores of *Clostridium botulinum* Type A; however, the inhibitory agent present in the culture fluid was not identified [36].

Table 1 Identification of surface microflora of smear cheese varieties

Cheese type	*Coryneform* species	*Brevibacterium* species	References
Gubbeen, Durrus, Ardrahan and Milleens	*Corynebacterium casei*, *Corynebacterium variabile*, *Arthrobacter arilaitensis*, *Arthrobacter* sp.	*Brevibacterium linens*	[1]
Tilsit and Chaume	*Corynebacterium casei*, *Microbacterium grubbeenense*	*Brevibacterium linens*	[48]
Gubbeen	*Corynebacterium flavescens*, *Corynebaterium casei*, *Corynebacterium mooreparkense*, *Microbacterium grubbeenense*	*Brevibacterium linens* BL2	[54]
Appenzeller	*Corynebaterium casei*, *Corynebacterium* species not identified, *Arthrobacter casei*, *Microbacterium grubbeenense*	*Brevibacterium linens*	[22]
Red smear soft cheeses (microbial consortia)	*Corynebacterium casei*, *Corynebacterium variabile*, *Arthrobacter nicotianae*, *Microbacterium grubbeenense*, *Microbacterium* sp.	*Brevibacterium linens*	[55]

A variety of inhibitory substances can be produced by bacteria. Some coryneform bacteria, including *B. linens*, have been already isolated from surface-ripened cheese for their ability to produce inhibitory substances against *L. monocytogenes*. The antimicrobial activity of 16 strains of *B. linens* was tested against *Listeria* species [9]. Although the nature of the inhibitory substances was not determined, they were heat-sensitive and their molecular sizes were greater than 12 kDa. *B. linens* do not produce acids from carbohydrates when growing in tryptic soy broth medium and the antimicrobial activity has been related with antimicrobial peptides [11]. These studies indicated that the inhibitory activities are associated with bacteriocin-like substances. Bacteriocins are antibacterial proteins produced by bacteria that kill or inhibit the growth of other bacteria. Generally, they have a relatively narrow killing spectrum and are only toxic to bacteria closely related to the producing strain. They are sensitive to the proteolytic enzymes and relatively heat-stable. Protease sensitivity is a key criterion for the characterization of an inhibitory substance such as a bacteriocin [6].

Among the gram-positive bacteria, lactic acid bacteria have been used as a reservoir for antimicrobial peptides with food applications. Nisin, produced by *Lactococcus lactis*, is approved for use in over 40 countries and has been used as a food preservative for over 50 years. Though nisin is currently the only bacteriocin approved for use in many countries, many bacteriocins have potential applications in food products [5, 7].

Biopreservation systems using bacteriocins have gained attention as a means of natural control in foods. Some studies suggest that bacteriocins may contribute as an additional barrier in the hurdle concept of food safety [6]. In addition, increased resistance or tolerance of *L. monocytogenes* strains to conventional lactic acid bacteriocins, like nisin and pediocin, has been observed [37]. Therefore, the identification and characterization of novel bacteriocins and the exploration of their potential use in biological control of pathogenic and spoilage microorganisms is an important research field. Production of bacteriocins is usually thought to provide an ecological advantage to the producer over other bacteria living in a particular habitat. Based on this idea, one would expect that a high percentage of isolates could produce bacteriocins.

Brevibacterium linens produced antimicrobial substances that inhibited the growth of many food poisoning bacteria, as well as yeast and moulds [27, 38]. An important aspect concerning the production of these antibacterial substances is the extrapolation of the inhibitory effects from a model buffer system to that observed in a smear surface-ripened cheese. The degree to which a bacteriocin produced at the surface of the cheese can diffuse toward the center obviously influences any inhibitory effect on microorganisms in the interior of the cheese. The molecular masses of the bacteriocins reported for *B. linens* are probably too large to result in significant diffusion from the surface to the interior, and their effects are, therefore, likely to be confined to the surface only [27]. The characteristics of bacteriocins produced by *B. linens* are shown in Table 2.

Bacteriocin production by two strains of *B. linens* was demonstrated [39]. The bacteriocin, Linecin, was found to be inhibitory to strains closely related to the producer strain. In a further investigation, the purification and characterization of Linecin A was reported. This bacteriocin consisted mostly of protein with an estimated molecular mass of 95 kDa [40]. Linecin A was thermolabile and sensitive to proteolytic attack, and was inhibitory to other strains of *B. linens*.

The bacteriocin Linocin M18, isolated from the culture supernatant of *B. linens* M18, exhibited a broad spectrum with activity against species of the genera *Bacillus*, *Arthrobacter*, *Corynebacterium*, *Micrococcus*, and *Listeria*. The inhibitory effect on the growth of *Listeria* spp. is most important for practical purposes because biopreservation systems have gained increasing attention as a means of natural control of the growth of pathogenic and spoilage organisms in foods [41]. In a model cheese ripening system, the antimicrobial activity of *B. linens* M18 toward *Lis-*

Table 2 Bacteriocins produced by *Brevibacterium linens*

Bacteriocin	Source	Mode of action	Antimicrobial spectrum	Molecular weight (kDa)	References
Linecin A	*Brevibacterium linens*	Undefined	Strains of *B. linens*	95	[40]
Linocin M18	*Brevibacterium linens* M18	Undefined	*Listeria* spp., coryneform and gram-positive bacteria	31	[41]
Antimicrobial agent	*Brevibacterium linens*	Undefined	*Listeria* spp. (*Listeria monocytogenes*)	Undefined	[10]
Linenscin OC2	*Brevibacterium linens* OC2	Cytoplasmic membrane-disruptive action, autolysis	Gram-positive, but not against gram-negative	285	[35]
Antibacterial peptide	*Brevibacterium linens* ATCC 9175	Bacteriostatic on *L. monocytogenes*	Gram-positive, but not against gram-negative	150	[11]

teria strains has been demonstrated with reduction of *L. ivanovii* and *L. monocytogenes* counts by 1–2 log units [42].

Oligonucleotide probes based on the N-terminal amino-acid sequence were used to locate the gene coding for Linocin M18. A single copy of the gene *lin* was located on chromosomal DNA. The amino acid composition, N-terminal sequence, and molecular mass derived from the nucleotide sequence of an open reading frame of 798 nucleotides coding for 266 amino acids, was found on a 3 kb *Bam*HI restriction fragment, and closely corresponds to those obtained from the purified bacteriocin [31].

Linenscin OC2, produced by *B. linens* OC2, inhibited gram-positive foodborne pathogens including *S. aureus* and *L. monocytogenes* but was not active against gram-negative bacteria. The biochemical mode of action of Linenscin OC2 is believed to be similar to that of bacteriocins such as nisin, which, in addition to their cytoplasmic membrane-disruptive action, induce autolysis [35].

The crude bacteriocin produced by *B. linens* inhibited the growth of *L. monocytogenes*, and *Corynebacterium fimi* but was inactive to gram-negative bacteria and the yeast tested. The antibacterial peptide was characterized, presenting potential for use as a preservative in foods systems [11]. The influence of temperature, NaCl concentration, and the use of the cheese whey media on the bacteriocin production by *B. linens* were studied. Bacteriocin production was higher at 25 °C and increased activity was observed in media containing 40 or 80 g L^{-1} NaCl. The addition of NaCl resulted in a significant increase in specific production rates of bacteriocin-like activity. Antimicrobial activity was also observed in cheese whey media [43].

Food and dairy products are prone to contamination with mycotoxigenic moulds, which may produce mycotoxins under favorable conditions. Aflatoxins, being potent hepatocarcinogens in mammals [44], are produced by *Aspergillus flavus*, *A. flavus* spp. *parasiticus*, and *A. nomius*. Factors such as biological (strain variability, inoculum size, competing microflora), chemical (substrate, nutrients and antifungal agents), and environmental (temperature, pH, water activity) affect the growth of the moulds, and consequently of biosynthesis of the aflatoxins [34].

The antifungal properties of *B. linens* against *A. flavus* were studied in relation to incubation temperature, time, light, addition of proteolytic enzymes, storage, heating, pH value, and enrichment of bacterial growth medium. The results indicated that *B. linens* produced antifungal compound when grown in nutrient broth medium at 27 °C. The antifungal compound seemed resistant to proteolytic enzymes, heat and storage, and was most active at alkaline pH. The results give a basis to recommend *B. linens* for use as a biological control agent in food industry [34].

The potential application of bacteriocins as food preservatives requires an in-depth knowledge of how they exert their bactericidal effect. Most bacteriocins whose primary mode of action is known to act at the plasma membrane. It has been proposed that these peptides form poration complexes that transverse the phospholipid bilayer; this provokes membrane permeabilization and hence depletion of the proton-motive force of sensitive cells [45].

The mode of action of linenscin OC2 on the *L. innocua* cytoplasmic membrane and the effects of environmental parameters were investigated. Addition of low doses of linenscin OC2 resulted in an immediate perturbation of the permeability properties of the cytoplasmic membrane and of the bacterial energetic state. Linenscin OC2 induced a loss of cytoplasmic potassium, depolarization of the cytoplasmic membrane, complete hydrolysis of internal ATP, efflux of inorganic phosphate, and transient increase in oxygen consumption [46]. The bacteriocin interacts with the cytoplasmic membrane and the permeability changes observed at low doses reflect the formation of pore-like structures in this membrane [47].

Potential applications

The commercial production of *B. linens* for use as a cheese-ripening agent, regarded as the organism responsible for the characteristic taste, aroma and color of surface smear cheese, is very important in the dairy industry. The use of defined surface starter cultures for the cheese varieties is possible and can replace the traditional "old–young" smearing. Appropriated surface cultures such as *B. linens*, should not only be sprayed onto the cheese surface, but inoculated into the cheese milk and cheese brines. This covers essential ecological niches with food grade culture strains and minimizes the risk of any microbial contamination [48]. *B. linens* contributes to breakdown of lipid and proteins during ripening cheeses where the organism is usually present. This species synthesizes highly active and multiple proteolytic enzymes during its growth. These enzymes are mainly proteases and peptidases [49]. *B. linens* also produces lipolytic enzymes. The lipase of *B. linens* releases volatile fatty acid components from milk triacylglycerols, thus influencing the characteristic taste of the ripened cheese. The lipolytic activity has been demonstrated to be cell-associated, with a maximal activity at alkaline pH [50].

The use of *B. linens* in wastewater treatment, vitamin production, and pharmaceutical process has been related [27]. Carotenoids are yellow to red pigments that occur widely in nature. These molecules have valuable applications in animal feeds, food technology, and pharmacy. *B. linens* synthesizes three aromatic carotenoids that are

responsible for its orange pigmentation, which distinguishes this species from other Brevibacteria [51].

By its numerous properties and importance in cheese technology, *B. linens* is a major cheese-ripening bacteria. To investigate the most interesting *B. linens* metabolic pathways, both at the biochemical and genetic level, genetic modifications in this species were realized. Interesting technological properties were observed, especially the production of sulfur compounds, which are important contributors of aroma [52].

The detection of the four novel native plasmids in *B. linens* has implications for the strain development of this industrially important bacterium. These native plasmids may further the study of the genetics of *B. linens* as well as contribute to the rational development of this species by the development of clonal vectors [53].

Conclusions

The genus *Brevibacterium* is a heterogeneous group of coryneform bacteria that have particular application in industrial production of vitamins, amino acids for fine chemical production, and are commonly used in cheese production [27, 46]. This genus contains species from diverse habitats, such as soil, poultry, fish, human skin, and food. Most research on *B. linens* has demonstrated that the physiology and metabolic activities of this bacterium are significantly strain-dependent.

Although bacteriocin production may play a role in preventing the growth of *Listeria* within a multiple hurdle concept, its production observed in single-strain-ripened cheeses cannot explain the striking effects observed with industrial wash-off cultures. Additional factors must be responsible for the inhibition of *Listeria*, the production of other inhibitory substances or unknown ecological interactions within the complex smear flora. The development of a defined surface-ripened culture will probably comprise a combination of reciprocally compatible strains with different bacteriocinogenic activity as well as suitable ripening sensory and antimycotic properties. Therefore, the investigation on antimicrobial substances produced by *B. linens* and the influence of environmental conditions on their biosynthesis is a subject of great interest and merits future studies.

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