Review

Bacillus Licheniformis—A Perspective for Medical Applications Producer of Variety of Antimicrobial Substances Including Antimycobacterials

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Abstract: *Bacillus licheniformis* produce several classes of antimicrobial substances which are mainly either peptides or proteins. Among of them bacteriocins - peptides or proteins of different structural composition including synthesized by bacteria ribosomally; non-ribosomally synthesized peptides and cyclic lipopeptides; exopolysaccharides. Different representatives of these classes act against Gram-positive, Gram-negative bacteria, fungal pathogens and amoebae cells. In this review, a detailed classification of antimicrobial substances produced by *B. licheniformis* based on their chemical structure and mode of the synthesis and activity is presented. For some (rather limited number) of secreted antimicrobials mechanism of their harmful effect on the target cells is established, however, for many of them it remains unknown. The antimicrobial activity for most substances was studied *in vitro* only, however some substances were characterized *in vivo* and are found practical applications in medicine and veterinary. The cyclic lipopeptides with surfactant properties are applied in industry. In this review, a special attention on antimycobacterials produced by *B. licheniformis* is made as a possible approach to combat multidrug resistant and latent tuberculosis. Indeed, a number of peptides and proteins revealed strong antimycobacterial activity. However, medical application of some bacteriocins with promising *in vitro* antimycobacterial activity is limited by their toxicity for animals and humans. In this connection, similarly with the enhancement of the antimycobacterial activity of natural bacteriocins using genetic engineering, reduction of the toxicity by the same approach looks feasible. A unique capability of *B. licheniformis* to synthesize and produce a bouquet of different antibacterial compounds allow to consider this organism as a universal natural vehicle for antibiotic substances in form of probiotic cultures strains to combat various types of pathogens including mycobacteria.

Keywords: *Bacillus licheniformis*; *Mycobacterium tuberculosis*; bacteriocin; antimicrobial peptides

1. Introduction

The spread of bacterial strains resistant to known antibiotics that cause severe infectious diseases dictates the need to develop and search for new approaches to combat these diseases [1]. The growing number of cases with multidrug resistant strains of the causative agent of tuberculosis *Mycobacterium tuberculosis* (*Mtb*) is probably most known and medically significant example which illustrates this problem. In addition to drug resistance, *Mtb* is able to persist asymptomatically in the host organism for many years, causing latent forms of tuberculosis. In this dormant state, *Mtb* cells are also resistant to known antibiotics [2–4].

The search and study of substances that have bactericidal or bacteriostatic properties against human and animal pathogens is necessary also for the development of new components of antibiotic therapy or disinfectants for objects and surfaces that have been in close contact with patients and may carry pathogenic bacteria. Currently, besides synthesis of new chemical substances, a significant attention is paid to exploration of the potential of the natural products of different origin as

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antimicrobials. The discovery of antibiotics that act against human pathogens is often based on the observation of the interaction between microorganisms, called antagonism. It manifests itself through the synthesis and release of substances that inhibit or completely suppress the growth of organisms of other species. Under natural conditions, a microorganism secreted substance(s) that inhibit the growth of another organism gains a competitive advantage in the struggle for environmental resources. Most of the antibiotics used for medical applications are secreted products or derivatives of microorganisms belonged to the order *Actinomycetalis* (among of them most known are *Streptomyces*). At the same time, bacterial world represents a huge reservoir of not yet discovered and used substances with antibacterial potential. In this regard, representatives of the genus *Bacillus* are known as producers of many enzymes and antimicrobial compounds. For example, *Bacillus amyloliquefaciens* is a source of the natural antibiotic barnase (ribonuclease), alpha-amylase used in starch hydrolysis, protease subtilisin used in combination with detergents, and the restriction enzyme BamH1 used in DNA research [5]. *Bacillus subtilis* produced 66 derived antimicrobials, *Bacillus brevis* - 23 peptide antibiotics [6]. There is a growing interest in considering these substances including bacteriocins as alternative antimicrobials for the treatment of human and animal infections [7–11].

Currently, the use of bacterial strains-probiotics and their metabolic products are also considered as a new approach for the control and prevention of various infectious diseases [12].Thus, many studies on animals demonstrated that probiotics from the *Bacillus* genus have antimicrobial properties. This conclusion also applies to humans [13,14]. The use of bacteriocins and antimicrobial peptides produced by probiotic strains is a good alternative to antibiotics, since their production is inexpensive and the occurrence of resistance to them is rare [15]. They exhibit a broad spectrum of activity against many Gram-positive and Gram-negative bacteria, but also against fungi. The efficacy and cost-effectiveness of many of these compounds make them attractive for clinical use [16]. A few natural peptides have shown potential and desirable therapeutic properties like antimicrobial, antiviral, anticancer and contraceptive activities. Also, they have been shown to protect against topical and systemic infections in combination with conventional antibiotics [17].

Among organisms belonged to *Bacillus* genus *Bacillus licheniformis* represents a unique specie which produces grate variety of antimicrobial substances. This bacterium is considered as a promising probiotic for use in the treatment of dysbacteriosis caused by various diseases [13]. The effectiveness of *B. licheniformis* as probiotic is associated with the ability to produce a significant amount of substances with antimicrobial, antioxidant and immunomodulatory activities [13], For example, a phosphorus-containing triene antibiotic – proticin [18,19]. The protective effect of *B. licheniformis* against zebrafish (Danio rerio) infected with *Vibrio parahaemolyticus* has been demonstrated. Due to the antagonistic activity of this probiotic, the complete survival of infected fish was observed, in contrast to fish not treated with *B. licheniformis* [20]. This probiotic in combination with *Bifidobacterium breve* significantly inhibits the adhesion of the pathogen *Kocuria rhizophila in vitro* [21] and revealed anti-vibrio activity against *Vibrio parahaemolyticus* [22]. The use of a crude extract from *B. licheniformis* resulted in a marked manifestation of antiviral activity against porcine epidemic diarrhea virus in Vero cells and reduced virus shedding in piglets [23]. After *B. licheniformis*-fermented products administration, the number of pathogenic bacteria including *Clostridium perfringens* decreased significantly in cats with chronic diarrhea [24]. Also in piglets, *B. licheniformis* treatment had positive effects against *Salmonella* [25]. Many works demonstrate that probiotic *B. licheniformis* produces antimicrobial substances and has a high ability to auto- and coaggregate against pathogenic bacteria [26]. Approaches are being developed to combat bacterial biofilms using silver nanoparticles and the probiotic *B. licheniformis* [27].

Bacteriocins from *B. licheniformis* are being considered as potential natural preparations for use in the food industry to preserve food [28,29].

In general, bacteriocins are a promising group of antimicrobial peptides that may represent a potential alternative to classical antibiotics in the fight against antimicrobial resistance in pathogenic microorganisms. There are many reports in the literature about numerous bacteriocins, many of which currently remain undiscovered due to the huge variety of their natural sources, which requires further research in this area[11].

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Taking in consideration of medical and industrial application of *Bacillus licheniformis* it needs thorough describing and characterization of variety of antimicrobial compounds produced and their use against resistant pathogens such as mycobacteria.

Therefore, this review focused on the current state of knowledge about classes of antibiotic substances produced by *B. licheniformis* and their structure and properties that may allow a more comprehensive perspective of their antimicrobial potential including antimycobacterial properties.

Antibacterial substances secreted by Bacillus licheniformis

The endospore-forming bacterium *Bacillus licheniformis* is capable of producing a significant amount of substances of different structures with different antibacterial activity [30]. When grown on the identical medium, ddifferent strains of *B. licheniformis* produce a different set of substances with antibacterial activity [31]. The secreted antimicrobial substances have molecular masses ranging from 1.4 to 20 kDa [28,29,32–38].

At the same time, variation in medium composition for *B. licheniformis* growth results in alteration of repertoire of secreted substances. Thus, on media containing iron, *B. licheniformis* is capable of synthesizing the red pigment pulcherrimin [39]. When growing on a medium with lactate and a high ratio of nitrogen and carbon, *B. licheniformis* Weigmann emend. Gibson can produce licheniformins, and when grown on a medium with glucose and a low nitrogen/carbon ratio, this strain produces bacitracins [37]. Several substances synthesized by *B. licheniformis* have been described and investigated as antibiotics against various types of bacteria. Their list and characteristics will be given below. Some of them (bacitracin) are used in combined antibacterial preparations intended for topical use. Others are used as oral antibiotics, but only in animals due to toxic effects.

When grown on the identical medium, different strains of *B. licheniformis* produce a different set of substances with antibacterial activity [31]. Among the antimicrobial components (Table 1, 2) that various strains of *B. licheniformis* can produce in a nutrient medium, there are several groups that differ in properties and structure.

Table 1. Substances produced by *Bacillus licheniformis* with antimicrobial activity.

1.Bacteriocins				
Class I - heat stable lanthionine-containing peptides smaller than 5 kD				
Substance(s) specific/unspecific name	**Producing strain**	**Molecular mass**	**Activity assay**	**Reference**
Sublichenin	B. licheniformis MCC 2512	3348 kDa	*Kocuria rhizophila ATCC 9341* *Pediococcus lolii MCC 2972* *Enterococcus durans B20G1* *Enterococcus faecalis MF3* *E. faecalis MM2* *E. faecalis CHL1* *E. faecalis CHL3* *E. faecalis CHL* *E. faecalis MCC 3063* *E. faecalis MCC 2773* *Enterococcus faecium MCC 2763* *Entercoccus avium CS32* *Enterococcus cecorum 1-40a* *Lactobacillus plantarum MCC 2774* *Listeria monocytogenes* *Staphylococcus aureus* *Staphylococcus aureus (MRSA)*	[53]

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			Escherichia coli *Klebsiella pneumoniae*	
Lichenicidin	*Bacillus licheniformis* DSM 13 (also produced by ATCC 14580, VK21, WIT 562, 564 and 566 strains, IMF20, IMF66, IMF69 and IMF80)	3 kDa and 3,25 kDa	*Bacillus cereus DSM 31* *Bacillus halodurans DSM 18197* *Bacillus megaterium KM (ATCC 13632)* *Bacillus subtilis 168 (DSM 402)* *Bacillus spec. HIL Y-85,54728* *Enterococcus faecium BM 4147–1* *Enterococcus faecium L4001* *Lactobacillus sake 790 E2* *Lactococcus lactis NCTC 497* *Micrococcus luteus DSM 1790* *Micrococcus luteus ATCC 4698* *Staphylococcus aureus ATCC 33592 (MRSA)* *S. aureus ATCC 29213 (MSSA)* *S. aureus 1450/94* *S. aureus Cowan (ATCC 12598)* *S. aureus Newman (NCTC 8178)* *S. aureus SG511* *S. aureus Wood 46 (ATCC 10832)* *Staphylococcus carnosus TM300* *Staphylococcus gallinarum Tu¨ 3928* *Staphylococcus saprophyticus DSM 20229* *Staphylococcus simulans 22* *S. aureus LT440/09 (community acquired MRSA)* *S. aureus LT420/09 (MRSA)* *S. aureus LT819/09 (MRSA, Rhine-Hessen epidemic strain)* *Enterococcus faecalis* *Streptococcus agalactiae*	[56,58,59,61, 219]
class II – heat stable non-lanthionine peptides smaller than 10 kDa				
Peptides active only against Gram-positive microorganisms				
Bacillocin 490	*B. licheniformis* 490/5	2 kDa	*Bacillus licheniformis 5 A2* *Listeria innocua our isolates* *Staphylococcus epidermidis our isolates* *Bacillus anthracis 7700* *Bacillus subtilis AZ56* *Bacillus cereus 6A2* *Bacillus stearothermophilus 9A19* *Bacillus smithii PRO/S*	[28]
bacteriocin-like substance	*Bacillus licheniformis* H1	3,5 kDa	*E. faecalis ATCC 19433* *L. monocytogenes ATCC 19111* *B. cereus ATCC 14579,* *B. subtilis ATCC 6633* *Lactobacillus species ATCC 33198* *Lactobacillus fermentum*	[64]

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			P. fluoresce	
Bacteriocin-like antibacterial peptides	*B. licheniformis* AnBa9	<10 kDa	*Staphylococcus aureus GCS1* *Bacillus cereus GCS2* *Staphylococcus epidermidis GCS4* *Kurthia gibsonii GCS6* *Micrococcus luteus GCS7* *Streptococcus mitis GCS9* *Bacillus subtilis B-4219* *L. lactis B-1821* *Staphylococcus epidermidis B-4268* *Bacilus smithii NRS-173* *Lactobacillus acidophilus B-4495* *Micrococcus luteus B-287* *Pediococcus acidilactici B-14958* *Leuconostoc mesenteriodes*	[35]
Lichenin	B. licheniformis 26L10/3RA	1400 Da	*Streptococcus bovis SB3* *Streptococcus bovis 26* *Ruminococcus avefaciens OF-2* *Ruminococcus avefaciens C94* *Ruminococcus albus B119* *Ruminococcus albus A-6* *Butyrivibrio fibrisolvens OR 12* *Eubacterium ruminantium GA-195 Lactobacillus casei ED-108*	[32]
Bacteriocin BL8	*B. licheniformis* BTHT8	1,4 kDa	*Clostridium perfringens* *Staphylococcus aureus* *Bacillus cereus* *Bacillus circulans* *Bacillus pumilus*	[65]
BSCY2	B.licheniformis CY2	6500 Da	*B.subtilis 6633*	[66]
Secondary Antimicrobial Metabolites VK1, VK2	*B. licheniformis* VK2 and VK21		*Pseudomonas putida I-97* *Staphylococcus sp. SA1* *Rhodococcus sp. SS1* *Bacillus megaterium VKM41* *Micrococcus luteus E509*	[67]
Licheniocin 50.2	B. licheniformis VPS50.2	3,25 KDa	*Bacillus subtilis ATCC 6633* *B. subtilis 168* *B. subtilis W23* *Enterococcus faecalis ATCC 29212* *Enterococcus saccharolyticus ATCC 43076* *Lactobacillus plantarum LMG92088 Lactobacillus zeae* *Lactococcus lactis IL1403* *Listeria monocytogenes ATCC 19111* *Micrococcus luteus ATCC 7468* *Staphylococcus aureus ATCC 25923* *Staph. aureus ATCC 33591* *Streptococcus agalactiae ATCC 12386*	[68]

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A89	*Bacillus licheniformis I89*	3249 Da	*Bacillus subtilis ATCC 14593* *Micrococcus luteus ATCC 9341* *Staphylococcus aureus ATCC 6538* *Staphylococcus aureus (hospital isolate)*	[69]
Peptides active against both Gram-positive and Gram-negative microorganisms				
Bacteriocin like inhibitory substance (BLIS)	*Bacillus licheniformis* IITRHR2 (FJ447354)	1,2 kDa	*Bacillus cereus MTCC1305* *Bacillus subtilis MTCC736* *Bifidobacterium bifidum NCDC235* *Enterococcus faecalis MTCC439* *Enterococcus faecalis NCDC114* *Lactobacillus casei NCDC017* *Lactobacillus lactis NCDC094* *Leuconostoc mesenteroides NCDC219* *Listeria monocytogenes MTCC387* *Listeria monocytogenes MTCC1143* *Pediococcus pentosaceus NCDC273* *Staphylococcus thermophilus NCDC074* *Escherichia coli MTCC1687* *Pseudomonas aeruginosa MTCC9027* *Shigella flexneri MTCC1457* *Shigella sonnei MTCC2957*	[70]
Bacteriocin MKU3	*B. licheniformis* MKU3	1,5 kDa	*Bacillus subtilis B4219* *Bacillus smithii NRS173* *Lactobacillus acidophilus B4495* *Lactobacillus fermentum B1840* *Lactobacillus lactis B1821* *Staphylococcus epidermidis B4268* *Micrococcus luteus B287* *Leuconostoc mesenteriodes B1118* *Pediococcus acidilactici B14958* *Staphylococcus aureus GCS1* *Bacillus cereus GCS2* *Bacillus cereus GCS3* *Staphylococcus epidermidis GCS4* *Staphylococcus epidermidis GCS5* *Kurthia gibsonii GCS6* *Micrococcus luteus GCS7* *Bacillus subtilis GCS8* *Streptococcus fecalis GCS9* *Bacillus cereus GCS10* *Bacillus cereus GCS11* *Lactobacillus acidophilus GCS12* *Escherichia coli DH5a* *Candida albicans MTCC 183* *Aspergillus niger MKU1*	[34]

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			Aspergillus fischeri FXN1 *Aspergillus fumigatus MKU3*	
Bacteriocin-like substance	*B. licheniformis B116*	4 kDa	*B. cereus CGMCC1.230* *Listeria monocytogenes CVCC1599* *Micrococcus luteus CMCC28001* *S. aureus CMCC26003* *S. aureus CICC21601* *S. aureus CVCC1885* *Streptococcus equi subsp. zooepidemicus CVCC1903* *E. coli CVCC245* *E. coli CICC21525* *E. coli CVCC195* *E. coli CVCC249* *S. enterica ser. Pullorum CVCC79301* *S. enterica ser. Typhimurium CVCC541d*	[71]
ppABP	*Bacillus licheniformis Me1 (MCC 2016)*	Between 3 and 3,5 kDa	*L. innocua FB 21* *L. murrayi FB 69* *M. luteus ATCC 9341* *L. monocytogenes Scott A* *Staph. aureus FRI 722* *B. cereus F 4433* *Salm. typhimurium MTCC 1251, FB 231* *Salm. paratyphi FB 254* *E. coli CFR 02* *Y. enterocolitica MTCC 859* *K. rhizophila ATCC 9341* *Shigella flexineri (clinical isolate)*	[72–74]
Licheniformins A,B,C		3800-4800 Da	*Mycobacterium phlei* *E. coli* *Staphillococcus aureus*	[37,75]
Antimicrobial compound	*Bacillus licheniformis MCC2514*	o6.4 kDa	*M. luteus* ATCC*9341* *S. aureus FRI722* *Klebsiella sp.* *A. hydrophila NRRL B445*	[31]
Peptides active against fungal pathogens				
Bacteriocin-like peptides	*Bacillus licheniformis ZJU12*	3 kDa	*Bacillius subtilis (bean curd isolate)* *Enterococcus faecium (clinical isolate)* *Micrococcus flavus (bean curd isolate)* *Staphylococcus aureus ATCC 25923* *Staphylococcus aureus (clinical isolate, MRSA)*	[33]

			Staphylococcus epidermidis (clinical isolate) *Xanthomonas oryzae pv.oryzae Zhe 173* *Alternaria brassicae (cabbage isolate)* *Fusarium oxysporum (cotton isolate)* *Guignardia sp. (shihu isolate)* *Pyricularia grisea (rice isolate)* *Rhizoctonia solani (rice isolate)*	
Antibiotics culture filtrate	*Bacillus licheniformis* strain MGrP1		Colletotrichum lindemuthianum C. kahawae Fusarium oxysporum f.sp. phaseoli Alternaria solani	[76]
Fungicin M-4	*Bacillus licheniformis M-4*	3600 Da	*Microsporum canis CECT 2797* *Mucor mucedo CECT 2653* *Mucor plumbeus CCM F 443* *Sporothrix schenckii CECT 2799* *Bacillus megaterium* *Corynebacterium glutamicum CECT 78*	[220]
Peptide A12-C	*B. licheniformis* A12	770 Da	*Microsporum canis CECT 2797* *Mucor mucedo CECT 2653* *M. plumbeus CCM F 443* *Sporothrix schenckii CECT 2799* *Trychophyton mentagrophytes CECT 2793* *Bacillus megaterium* *Corynebacterium glutamicum CECT 78* *C. glutamicum CECT 80* *Sarcina sp.* *Mycobacterium phlei*	[46]
QSM (ComX pheromone)	*B. licheniformis* NCIMB 8874	ND	A. flavus NRL 3375 and ESP 15	[78]
Amoebolytic substances from B. licheniformis				
Peptide A12-A и A12-B	*Bacillus licheniformis A12*	1,430-1,600 Da	*Acanthamoeba sp. strain Gr-1* *N. fowlen S-3 (= ATCC 30809)* *N. fowlen HB-1 (= ATCC 30174)* *N. lovaniensis Aq/9/1/45D* *N. gruberi CCAP 1516/le* *Candida albicans CECT 1394* *Cryptococcus neoformans CECT* *Saccharomyces heterogenicus* *Aspergillus niger CECT 2089* *Microsporum canis CECT 2797* *Mucor mucedo CECI 2653* *Mucorplumbeus CCM F443* *Sporothrix schenchii CECT 2799*	[79]

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			Ttychophyton mentagrophytes *CECT 2793* *B. megaterium* *Cotynebactenum glutamicum* *CECT 78* *Sarcina sp.*	
Amoebicins M4-a,b,c	*Bacillus licheniformis M-4*	3 kDa-3,2 kDa	*Acanthamoeba sp. Gr-1* *Naegleriafowleri S-3 (= ATCC 30809)* *N.fowleri HB-1 (= ATCC 30174)* *Naegleria lovaniensis Aq/9/1/45D* *Naegleria gruberi CCAP 1516/le* *Aspergillus niger CECT 2089* *Candida albicans CECT 1394* *Cryptococcus neoformans CECT 1075* *Microsporum canis CECT 2797* *Mucor mucedo CECT 2653* *Mucorplumbeus CCM F443* *Penicillium sp.* *Rhizopus oryzae CECT 2340* *Saccharomyces cerevisiae* *Sporothrix schenckii CECT 2799* *Trychophyton mentagrophytes CECT 2793* *Alcaligenes faecalis* *Bacillum megaterium* *B. megaterium (spores)* *Corynebacterium glutaminicum CECT 78*	[80]
Amoebicins d13-A, d13-B and d13-C	*B. licheniformis strain D-13*	1,870 Da	*Acanthamoeba sp. strain Gr-1* *N. fowleri S-3 (= ATCC 30809)* *HB-1 (= ATCC 30174)* *Naegleria lovaniensis Aq/9/1/45D* *Naegleria gruberi CCAP 1516/le* *Alcaligenes facecalis* *B. licheniformis M-4, A12* *Bacillus megaterium* *Corynebacterium glutamicum CECT 78* *Enterococcus faecalis S-13, S-14, S-48, S-86* *Micrococcus luteus* *Mycobacterium phlei* *Pseudomonas reptilovora N5*	[81]
class III – heat-labile proteins larger than 10 kDa				
BLIS_SXAU06	*B. licheniformis* SXAU06	14 kDa	*S. aureus* *S. epidermidis* *M. luteus* *L. monocytogenes*	[82]
BL-DZ1 (BL00275)	*B. licheniformis* strain D1	14 kDa	*Candida albicans* BH	[83]

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			Pseudomonas aeruginosa PAO1 biofouling *Bacillus pumilus* TiO1	
Antifungal protein	*B. licheniformis* HS10	55 kDa	*Phytophthora capsici* *Botrytis cinerea* *Sclerotinia sclerotiorum* *Bipolaris maydis* *Fusarium graminearum* *Bipolaris sorokinianum* *Gaeumannomyces graminis*	[84]
YbdN Protein	*B. licheniformis* (seaweed isolate)	30,7 kDa	*MRSA 9551* *MRSA J2407* *VRE 788* *VRE 1349* *L. monocytogenes NCTC 7973,NCTC 10357*	[85]
Chitinase	B. licheniformis MY75 (also produced by Mb-2,TP–1, S213, SSCL-10, B307 strains)	55 kDa	*G. saubinetii* *A. niger*	[86]
Antifungal Protein F2	*B.licheniformis* BS-3	31 kDA	*Aspergillus niger* *Magnaporthe oryzae* *Rhizoctonia solani* *Fusarium oxysporum (schl.)f.sp. momordicae.*	[92]
Antimicrobials protein	*B. licheniformis JS*	16 kDa	*Bacillus cereus* *Bacillus subtilis* *Shigella dysenteriae* *Salminella typhimurium*	[93]
AMS	B. licheniformis T6-5	20 kDa	*Desulfovibrio alaskensis NCIMB 1349*	[94,221]
AMS	*B. licheniformis* H2O-1	between 90 and 120 kDa	*Desulfovibrio alaskensis NCIMB 1349* *SRB-containing consortium T6-lab*	[94,221]
class IV – complex with a single lipid or carbohydrate moiety				
F4, F5 and F6	*B. licheniformis* BFP011	Less than 45 kDa	*B. amyloliquefaciens* TISTR 1045 *B. licheniformis* TISTR 1010 *B. subtilis* ATCC 6633 *B. subtilis* TISTR 008 *B. pumilus* TISTR 905 *B. cereus* ATCC 11778 *B. megaterium* (clinical isolate) *S. aureus* ATCC 25923 *E. coli* O157: H7 *S. typhi* ATCC 5784 *K. pneumonia* ATCC 17736 *V. cholarae* (clinical isolate) *C. capsici*	[95]
Ieodoglucomides A and B	*B. licheniformis 09IDYM23*	ND	*S. aureus* *B. subtilis*	[96]

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			B. cereus *S. Typhi* *E. coli* *P. aeruginosa* *C. albicans* *A. niger*	
Ieodoglucomide C and ieodoglycolipid	*B. licheniformis* 09IDYM23	ND	*Staphylococcus aureus* *Bacillus subtilis* *Bacillus cereus* *Salmonella typhi* *Escherichia coli* *Pseudomonas aeruginosa* *C. albicans* *A. niger* *R. solani* *C. acutatum* *B. cenerea*	[97]
class V - Bacteriocins with undetermined molecular weight				
Antipathogenic Metabolites	*Bacillus licheniformis (Upper arm skin isolate)*	ND	*Staph. aureus (ATCC 6538)* *Kl. pneumoniae subsp. Pneumonia (CMSOGH)*	[222]
Antipathogenic Metabolites	*Bacillus licheniformis (Upper arm skin isolate)*	ND	*Kl. pneumoniae subsp. pneumoniae*	[223]
Antimicrobial substance	B. licheniformis A-1-5B-AP	ND	*Porphyromonas gulae* 3/H *Prevotella intermedia* 1/P *Streptococcus mutans* ATCC 35668. *Micrococcus luteus* DSM 1790	[100]
Bacteriocin	*B. licheniformis* HJ2020 MT192715.1	ND	*Escherichia coli* 0157:H7 *Staphylococcus aureus* *Salmonella typhi* *Pseudomonas aeruginosa* *Bacillus cereus* *Candida albicans* *Bacillus subtilis*	[101]
2. Non-ribosomal biosynthesized peptides				
Bacitracin				
Bacitracin/Ayfivin	*Bacillus licheniformis* strain EI-34-6, NH-5	1,42 kDa	*M. tuberculosis* *M. smegmatis* *Actinomyces israeli* *Pantoea ananatis* *gram-positive cocci* *staphlococci* *streptococci* *corynebacteria* *Treponema pallidum* *T. vincenti,, anaerobic cocci* *clostridia* *neisseria* *Gonococci* *meingococci*	[106,110,124, 224]

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Antimicrobial compound (a variant of subpeptin and bacitracin)	*B. licheniformis* IMF1, IMF2, IMF5, IMF6, IMF22 and IMF78	1,42 kDa	*L. lactis* HP *L. bulgaricus* LMG 6901 *S. aureus* ST528 *S. agalactiae* ATCC 13813 *L. innocua* FH2333 *L. monocytogenes* LO28	[61]
Cyclic lipopeptides (Biosurfactants)				
Surfactin homologues				
Surfactin and lichenysin isoforms	*Bacillus licheniformis HSN 221*	1048-1063 Da	ND	[143]
Surfactin	*B. licheniformis* BC98	1035 Da	*Sclerotium sclerotinii* *Phomopsis phyllanthi* *Rhizoctonia bataticola* *Aspergillus niger* N 573 *Curvularia lunata* *Magnaporthe grisea* *Helminthosporium sp.* *Chaetomium sp.* *Fusarium verticillioides* *Pestaliopsis magnifera* *Gleosporium magnefera*	[145]
Lipopeptides	*B. licheniformis (soil isolate)*	1022 and 1036 Da	*B. licheniformis* *Pseudomonas aeruginosa* *Escherichia coli* *Candida utilis* *C. tropicalis* *Trichosporon cutaneum* *Saccharomyces cerevisiae* *Trichoderma reesei* *Penicillium oxalicum*	[225]
Lipopeptide biosurfactants	*B. licheniformis* MB01	994, 1008, 1022, and 1036 Da	*Escherichia coli* *Vibrio cholerae* *Vibrio parahaemolyticus* *Vibrio harveyi* *Pseudomonas aeruginosa* *Staphylococcus aureus* *Proteus species*	[146]
Lipopeptide biosurfactants (surfactin homologues and fengycin A,B)	*B. licheniformis* V9T14 (DSM 21038)	ND	*E. coli* CFT073 (biofilm formation)	[139,147]
Surfactin (major isoform – surfactin C)	*B. licheniformis* ATCC 12713	ND	*B. hyodysenteriae* ATCC *27164* *C. perfringens* ATCC*13124* *Staphylococcus aureus BCRC10780* *Eimeria* species	[149,150]

Surfactant BL86	*Bacillus licheniformis BL86*	from 979 to 1091 Da and varying in increments of 14 Da		[154]
BL1193 (non-lipopeptide type biosurfactant together with lipopeptides, plipastatin, and surfactin)	*B. licheniformis* F2.2	1,193 Da	*B. subtilis* *Pseudomonas aeruginosa* *Escherichia coli* Aspergillus niger, Penicillium sp. Fusarium sp. Cladosporium sp. (inhibited by plipastatin)	[156]
		Lichenysins		
Lichenysin	*B. licheniformis* NBRC 104464	ND		[161]
Lichenysins A	*B. licheniformis* BAS50	1,006-1,034 Da	*Acinetobacter calcoaceticus* *Alcaligenes eutrophus* *Bacillus sp. strain ATCC 39307* *Bacillus subtilis* *Escherichia coli* *Enterobacter sp. strain 306* *Pseudomonas fluorescens* *Pseudomonas proteofaciens* *Staphylococcus aureus*	[131]
Biosurfactant lichenoformin	*B. licheniformis* MS3	1438 Da		[226]
BLS	*B. licheniformis P40*	800 Da	*Bacillus cereus ATCC 14579* *Bacillus cereus (food isolate)* *Bacillus subtilis (food isolate)* *Corynebacterium fimi NCTC 7547* *Enterococcus faecalis (clinical isolate)* *Lactobacillus acidophilus ATCC 4356* *Listeria monocytogenes ATCC 7644* *Listeria inoccua (food isolate)* *Rhodococcus sp.* *Staphylococcus intermedius (clinical isolate)* *Streptococcus sp. (b-haemolytic)* *Streptococcus sp. (clinical isolate)* *Streptococcus sp. (clinical isolate)* *Aeromonas hydrophila (clinical isolate)* *Aeromonas sp. (clinical isolate)*	[29,166]

 doi:10.20944/preprints202305.2001.v1

			Enterobacter aerogenes (food isolate) *Erwinia carotorovora (food isolate)* *Erwinia carotorovora 309 (food isolate)* *Erwinia carotorovora A325 (food isolate)* *Pasteurella haemolytica (clinical isolate)* *Salmonella Gallinarium (clinical isolate)*	
Fengycins				
Fengycins A,B (and surfactin homologues)	*B. licheniformis V9T14 (DSM 21038)* (also produced by *Bacillus licheniformis* B6)	ND	*E. coli* CFT073 (biofilm formation)	[139,147,148]
Lipopeptides (fengycins A and B, iturin, kurstakin, surfactin isophorms)	*Bacillus licheniformis B6*	1200-1650 Da	*Staphylococcus aureus* *Escherichia coli* *Klebsiella* sp. *E. coli*	[148]
Other lipopeptides				
Biosurfactant M104			*Bacillus subtilis ATCC 6633* *Bacillus thuringiensis var. kurstaki ATCC 19266* *Bacillus thuringiensis ATCC 10792* *Bacillus cereus ATCC 9634* *Staphylococcus aureus ATCC 25928* *Methicillin-resistant Staphylococcus aureus (MRSA), ATCC 25928* *Pseudomonas aeruginosa ATCC 10145* *Escherichia coli ATCC 11775* *Escherichia coli ATCC 11246* *Salmonella typhimurium ATCC 14028* *Proteus vulgaris ATCC 13315* *Candida albicans ATCC 70014*	[227]
Antiadhesin (I)	*B. licheniformis* 603	ND	*Corynebacterium variabilis Ac1122* *Acinetobacter sp.*	[177]
CB-1	*Bacillus licheniformis*	42 kDa	Pyriculariz oryzae *MAFF 101002* Rhizoctonia solani *CF-1* Corticium rolfsii *MAFF 712043* Tyromyces palustris *MAFF 420001* *Botrytis cinerca MAFF 712057* Coriolus versicolor *CF-2*	[178]

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			Fusarium oxysporum NFRI 1011 *Saccharomyces cerevisiaeY02587* *Escherichia coli K-12* *Bacillus cereus NFRI 8004*	
NIOT-AMKV06	*Bacillus licheniformis NIOT-AMKV06*	ND	*Proteus mirabilis MTCC142* *Vibrio cholerae MTCC3904* *Klebsiella pneumoniae MTCC109* *Enterococcus faecalis MTCC439* *Bacillus subtilis MTCC441* *Staphylococcus aureus MTCC96* *Shigella flexineri MTCC1457* *Micrococcus luteus MTCC1541* *Salmonella typhi MTCC734*	[179]
3.Exopolysaccharides				
Levan (fructan)	*B. licheniformis* BK1, BK2	~2-100 × 10^6 Da	*Staphylococcus aureus* *E. coli* *Pseudomonas aeruginosa*	[186]
EPS1	*B. licheniformis* 24		*Vibrio cholerae* non-O1	[185]
Bl-EPS	*B. licheniformis* Dahb1		*B. subtilis KT763078.1* *B. pumilus* Dahb3 *HQ693273.1* *P. aeruginosa Dahp1 (HQ400663.1)* *P. vulgaris Dahp1 (HQ116441.1)* *C. albicans*	[189]
EPS-T14	*B. licheniformis* T14	1000 kDA	*multiresistant clinical strains:* *Escherichia coli* *Klebsiella pneumonia* *Pseudomonas aeruginosa* *Staphylococcus aureus*	[190]
Exopolysaccharide	*B.licheniformis SP1*	1800 kDa	*Escherichia coli PHL628* *Pseudomonas fluorescens (only biofilms formation)*	[191]

Table 2. Substances produced by Bacillus licheniformis with antimycobacterial activity.

Substance name	Molecular mass	Sensitivity to enzymes	Sensitivity to temperature	Reference
Bacitracin/Ayfivin	1.42 kDa	ND	Resistant to temperature under 60 °C.	[104,110]
Proticin	560.666 Da	ND	ND	[38]
Peptide A12-C	770 Da	resistant to trypsin, pronase and proteinase K, carboxypeptidase A, alkaline phosphatase, lipase, lysozyme, β-glucosidase and β-glucuronidase	resistant to heat (100°C for 30 min at pH 7.0)	[46]
Licheniformins	3800-4800 Da	ND	ND	[37]

 doi:10.20944/preprints202305.2001.v1

Amoebicins d13-A, d13-B, and d13-C	1870 Da	Resistant to trypsin, pronase, proteinase , lipase and β-glucuronidase	retained 100% of the activity after being heated at 100°C for 30 min and also after being stored at -20°C for 6 months	[81]

1. Bacteriocins

Bacteriocins - substances represented by an amino acid sequence (peptides or proteins) and acting against other strains of bacteria or closely related species. They demonstrated both bactericidal and bacteriostatic effects. Bacteriocins are natural antimicrobial peptides that are synthesized by bacteria ribosomally [10,11,40].Genes whose expression leads to the synthesis of bacteriocins are organized into clusters of operons and can be located in the genome, plasmids, or other mobile genetic elements. These genes are inducible; peptide secretion and accumulation outside the cell is required for their induction. More details of bacteriocins biosynthesis described in review Nishie et al.[9]. Bacteriocins are heterogeneous substances that demonstrate variable biochemical properties, molecular weights, inhibitory spectra and mechanisms of action [10,41] Due to the wide spectrum of antagonistic activity inherent in bacteriocins of some strains of microorganisms, they have the potential for use as part of antibacterial drugs. Many antimicrobial peptides produced by *Bacillus spp*. have different resistance to enzyme activity, with stability over a wide range of pH and temperature. The most of these peptides have high specificity against microbial pathogens and low cytotoxicity against human cells [42]. The sensitive bactericidal mechanisms include the pore-forming type, a nuclease type with DNase and RNase function, and peptidoglycanase type etc. [10].

As a result, the formation of pores occurs, which leads to the rapid removal of small cytoplasmic molecules, ions from target cells and the collapse of the proton motive force, which results in the death of bacterial cells [9,43]. However, other antimicrobial mechanisms of bacteriocins have been also proven [11]. Despite the popularity of research on the properties of bacteriocins in recent years and their usage in medicine, veterinary and food industry [10,11]. Many bacteriocins have not yet been studied, and this line of research is relevant.

The production of several bacteriocin-like substances (Table 1) with different characteristics and a wide spectrum of activity against pathogenic bacteria was recorded for *B. licheniformis* strains [44]. For example, *B. licheniformis* SMIA-2, a thermophilic and thermostable enzyme-producing strain, is demonstrated to be active against some strains of *Staphylococcus aureus* and *Bacillus sp*. Genome annotation of this strain detected gene clusters responsible for antimicrobial component production (lichenysin, fengycin, lichenicidin and bacillibactin biosynthetic gene clusters) [45].

B. licheniformis produces various bacteriocins ranging in molecular weight from 1.4 kDa to 55 kDa, but the expression of particular antimicrobial agent may depend on environmental conditions, growth period, and the specific strain of this bacterium [28,32,33,36,46].

In general, based on thermostability, size and chemical moieties, bacteriocins are classified into four major groups [47]: class I – heat stable lanthionine-containing peptides smaller than 5 kDa, class II – heat stable non-lanthionine peptides smaller than 10 kDa, class III – heat-labile proteins larger than 30 kDa, and class IV – complex with a single lipid or carbohydrate moiety [48]. In this review, for the description of antimicrobial substances produced by different strains of *B. licheniformis* we used the Cotter's classification with a slight modification: heat-stable and heat-labile proteins larger than 10 kDa were assigned to class III and added class V - proteins with undetermined molecular weight.

1.1. Class I - heat stable lanthionine-containing peptides smaller than 5 kDa

Lantibiotics are antimicrobial peptides that undergo post-translational modification. They contain non-standard amino acids: lanthionine, β-methyl lanthionine and dehydrated residues (dehydrated amino acids) [49]. Their molecular weight does not exceed 5 kDa. Lantibiotics are

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active at low concentrations and therefore are attractive antimicrobials. They mainly target important targets such as lipid II. A number of lantibiotics interact with the cell wall precursor lipid II (undecaprenyl-Pyrophosphoryl-MurNAc-(pentapeptide)-GlcNAc), which prevents cell wall biosynthesis and contributes to the destruction of the bacterial membrane [50]. Thus, the most well-studied lantibiotic nisin interacts with the pyrophosphate fragment of lipid II. Critical to this binding are the two N-terminal rings of the lantibiotic [51]. Formation of the pore complex results in cell membrane permeabilization and dissipation of the proton motive force [50].

In general, lantibiotics are synthesized and secreted by Gram-positive microorganisms and their activity is most often manifested in relation to closely related Gram-positive bacteria. In relation to Gram-negative bacteria their activity is rather limited [49]. Since the cell wall of Gram-negative bacteria is an effective permeability barrier due to the presence of an outer membrane, which creates an obstacle to access to the peptidoglycan layer (localization of lipid II) and the cytoplasmic membrane. Moreover, the anionic cell surface of Gram-negative bacteria promotes the binding of cationic lantibiotics, where such an interaction potentially increases the stability of the outer membrane through electrostatic interactions [52].

B. licheniformis produced two lantibiotics which may be important for applications in various industries.

The first one is sublichenin **-** subtilin-like lantibiotic of probiotic bacterium *Bacillus licheniformis* MCC 2512 has a molecular weight 3348 Da and the succinylated form of sublichenin with molecular weight 3448 Da [31,53]. Subtilin - a natural variant of nisin refers to linear pentacyclic class-I antibiotics [54]. Sublichenins from *B. licheniformis* are almost identical to subtilin JS-4 from *B. subtilis*. Subtilin JS-4 retained >90% and 86.1% of its antibacterial activity even after a 30 min exposure to 80-100^{0}C and 121^{0}C respectively, indicating considerable thermostability. Subtilin JS-4 was also rapidly inactivated by proteolytic enzymes including proteinase K, trypsin, papain and pepsin [55]. It also showed a broad antimicrobial spectrum against Gram-positive bacteria. Subtilin JS-4 inhibited the growth of foodborne bacteria *L. monocytogenes* by increasing cell membrane permeability, triggering pore formation and K^{+} leakage, and damaging cell membrane integrity, which eventually disrupted the membrane and cellular structure [55].

The second antibiotic is lichenicidin - a dipeptide lantibiotic consisted of synergetic lantibiotic pair, Licα (3251.699 Da) and Licβ (3021.762 Da) that was described for *B. licheniformis* DSM 13. This substance demonstrated activity against the growth of Gram-positive bacteria as *Bacillus subtilis*, *Micrococcus luteus*, *Staphylococcus aureus*, Streptococcus pyogenes, *Staphylococcus simulans* and *enterococci* but neither caused hemolysis nor inhibited the growth of Gram-negative bacteria. Lichenicidin is associated with the cell surface and shows stability against trypsin, chymotrypsin and the proteases [56]. Moreover, it has been shown that lichenicidin can be produced by other strains of *B. licheniformis*, and the structure of its peptides may differ depending on the producing strain. Lichenicidin was not cytotoxic to human erythrocytes and fibroblasts [57]. *B licheniformis* strain ATCC 14580 produced lichenicidin with activity against a range of pathogenic microorganisms including *Listeria monocytogenes*, *Staphylococcus aureus*, vancomycin-resistant enterococci, *Bacillus cereus*, *Streptococcus pneumoniae* and Streptococcus mutants [58]. Lichenicidin also has been demonstrated to be produced by *B. licheniformis* strain, VK21 [59]; WIT 562, 564 and 566 [60]. Also, lichenicidin production was found for *B. licheniformis* isolates (isolated from retail infant milk formulae) - strains IMF20, IMF66, IMF69 and IMF80. These strains demonstrated antimicrobial activity against the Gram-positive target organisms. No activity was observed against the Gram-negative bacteria *E. coli* and *S. typhimurium* [61].

The lichenicidin consists from the two mature peptides, Bliα and Bliβ, the synergistic activity of their is required for full activity. The lichenicidin acts through a dual mode of action that involves Bliα recognition of lipid II, providing specificity and stability for the interaction of Bliβ that induce leakage of the intracellular contents of bacteria [62,63].

1.2. Class II – heat stable non-lanthionine peptides smaller than 10 kDa

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This class includes unmodified peptides with a molecular weight up to 10 kDa. The overwhelming majority of them are thermostable membrane-active peptides. Among them, peptides that are active only against Gram-positive microorganisms and active against both Gram-positive and Gram-negative microorganisms can be distinguished. Peptides with antifungal and amoebolytic activity were also identified.

1.2.1. B. licheniformis secreted peptides active only against Gram-positive microorganisms

Since antagonism provides a survival advantage in the suppression of related species of microorganisms, it is not surprising that most bacteriocins secreted by different strains of *B. licheniformis* are active only against Gram-positive bacteria. Among them there are peptides insensitive and sensitive to the action of proteolytic enzymes. However, the vast majority of identified bacteriocins that are active only against Gram-positive microorganisms are sensitive to the action of proteinases.

So, Bacillocin 490, a bacteriocin with low molecular mass (2 kDa) produced by a thermophilic strain *B. licheniformis* 490/5 isolated from dairy foods, shows high thermal stability, with 46.4% residual activity after 1 h of exposure to 100°C. This bacteriocin was inactivated by pronase E and proteinase K. Bactericidal activity was kept during storage at 4°C and was remarkably stable in a wide pH range. The activity range of bacillocin 490 was limited to some Gram-positive bacteria. Highest antimicrobial activity was against *Bacillus stearothermophilus*, *B. smithii*, *B. subtilis* and *B. anthracis*. It was observed moderate inhibition of *B. cereus*, very low inhibition of *Listeria innocua* and *S. aureus* and no inhibition of *B. thuringe*nsis and *Streptococcus thermophilus*. This activity spectrum clearly shows that bacillocin 490 is active principally against species phylogenetically related to the producer strain. Incubation of *B. smithii* in the presence of bacillocin 490 resulted in 96% killing in 30 minutes, indicating that the bacteriocin has a bactericidal effect [28]

The supernatant of thermophilic strain *B. licheniformis* H1 exhibited antagonistic activity against various species of Gram-positive bacteria such as *Listeria monocytogenes* but not against Gram-negative bacteria except *Pseudomonas fluorescens*. Inactivation of this bacteriocin-like activity by a-chymotrypsin, trypsin, and papain was highly significant. There was no significant decrease in antimicrobial activity after incubation of bacteriocin-containing supernatant from *B. licheniformis* H1with pepsin or lipase. The bacteriocin-like substance was found to be stable at temperatures up to 75°C for 60 min, but it lost activity after being autoclaved at 121°C for 15 min. The concentrated antimicrobial activity was found in the protein fraction obtained with 60% ammonium sulfate saturation. Sodium dodecyl sulfate – polyacrylamide electrophoresis analysis of concentrated partially purified supernatants collected after resting the bacterial cells at 55°C revealed a bacteriocin-like protein with a molecular mass of approximately 3.5 kDa [64].

B. licheniformis AnBa9 produced antibacterial peptides of bacteriocin type with the molecular mass of <10 kDa. Production of these peptides was 25-fold higher under optimized condition for producer growth than under un-optimized condition. The level of this bacteriocin production and its specific activity were gradually decreased by increasing the concentration of lactose and NH_4NO_3. High concentration of yeast extract, alkaline pH and elevated temperature improved the production of antibacterial peptide by *B. licheniformis* AnBa9. *B. licheniformis* AnBa9 inhibited several Gram-positive bacteria *Staphylococcus aureus*, *Bacillus cereus*, *Staphylococcus epidermidis*, *Kurthia gibsonii*, *Micrococcus luteus*, *Streptococcus mitis*, *Bacillus subtilis*, *L. lactis*, *Bacillus smithii*, *Lactobacillus acidophilus*, *Pediococcus acidilactici* and *Leuconostoc mesenteriodes*. However, these bacteriocins did not inhibit *Listeria* strains and Gram-negative bacteria. Loss of antibacterial activity of permeate after the treatment with Proteinase K, Pronase E and Trypsin, suggested that these bacteriocins are sensitive to proteolytic enzymes. They are resistant to temperature up to 100 ºC for 30 min and wide range of pH from 4 to 12 [35].

Under anaerobic conditions *B. licheniformis* 26L10/3RA produced inhibitory bacteriocin-like component called Lichenin. This peptide was purified to homogeneity and having an estimated molecular mass of approximately 1400 Da. Lichenin was found to be hydrophobic, sensitive to atmospheric oxygen, retained biological activity even after boiling for 10 min and was active over a

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pH range of 4.0-9.0. It was active against *Streptococcus bovis*, *Ruminococcus albus*, *Ruminococcus avefaciens*, *Eubacterium ruminantium*. The biological activity of this peptide was completely inactivated by proteinase K treatment but the same was resistant to trypsin. Lichenin production was observed only upon *B. licheniformis* anaerobic growth and the antibacterial activity was also demonstrated only for the reference strains grown under anaerobic conditions. Inability of Lichenin to inhibit aerobically grown bacteria was explained either by inactivation of it by atmospheric oxygen or by the target bacteria due to oxidative respiration. No N-terminal block was observed in the sequence and the peptide did not show any characteristics of cyclicity. But the seventh amino acid residue could not be identified and it did not belong to any of the natural amino acids [32].

Strain BTHT8, identified as *B. licheniformis*, inhibited the growth of Gram-positive test organisms. The active component labelled as bacteriocin BL8 was purified from supernatant of strain *B. licheniformis* BTHT8. The molecular mass was determined as 1,4 kDa. N-terminal amino acid sequencing of BL8 gave a 13 amino acid sequence stretch. Bacteriocin BL8 was stable even after boiling at 100°C for 30 min and over a wide pH range of 1–12 [65].

A bacteriocin from *B. licheniformis* cy2 named as BSCY2 was stable in the pH range of 2.5-9.5. It is active against *B. subtilis*. BSCY2 was stable below 40^{0}C and it retained its antimicrobial activity during long tern storage at -20^{0}C and -70^{0}C. BSCY2 was inactivated 15 min exposure to temperatures over 80^{0}C and lost 50% of its antimicrobial activity within 2 hr at 70^{0}C. BSCY2 was inactivated by proteinase K treatment, which indicates its proteinous nature. Direct detection of the BSCY2 band showing antimicrobial activity on Tricine-SDS-PAGE suggested an apparent molecular mass of about 6,500 Da [66].

Strains *B. licheniformis* VK2 and VK21 isolated from thermal springs of the Kamchatka Peninsula produced peptides with antimicrobial activity against several gram-positive bacteria (*Staphylococcus sp.*, *Rhodococcus sp.*, *Bacillus megaterium*, *Micrococcus luteus*). Active substances were extracted with n-butanol. They were resistant after boiling for 30 min and action of trypsin and chymotrypsin but were partly hydrolyzed by pronase. They were stable at a pH range of 2.0–9.0 [67].

In contrast to above mentioned bacteriocins of this group there are bacteriocins which retain their activity after treatment with proteolytic enzymes.

The strain *B. licheniformis* VPS50.2 produced bacteriocin licheniocin 50.2 (molecular mass about 3.25 kDa) effective against Gram-positive bacteria, including *Listeria monocytogenes*, methicillin-resistant *Staphylococcus aureus* and b-haemolytic streptococci. The bacteriocin activity was insensitive to lysozyme and proteinase K, heat stable after incubation at 100°C for 30 min and over wide range of pH (2–12). The inhibitory spectrum recorded in this work was limited to Gram-positive bacteria only. The maximum antagonistic activity was found in the precipitate with 60% saturation of ammonium sulfate [68].

B. licheniformis strain I89 produced compound A89, which exhibited antimicrobial activity against a number of Gram-positive bacteria. The molecular weight of A89 was 3249.7 Da. A89 was resistant to proteolytic degradation because none of the tested proteases (aspartic (cardosin A and cardosin B) and serine proteinases (α-chymotrypsin, trypsin and endoproteinase Glu-C)) reduced its antimicrobial activity. The thermal stability of A89 was estimated from 37°C to 100°C [69].

Despite varying degrees of sensitivity to the action of proteolytic enzymes, bacteriocins of this group are resistant to elevated temperatures and wide pH values that makes them especially perspective for medical applications.

1.2.2. B. licheniformis secreted peptides active against both Gram-positive and Gram-negative microorganisms

Bacteriocins secreted by *B. licheniformis* and showing activity against both Gram-positive and Gram-negative microorganisms in the vast majority are also sensitive to the action of proteolytic enzymes, but resistant to elevated temperatures. At the same time, they have different sensitivity to a wide range of pH. All bacteriocins of this sub-group were sensitive to the action of proteinases.

Strain of *B. licheniformis* IITRHR2 produced bacteriocin like inhibitory substance (~1.2 kDa) which was thermostable (up to 80°C but showed decreased activity at higher temperatures) and pH

resistant but lost activity when subjected to proteinase treatment (proteinase K and pronase E). This bacteroicin inhibited various gram-positive bacterial strains such as *B. subtilis*, *B. cereus*, *Streptococcus thermophilus*, *Pediococcus pentosaceus*, *Leuconostoc mesenteroides*, *L. monocytogenes*, *Bifidobacterium bifidum*, *Enterococcus faecalis*. Growth of gram-negative bacteria *Shigella flexneri*, *Shigella sonnei* and *Pseudomonas aeruginosa* was also inhibited by this compound [70].

The culture supernatant of *B. licheniformis* MKU3 exhibited bacteriocin-like activity against of several type strains of Gram-positive bacteria such as *Bacillus subtilis*, *Bacillus smithii*, *Staphylococcus epidermidis*, *Micrococcus luteus*, *Leuconostoc mesenteriodes* and *Pediococus acidilactici*, *B. cereus*, *B. megaterium*, *K. gibsonii*, *Staphyloccus* sp., *Streptococcus* sp., *Micrococcus caseolyticus* (but not *Listeria sp.)*. On the other hand, Gram-negative bacteria such as *Serratia marcescens* and *Pseudomonas fluorescens* B10 were not inhibited by this bacteriocin excluding *Escherichia coli*. The extract showed significant activity against different fungi including *Aspergillus niger*, *A. versicolor*, *A. fischeri* and *A. fumigatus* and the yeast *Candida albicans*. The active substance apparently is a bacteriocin-like protein with a molecular mass of 1.5 kDa. This bacteriocin activity was found to be stable under a pH range of 3.0–10.0 and at temperatures up to 100^{0}C for 60 min, but inactivated by proteinase K, trypsin or pronase E. The bacteriocin lost its activity after incubation at 121^{0}C for 15 min. The composition of the medium affects the production of this bacteriocin [34].

A strain *B. licheniformis* B116 showed strong antimicrobial activity against *Staphylococcus aureus* and *Salmonella enterica ser. Pullorum*. The bacteriocin was precipitated by ammonium sulfate and its molecular mass was determined as ~4 kDa. Culture supernatant of this strain exhibited antimicrobial activity against both Gram-positive and Gram-negative bacteria, including *Bacillus cereus*, *Staphylococcus aureus*, *Listeria monocytogenes*, *Micrococcus luteus*, *Escherichia coli*, *Streptococcus equi* and *Salmonella spp*. The bacteriocin was resistant to heat, acid and alkaline treatment. Activity of the bacteriocin was totally lost after digestion by pronase and partially lost after digestion by papain and lipase. Inactivation by lipase indicated that the bacteriocin may contain a lipid moiety [71].

B. licheniformis MCC 2016 (strain was also named Me1) produced the antibacterial peptide ppABP that was completely abolished by proteinase K. The culture isolated from milk is able to produce a proteinaceous antibacterial peptide with molecular weight falling in the range of the antibacterial peptide is low-molecular weight and the size is between 3.0 and 3.5 kDa, which exhibits broad spectrum of inhibitory activity and is stable over a wide range of temperature and pH. The ppABPs were found to be thermally stable for 15 min at 80 ^{0}C. The SN of this strain exhibited inhibitory activity against both Gram-positive and Gram-negative food-borne and human pathogens [72,73]. The activated films with ppABP from *B. licheniformis* Me1 showed a zone of inhibition that did not confine to the film area, indicating that the ppABP diffused from the films into the medium [74].

Strains *B. licheniformis* Weigmann emend. Gibson produced antibacterial agents licheniformins with *in vitro* bacteriostatic activity against many organisms, including *Mycobacterium tuberculosis*. In addition to inhibiting the growth of mycobacteria, they showed efficacy against *Staphylococcus aureus* and *Escherichia coli* [75]. Peptides has molecular mass 3800, 4400 and 4800 Da.[37]

Strains B. *licheniformis* MCC2512 and MCC2514 exhibited inhibitory activity against *Micrococcus luteus*, *Staphylococcus aureus*, *Klebsiella sp*. and *Aeromonas hydrophila*. In addition to these pathogenic strains, B. *licheniformis* strain MCC2512 also had inhibitory activity against *Listeria monocytogenes* and *Salmonella typhimurium*. The activity of the bacteriocins from both cultures was completely lost on exposure to proteinase K, indicating the proteinaceous nature of the compound. Upon treating the sample with trypsin and pepsin, 100% activity was retained, but with a-amylase, 50% activity was lost. The isolated bacteriocins varied in their mechanisms of action and stability. The molecular weight of inhibitor components from MCC2514 and MCC2512 was found to be 6.5 and 3.5 kDa, respectively. *B. licheniformis* MCC2512 produced a subtilin-type antimicrobial compound that acts on cell wall synthesis. Whereas MCC2514 inhibited RNA synthesis [31]. Active substance produced by *B. licheniformis* MCC2512 was identified as sublichenin [53].

1.2.3. B. licheniformis peptides active against fungal pathogens

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An important characteristic of some bacteriocins is the ability to exhibit antifungal activity, which significantly expands the horizons of their application both in medicine and in agriculture, as well as in the food industry.

The cell-free supernatant of *B. licheniformis* ZJU12 isolated from soil exhibited pronounced antibacterial (for Gram-positive bacteria) activities. The bacteriocin-like peptides produced by *B. licheniformis* ZJU12 showed no activity against Gram-negative bacteria, but shows inhibitory activity against fungi (*Xanthomonas oryzae pv.oryzae, Alternaria brassicae*, *Fusarium oxysporum* and others). After treatment with proteinase K and trypsin, the antagonistic activity was lost completely. Estimated molecular mass by Tricine-SDS-PAGE of the antagonistic compound were approximately 3 kDa. These characteristics indicated that the antagonistic substances produced by this strain had the nature of bacteriocin. The activity was stable following temperature exposure up to 100 °C for 30 min, but lost completely at 121 °C for 15 min. The maximum antagonistic activity was found in the resolved precipitate of supernatant with 60% saturation of ammonium sulfate. It has low toxicity since no adverse effects to mice were detected at a dose of up to 0.8 mg/20 g in the acute toxicity tests [33].

B. licheniformis strain MGrP1 produced antibiotics in liquid media containing soyabean meal and mannitol that inhibited the growth of the plant fungal pathogens of agricultural importance, namely: *Colletotrichum lindemuthianum* (Bean anthracnose), *Colletotrichum kahawae* (Coffee berry disease), *Fusarium oxysporum f.sp. phaseoli* (Fusarium yellow) and *Alternaria solani* (Early blight). Paper chromatography combined with bioautography revealed two thermostable active compounds whose activity was optimal at pH 6. Low pH ranges and autoclaving temperatures significantly reduced the activity of the antibiotics [76].

The fungicin M–4 produced by *B. licheniformis* M–4 is composed of 34 amino acid residues of seven different amino acids, including four residues of ornithine per molecule. The same producing strain shows inhibitory activity against the human pathogenic amoeba *Naegleria fowleri*. Purified fungicin M-4 demonstrate antifungal activity against the pathogenic fungi *Sporothrix schenckii* and *Microsporum canis.* Fungicin M-4 was resistant to proteolytic enzymes and to lipase. Antifungal activity was fairly resistant to heat, although incubation at 80°C for 30 min caused 30% inactivation. Activity was stable in the range of pH from 2.5 to 9.0. Its molecular weight was 3600 Da. Attempts to deduce an amino acid sequence ware unsuccessful, suggestinf what fungicin may be a cyclic peptide or blocked at its amino-terminal end [77].

Peptide A12-C from *B. licheniformnis* A12 has a pronounced antifungal effect and is an acidic hydrophilic peptide with a mass of 770 Da, containing only six different amino acids. Peptide A12-C was resistant to such proteolytic enzymes as trypsin, pronase and proteinase K. It is resistant to carboxypeptidase A, alkaline phosphatase, lipase, lysozyme, β-glucosidase and β-glucuronidase. Peptide A12-C is resistant to heat (100°C for 30 min at pH 7.0) and incubation at room temperature under acidic conditions (pH 2.5), but loses 75% of activity after incubation at pH 9.5 for 30 min at room temperature. Peptide A12-C is active against several fungi (*Microsporum canis*, *Mucor mucedo*, *M. plumbeus*, *Sporothrix schenckii* and *Trichophyton mentagrophytes*) and bacteria (*Bacillus megaterium*, *Corynebacteriurn glutamicum*, *Sarcina* and *Mycobacterium - Mycobacterium phlei*) [46].

B. licheniformis NCIMB 8874 produced peptide ComX with antifungal activity against the fungal leaf pathogen *Alternaria alternata*. ComX consist from 13-amino-acid residue, Glu-Ala-Gly-Trp-Gly-Pro-Tyr-Pro-Asn-Leu-Trp-Phe-Lys [78].

1.2.4. Amoebolytic substances from B. licheniformis

Bacteriocins with amoebolytic activity have been identified. All of them were resistant to the action of proteolytic enzymes and elevated temperatures.

B. licheniformnis A12 produces two amoebolytic substances (amoebicins A12-A and A12-B) in liquid media during sporulation. Both substances are heat- and protease-resistant peptides containing aspartic acid, glutamic acid, serine, proline, and tyrosine in a molar ratio of 5:2:2:2:2. No fatty acids or carbohydrates have been detected. Both amoebicins retained 100% of their activity after being heated at 100°C for 30 min at pH 7.0. They were also resistant to incubation at room temperature under acidic conditions (pH 2.5), but lost 75% of their activity upon incubation at pH 9.5 for 30 min.

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The crude supernatants, as well as the purified substances, retained 100% of their activity after storage for 1 month at 4°C or for 6 months at -20°C. Amoebicins A12-A and A12-B were resistant to the enzymes trypsin, pronase, proteinase K, alkaline phosphatase, lipase, lysozyme, α-glucosidase, and 3-glucuronidase. They were also resistant to carboxy peptidase A, suggesting that a free carboxyl terminus was not present. Their molecular weight is 1,430-1,600 Da. Purified amoebicins A12-A and A12-B exhibit amoebolytic action against *Naegleria fowleri*. They also exhibit antibiotic action against yeasts (*Saccharomyces heterogenicus* and *Cryptococcus neoformans*) and several fungal species (*Aspergillus niger*, *Microsporum canis*, *Mucor plumbeus*, and *Trychophyton mentagrophytes*). Their antibacterial spectrum appears to be restricted to *Bacillus megaterium*, *Corynebacterium glutamicum*, and *Sarcina sp.* The amoebolytic effect was studied by electron microscopy. At 10 min after addition the characteristic shape of the cells changed. Firstly, they developed abnormal globular pseudopodia, and then they became rounded. After 30 min of incubation the cell membrane ruptured, with the release of abundant cytoplasmic material. All of this was followed by complete cellular destruction within 1 h. [79].

B. licheniformnis M-4 produced three antibiotic peptides (m4-A, m4-B, m4-C) with amoebolytic activity. They were active against human pathogenic and non-pathogenic strains of *Naegleria fowleri* - the causative agent of primary amoebic meningoencephalitis. The amoebicidal activity of these peptides was resistant to action of trypsin, proteinase K or carboxypeptidase A. They are cyclic peptides with molecular weights ranged from 3,000 to 3,200 Da. These peptides are composed of six different amino acids (Asp, Glu, Ser, Thr, Pro, Tyr), and there were only differed in the number of Asp residues. The three amoebicins had a broad antifungal spectrum, although peptide m4-C showed a two-fold higher specific activity against a variety of fungi and yeasts then others. The three peptides showed a narrow antibacterial spectrum, but *Bacillus megaterium* (not spores) was highly sensitive [80]. The amoebicins from *B. licheniformis* M-4 differ from those produced by strain A12 in molecular weight, in their amino acid composition (A12-A and A12-B contain threonine), in the number of residues per molecule and different solubility in water (A12-A and A12-B are not water soluble) [79,80].

B.licheniformis D-13 produces three hydrophobic peptides (amoebicins d13-A, d13-B, and d13-C) that elicit antiamoebic activity against human-pathogenic and nonpathogenic species of Naegleria and have a broad spectrum of antibacterial activity. The three amoebicins have the same amino acid composition and molecular weight 1,870 Da. The three amoebicins were stable in a pH range from 2.5 to 9.5, and they retained 100% of the activity after being heated at 100°C for 30 min and also after being stored at -20°C for 6 months. Since purified amoebicins were not soluble in aqueous buffers, a mixture of partially purified amoebicins in 20 mM Tris-HCl (pH 7.2) was tested for sensitivity to various enzymes. The mixture retained 100% of its activity after being treated for 1 h with proteases (trypsin, pronase, and proteinase K), lipase, or β-glucuronidase. Amoebicin d13-B causes lysis of amoebae through disorganization of the cell membrane. No amino acid residues were detected after the N-terminal sequence of amoebicin d13-B, suggesting that this peptide is cyclic or blocked at its amino terminus. [81]

1.3. Class III – Proteins larger than 10 kDa

This class includes unmodified peptides with a molecular weight larger than 10 kDa. In most cases, these are thermostable membrane-active peptides sensitive to proteinase treatment.

B. licheniformis SXAU06 produced a bacteriocin-like substance (BLIS) with an approximate molecular weight of 14 kDa designated as BLIS_SXAU06. It was active against *Escherichia coli*, *Salmonella enterica*, *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Micrococcus luteus* and *Listeria monocytogenes*. BLIS_SXAU06 exhibited high resistance to treatment of high temperature, high acidity and alkalinity, proteinase K, but it was fully inactivated by pronase E and partially inactivated by trypsin and pepsin. BLIS_SXAU06 was heterologously expressed in *E. coli* and the recombinant BLIS_SXAU06 exhibited effective antibacterial activity against *S. aureus*, *S. epidermidis*, *M. luteus*, and *L. monocytogenes* [82].

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When the tropical marine strain of *B. licheniformis* D1 grown in Luria Bertani (LB) broth containing tryptone medium it produces a 14 kDa protein BL-DZ1 (BL00275) with antimicrobial activity against pathogenic *Candida albicans* BH, *Pseudomonas aeruginosa* PAO1 and biofouling *Bacillus pumilus* TiO1 cultures. The antimicrobial activity was lost after treatment with trypsin and proteinase K. The protein was stable at 75ºC for 30 min and over a pH range of 3.0 to 11.0. The protein BL-DZ1 was able to inhibit both biofilm growth and disrupted pre-formed biofilms of *C. albicans*, *P. aeruginosa* and *B. pumilus* [83].

B. licheniformis HS10 produced the antifungal protein with molecular weight of about 55 kDa, identified as carboxypeptidase. It had significantly inhibition activity in respect to eight different kinds of plant pathogenic fungi, and it was stable with good biological activity at as high as 100 ºC for 30 min and in pH value ranged from 6 to 10. The biological activity was negatively affected by protease K. The protein had a broad spectrum antifungal activity against seven kinds of plant pathogenic fungi [84].

Isolated from seaweed *B. licheniformis* produced a protein with antibacterial activity against methicillin-resistant *Staphylococcus aureus*, vancomycin-resistant enterococci, and *Listeria monocytogenes.* The antibacterial activity was maximal in cultures grown under shaking at 210 to 230 rpm. Antibacterial activity was not found in cultures grown statically or with other speeds of rotary shaking. The antibacterial compound was sensitive to proteinase K, pronase, and trypsin, but was not affected by Tween-20, -40, -60, or -80, or a- or b-amylase. Activity was not adversely affected by heating up to 40ºC or treatment at pH range from 5 to 14. The bioactive compound was determined to be associated with a protein of 30.7 kDa, which had homology to the secreted YbdN protein of *B. licheniformis* ATCC 14580 [85].

B. licheniformis MY75 secreted high levels of extracellular chitinase with molecular weight of 55 kDa and inhibited the growth of pathogenic fungi *Gibberella saubinetii* and *Aspergillus niger.* Secretion of this protein was induced by the chitin powder [86]. It was demonstrated that chitinase proteins present in the culture supernatant of *B. licheniformis* Mb-2 [87], *B. licheniformis* TP–1[88], *B. licheniformis* S213 [89], *B. licheniformis* SSCL-10 [90], *B. licheniformis* B307 [91].

B. licheniformis BS-3 produced antifungal 31 kDa protein F2 that inhibited the growth of *Aspergillus niger*, *Magnaporthe oryzae* and *Rhizoctonia solani.* F2 protein was moderately resistant to hydrolysis by trypsin, proteinase K. A higher antifungal activity of F2 was observed in a range of pH 6.0 to pH 10.0, and at a temperature below 70 °C for 30 min [92].

As in other cases, this group of bacteriocins contains proteinase-resistant ones. This property probably makes these proteins applicable for the administration through digestive system.

B. licheniformis strain JS has been shown to produce 16 kDa antimicrobial protein (AMP) which demonstrated more activity against Gram-positive bacteria *Bacillus cereus* as compared to other bacteria. AMP was less active against Gram-negative (*S. dysenteriae*, *S. typhimurium*) bacteria. The purified peptide also increases the effectiveness of antibiotics such as kanamycin, neomycin and streptomycin. So, it could be important because of AMPs produced by *B. licheniformis* may facilitate entry of these antibiotics inside the pathogens and increase their efficiency. The antimicrobial activity was 100% after AMP incubation at temperature range between 10 and 90 °C. The trypsin digestion study reveals that the AMP retains its 100% activity [93].

B. licheniformis T6-5 inhibited more than 65% of the 40 *Bacillus* strains and sulphate-reducing bacteria *Desulfovibrio alaskensis*. Treatment of supernatant with organic solvents led to total (acetone, ethanol and methanol) or partial (chloroform) inactivation of the inhibitor component. Probably, inhibitor contains a lipidic portion as a part of its structure. This substance was heat stable after incubation at 100ºC for 1 h and maintained its activity after being autoclaved at 121ºC for 15 min. It was active in a wide range of pH values (3.5–9.5). The inhibitory component is resistant to the action of Pronase E, Proteinase K, Trypsin, RNase, Chitinase, b-Galactosidas, a-Galactosidase, Manosidase. The substance produced by strain T6-5 was estimated by dialysis to be bigger than 12 000 Da. According to the SDS-PAGE analysis, the strain T6-5 showed an inhibitory zone related to a region of ca 20 kDa, corresponding to the molecular weight suggested by the dialysis membrane approach. [36]. *B. licheniformis* H2O-1 antimicrobial substance inhibitory zones were related to a region of high

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molecular mass (90–120 kDa) [36]. Strains *B. licheniformis* T6-5 and H2O-1 prevented the formation of *B. pumilus* LF4 biofilm and also eliminated pre-established LF4 biofilm [94]. The nature and precise structure of the above inhibitory substances are still unclear.

1.4. Class IV – complex with a lipid moiety or carbohydrate moiety

B. licheniformis BFP011 isolated from papaya (Thailand) could produce extracellular antimicrobial substances which were active against some important phytopathogens, pathogenics and spoilage microorganisms such as *Colletotrichum capsici*, *Escherichia coli* O157: H7 and *Salmonella typhi* ATCC 5784. The 3 types of antimicrobial substances (F4, F5 and F6) produced by *B. licheniformis* BFP011 were not sensitive to pronase and revealed in stationary phase cultures. The antimicrobial substances of this bacterium were stable at 37 and 70°C and also partly resistant to the temperature 121°C. The most of antimicrobial protein substances from culture supernatant were extracellular compounds having low molecular weights of less than 45 kDa. The antimicrobial substances of *B. licheniformis* BFP011 contain peptides and unsaturated fatty acids, however, precise structural organization of these compounds are not known. They exhibited a broad spectrum of antimicrobial activity against both Gram–positive and Gram–negative bacteria and fungus *C. capsici*. These substances differed from iturin A (commercial), bacitracin (commercial) and bacteriocin-like substance of *B. licheniformis* P40 [95].

From Marine-derived *Bacillus licheniformis* 09IDYM23 were isolated two glycolipopeptides, ieodoglucomides A and B. They are consisting of an amino acid, a new fatty acid, a succinic acid, and a sugar. Glycolipopeptides were found to have moderate antimicrobial activity when tested against both Gram-positive and Gram-negative bacteria and fungi such as *S. aureus, P. aeruginosa, E. coli, B. cereus, A. niger. Ieodoglucomides A molecular formula was assigned as* $C_{30}H_{53}NO_{12}$*, Ieodoglucomides B -* $C_{29}H_{51}NO_{12}$[96].

The same strain 09IDYM23 produces a glycolipopeptide, ieodoglucomide C and a new monoacyldiglycosylglycerolipid, ieodoglycolipid. Compounds shows antimicrobial activity against fungi *C. albicans, A. niger, R. solani, C. acutatum, B. cenerea* and bacteria *S. aureus, B. subtilis B. cereus, S. typhi, E. coli, P. aeruginosa*. Molecular formulae of each isolated component were determined to be $C_{29}H_{51}NO_{12}$ and $C_{30}H_{56}O_{14}$[97].

Hereby, the bacteriocins produced by *B. licheniformis* are characterized by resistance to various pH values, thermal stability, and, in some cases, sensitivity to proteolytic enzymes. However, they differ in the spectrum of antibacterial activity for different strains of *B. licheniformis*. For example, a bacteriocin produced by *B. licheniformis* MKU3 isolated from slaughterhouse sediments did not inhibit *L. monocytogenes*, *P. fluorescens* or *S. marcescens*, but inhibited *E. coli* [34]. A bacteriocin-like peptide produced by *B. licheniformis* ZJU12 isolated from soil exhibited antagonistic activity against *S. aureus* [33], and *B. licheniformis* P40 inhibited *E. aerogenes* but did not inhibit *P. fluorescens* [29]. Anaerobiosis specific expressed Lichenin demonstrated a narrow spectrum of activity against the ruminal anaerobs [32].

1.5. Bacteriocins with undetermined molecular weight

A skin isolate of *B. licheniformis* showed most potent antibacterial activity at pH 7, at an incubation period of 48 h and at an incubation temperature of 25^0C. Antipathogenic metabolites was then detected as bacteriocin like substances. It demonstrated heat stability up to 80^0C for 30 minutes. Papain treated cell-free supernatant did not show any bacteriocin activity, suggesting that the substances could be antimicrobial peptides. This bacteriocin inhibited growth of *Staph. aureus* and *Kl. pneumoniae subsp. Pneumonia* [98].

Skin isolate *B. licheniformis* UpA was observed producing antimicrobial metabolite which was effective against *Klebsiella pneumoniae subsp. pneumoniae*. It was detected as bacteriocin like substances which was further confirmed as antimicrobial peptide through papain treatment. Produced bacteriocin was stable to heat-treatment up to 80 °C for 30 min and up to pH 7 [99]

The supernatant of *B. licheniformis* A-1-5B-AP significantly reduced the growth of oral pathogenic strains *Porphyromonas gulae* 3/H, *Prevotella intermedia* 1/P and *Streptococcus mutans* ATCC

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35668. On the other hand, *B. licheniformis* A-2-11B-AP only significantly inhibited the growth of *P. intermedia* 1/P and *S. mutans* ATCC 35668. However, enzyme-treated SN of *B. licheniformis* A-1-5B-AP did not lose its antimicrobial effect and significantly inhibited the growth of *Micrococcus luteus* DSM 1790. Proteinase K, lipase or α-amylase did not affect the antimicrobial activity present in the SN of strain of *B. licheniformis* A-1-5B-AP .The presence of genes associated with the synthesis of lichenysin was detected, although its presence in medium was not confirmed [100].

B. licheniformis HJ2020 MT192715.1 produced bacteriocin active against many species of food spoilage microorganism. Residual inhibition activity of bacteriocin were varied according to conditions of incubation and type of treatment. The inhibitory activity was attained to 220 and 360 U ml^{-1}against to pathogenic strains, including clinical isolates of *Escherichia coli* and *Salmonella typhi* respectively, while it attained to 42, 60, and 80 U/ml against to *B.subtilis*, *B. cereus* and *Candida albican* respectively [101]. No activity was detected against *Lactobacillus* and *Bifidobacterium*. These results were similar to those shown by *B. licheniformis* P40 [29]. Bacteriocin lost about 25-40% of its activity when incubated in acidic pH (between 3-5), while it lost about 80 % of its activity at pH 10 and there is no activity at pH 12. Heat stability of bacteriocin also was tested and the results show that it retained all activity when incubated at 5 - 35 ^{o}C for 30 min. It lost about 25-50 % of its activity after incubation at 50- 80 ^{o}C and lost all activity when incubated at 100^{o}C for 30 min or treated with autoclave at 121 ^{0}C for 15 min at 15 psi. Reduction of bacteriocin activity and lost all of its activity at high temperature attributed to denaturation indicating proteinaceous nature of bacteriocin. Results also revealed that bacteriocin was stable when treated with α- amylase and lipase pointing absence of glycosidic or lipidic residuals [101].

2. Non-ribosomal biosynthesized peptides

Non-ribosomal peptides are synthesized by sequential condensation of amino acids, carried out by special non-ribosomal multimodular peptide synthetases, which mainly found in bacteria and fungi. Many peptides not produced by ribosomes contain unnatural amino acids and other molecules that are not found in peptides synthesized by ribosomes [102]. Such peptides include many well-known substances such as antibacterial drugs (penicillin, vancomycin), antitumor compounds (bleomycin) and immunosuppressants (cyclosporine) [103].

2.1. Bacitracin

Bacitracin, the first non-ribosomal peptide antibiotic isolated so far from *B. licheniformis* cultures [104], is actively used in medicine and veterinary medicine with sufficient safety [105]. It is part of topical medicines for disinfection of wound surfaces. Bacitracin is a polypeptide of about 1.42 kDa. It is a non-ribosomally synthesized docapeptide antibiotic produced by certain strains of *B. subtilis* and B. *licheniformis* [106]. Bacitracin contains 12 amino acids, four of which are the D-isomers of glutamic acid, aspartic acid, phenylalanine, and ornithine [107]. The synthesis of this peptide is rare in other species of the genus *Bacillus*, that indicates the importance of its discovery in *B. licheniformis*. This antibiotic inhibits cell wall synthesis of many Gram-positive and some Gram-negative bacteria [108]. In addition, due to its fast elimination rate and low absorption, it can be used as an additive in animal feed [109]. Bacitracin from *B. licheniformis* is also known as Ayficin [110]. This antibiotic is a mixture of at least 5 polypeptides, and consists of 3 separate compounds, bacitracin A, B and C [111]. This antibiotic is released from bacteria only under cultural conditions that will eventually support spore formation [107]. Bacitracin begins to be synthesized in the early exponential phase of vegetative growth, reaching a constant rate in the stationary phase of growth in a synthetic medium without glucose. The addition of glucose inhibits the synthesis of bacitracin, however, this inhibition is not the result of catabolite repression, but a decrease in the pH of the growth medium, presumably due to the accumulation of pyruvate and acetate [112].

Bacitracin had a potent antibiotic activity against Gram-positive cocci, staphlococci, streptococci, corynebacteria, *Treponema pallidum*, *T. vincenti*, *Actinomyces israeli*, anaerobic cocci, clostridia, neisseria, most gonococci and meingococci, but it is relatively ineffective against most other Gram-negative bacteria [112]. It influences the transport of metal ions, the synthesis of peptidoglycan, the

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permeability of membranes and the biosynthesis of enzymes in the cell and it can also inhibit biofilm formation in cariogenic *Streptococcus mutans* [113]. It is not used as an antibiotic in humans, because it has a toxic effect [114]. Bacitracin A shows activity against rice pathogen Pantoea ananatis[106].

It has been demonstrated that bacitracin is able to inhibit activity of subtilisin-like serine endopeptidases, porcine glutamyl and neutral aminopeptidases [115], and protein disulfide isomerase [116]. Bacitracin inhibits the activity of a highly glycosylated cell surface membrane serine aminopeptidase (porcine dipeptidyl peptidase-IV) that plays a relevant role in tumor progression and glucose metabolism [117]. In addition, bacitracin has shown dual specificity: as a metal-ion-independent RNase and as a magnesium-dependent DNase. It was able to degrade nucleic acids, being especially active against RNA molecules [118].

The six isolates *B. licheniformis* from retail infant milk formulae (strains IMF1, IMF2, IMF5, IMF6, IMF 22 and IMF78) demonstrated a higher antimicrobial potency than lichenicidin-producing strains. Further analyses identified a peptide of 1,422 Da. This peptide shows a high homology to the non-ribosomal peptides bacitracin and subpeptin, known to be produced by *Bacillus spp*. Strains IMF20, IMF66, IMF69 and IMF80 are also able to produce two-peptide antibiotic lichenicidin [61].

Two antimicrobial peptides, subpeptin JM4-A and subpeptin JM4-B, with molecular masses of 1,422.71 Da and 1,422.65 Da have been reported to be produced by the soil isolate *Bacillus subtilis* JM4 [119,120].

Bacillus licheniformis strain EI-34-6 was isolated from the surfaces of the seaweed *P. palmata*, was grown in air-membrane surface (AMS) bioreactor, and it was observed that cells produced antimicrobial compounds which they did not produce when they were grown in shake flask cultures. Inhibitory compounds were active against *Staphylococcus aureus* strains MRSA9551 and MRSA14986 and vancomycin-resistant *985558476* strains VRE788 and VRE1349. Glycerol and ferric iron were important for the production of antimicrobial compounds and the red pigment, similar to pulcherrimin. The release of these secondary metabolites and bacitracin was not due to the onset of sporulation. Cell-free spent medium recovered from beneath the reactor membrane could induce production of antimicrobial compounds and red pigment in shake flask cultures. Antimicrobial compound was purified, and on the basis of its chemical structure it was determined to be bacitracin [121]. Supernatant produced by bacteria also capable to dispersing bacterial biofilms. The source of this activity is an extracellular DNase (NucB), enzyme rapidly breaks up the biofilms of both Gram-positive and Gram-negative bacteria. Produced ribonuclease (Barnase) may does have an important role on dispersal efficacy.[122]

2.2. Cyclic lipopeptides (Biosurfactants)

Biosurfactants include amphiphilic compounds produced by microorganisms with significant surface and emulsifying activity. These are microbial surfactants which are chemically active compounds of amphiphilic structure with hydrophilic (peptides or amino acids, polysaccharides) and hydrophobic (fatty acids) fragments. They are able to localize between liquids of different polarity, thereby reducing surface and interfacial tension at the surface and interface, respectively, with very low critical micelle concentration, no toxicity, high biodegradability and resistance to extreme conditions such as high temperatures, extreme pH and high salinity [123]. Surfactants are used as cleaning agents, detergents, dispersants, moisturizers, emulsifiers, and in the bioremediation of oil-contaminated sites[124]. Due to their antimicrobial and antiviral activity, they have been used to combat microbial and viral infection of plants[125] . A number of studies have shown the effectiveness of the use of surfactants in the composition of antitumor drugs[126–128]. Microbial surfactants have a number of advantages, such as biodegradability, operation in a wide range of pH, temperature, resistance to high concentrations of NaCl, higher selectivity and stability, and also exhibit antibacterial and antifungal activity [129].

Several lipopeptide biosurfactants produced by *B. licheniformis* have been demonstrated to have antimicrobial activity [129–132]. *B. licheniformis* is able to secrete biosurfactants (Table 1), such as lipopeptides, under various growth conditions - in the presence and absence of oxygen, under conditions of high salinity and temperature [133]. They can be a useful tool to combat biofilm-forming

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bacteria. Lipopeptides are of particular interest because of their high surface tension activity and antibiotic potential [134].

A lipopeptide biosurfactant generally consists of a fatty acid chain and a peptide chain with several amino acids [134]. In lipopeptides a fatty acid residue is covalently linked to a peptide chain. Typically, this family includes the members of surfactin, lichenysin, iturin and fengycin [135]. The relationship between the structure and functions of lipopeptides is expressed by varying degrees of antagonistic action depending on the pathogen, although in general they all cause the appearance of pores in cell membranes. *B. licheniformis* is capable of producing cyclic lipopeptides related to biosurfactants [136]. Analysis of *B. licheniformis* lipopeptides isolated in seven different geographic areas showed a difference in their content depending on the locality [137].

2.2.1. Surfactin homologues

Surfactin – a well characterized cyclic lipoprotein isolated from *Bacillus subtilis* and one of the most effective and powerful biosurfactant [138]. Surfactin family is a mixture of cyclic lipopeptides built by variants of a heptapeptide and a β-hydroxy fatty acid with chain length of 13–18 carbon atoms. A lactone bridge between the β-hydroxyl function of the acid and the carboxy-terminal function of the peptide confers a cyclic structure to the molecule [139]. When this lipopeptide interacts with gram-positive bacteria, cell lysis is observed [140]. Surfactin is able to form pores in biological membranes and destabilize lipid packaging. Due to hydrophobic interactions, it binds to the membrane and affects the ordering of the hydrocarbon chain, which affects the thickness of the membrane [141]. Surfactin biosynthesis is catalysed non-ribosomally by the action of a large multienzyme complex consisting of four modular building blocks, called the surfactin synthetase [142].

B. licheniformis HSN221 produced nine variants of surfactin and lichenysin lipopeptides. The medium components with glucose, ammonium chloride and yeast extract were especially suitable for the production of surfactin homologues [136,143]. Two produced surfactin monomethyl esters' and one lichenysin monomethyl esters' molecular masses detected by ESI-MS were 1048, 1049, and 1063 Da[144].

B. licheniformis BC98 inhibited the growth phytopathogens such as *Magnaporthe grisea*, *Curvularia lunata* and *Rhizoctonia bataticola.* Active component had a molecular mass 1035 Da. The active lipopeptide was identified as surfactin. The activity of antagonistic lipopeptide was found to be highly stable at extreme pH and temperature and it was also resistant to protease treatment. Microscopic analysis of the effect of the antagonist on *M. grisea* revealed bulbous hyphae showing patchy and vacuolated cytoplasm when observed under the electron microscope. This lipopeptide was highly potent in its antagonistic activity as it completely inhibited the growth of *M. grisea* at a concentration as low as 1 μg ml^{-1} [145].

Lipopeptides isolated from *B. licheniformis* supernatant [130] shows the highest structural analogy with surfactin produced by *B. subtilis* [138]. The lipophilic part consisting of C_{14} or C_{15} branched and hydroxy saturated fatty acids was linked to the hydrophilic peptide moiety, which contained seven amino acids (Glu, Asp, Val, three Leu and Ile) by a lactone linkage. Antibiotic activity was demonstrated against Gram-negative bacteria (Pseudomonas aeruginosa and *Escherichia coli*), yeasts and some fungi (*Trichoderma reesei* and *Penicillium oxalicum*). Two molecular weights, 1022 and 1036, were determined. The mass difference of 14 units characterizes the lipopeptide as a mixture of closely related molecules varying in their fatty acid residues [130].

The lipopeptides produced by *B. licheniformis* MB01 were determined as cyclic surfactin homologs with molecular weight 994, 1008, 1022, and 1036 Da. The lipopeptides demonstrated well resistance to UV light and the change of pH and temperature. This surfactins are active against the gram positive and negative bacteria (*Escherichia coli*, *Vibrio cholerae*, *Vibrio parahaemolyticus*, *Vibrio harveyi*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, and *Proteus species*) [146].

B. licheniformis V9T14 produced C_{13}, C_{14} and C_{15} surfactin homologues, whose structures were confirmed by the product ion spectra of the sodiated molecules at m/z 1030, 1044 and 1058 [139]. The V9T14 biosurfactant active against the *Escherichia coli* CFT073 biofilm formation [147].

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B.licheniformis B6 produces surfactin among other lipopeptides.[148]

B.licheniformis ATCC 12713 produces surfactin with a strong antibacterial activity against *C. perfringens* and *Brachyspira hyodysenteriae,* pathogens causing necrotic enteritis and swine dysentery. It was also demonstrated for the first time that the major isoform of surfactin in *B. licheniformis* was surfactin C [149]. The fermented products obtainted from the same strain were able to inhibit the growth *of Staphylococcus aureus in vitro a*nd adding them in dietary feed can ameliorate *Clostridium perfringens*-induced intestinal necrotic lesions in broilers [150,151]. It was found that this substance is surfactin, which shows showed stronger bacterial killing activity against C. perfringens but not against the causative agent of swine dysentery - *Brachyspira hyodysenteriae* unlike surfactin from *Bacillus subtilis* [152]. Furthermore, *B. licheniformis* ATCC 12713-derived surfactin exhibited anti-coccidial activity by inhibiting the life cycle of *Eimeria* species. It was shown that this surfactin directly inhibit *E. tenella* oocyst growth *in vivo*, thereby preventing coccidiosis in broilers [153].

B. licheniformis 86 produced a mixture of lipopeptides with the major components ranging in size from 979 to 1091 Da and varying in increments of 14 Da. The most abundant components are of 1021, 1035 and 1049 Da. Data on the structure of this surfactant indicate its surfactin-like nature [154,155].

B. licheniformis F2.2 produced a non-lipopeptide type biosurfactant BL1193 together with lipopeptides, plipastatin, and surfactin in an amino acid depleting medium. Plipastatin inhibited the growth of Gram-positive bacteria (*B. subtilis*), Gram-negative bacteria (*Pseudomonas aeruginosa, Escherichia coli*), and Eumycetes (Aspergillus niger, Penicillium sp., Fusarium sp., and Cladosporium sp.). Plipastatin and surfactin were abundantly produced in nutrient rich medium. In addition, a non-lipopeptide type biosurfactant BL1193 was produced upon growth of the producer in a synthetic medium, but not in rich medium [156].

2.2.2. Lichenysins

A surface-active substance known as lichenysin is produced by *B. licheniformis* as a secondary metabolite, and its biosynthesis is catalyzed by non-ribosomal peptide synthetases. Its structure is very similar to that of surfactin. Both compounds can be produced under aerobic or anaerobic conditions [157]. Lichenysin has a higher surfactant power and a much higher hemolytic activity compared to surfactin [158,159]. The main differences between lichenysin and surfactin are the presence of glutamine residue (Gln) at position 1 of the lichenysin peptide sequence in place of glutamic acid (Glu) of surfactin and the resulting changes in the physicochemical properties. Lichenysin is a better chelating agent toward Ca^{2+} than is surfactin [159]. Some strains of *B. licheniformis* produced lichenysins and were mostly detected as sodium adducts at m/z 1029 and 1057 Da [160].

Thus, *B. licheniformis* NBRC 104464 produces a cyclic lipopeptide different from surfactin – lichenysin with m/z 1029.5, 1043.5, and 1057.5. The association constant of this lichenysin with Ca^{2+} is four-fold higher than that of surfactin [161].

Both aerobically and anaerobically *B. licheniformis* BAS50 produced lichenysin A with the major components ranging in size from 1,006 to 1,034 Da. Lichenysin A has an isoleucine as the C-terminal amino acid instead of the leucine of surfactin and lichenysin B and an asparagine residue instead of the aspartic acid residue of surfactin, lichenysin B, and lichenysin C. Glucose and sucrose but not arabinose, fructose, or maltose supported the best surfactant production. Inhibitory activity observed against *Acinetobacter calcoaceticus*, *Alcaligenes eutrophus*, *Bacillus cereus*, *Bacillus sp.* strain ATCC 39307, *Escherichia coli*, *Enterobacter sp.* strain 306, *Pseudomonas fluorescens*, *Pseudomonas proteofaciens*, *Staphylococcus aureus*. No growth inhibition by lichenysin A was detected for *B. licheniformis* BAS50 itself, *B. subtilis* and *Rhodococcus globerulus* [131].

Eight types of lichenysin commonly produced by *B. licheniformis* are lichenysin A, lichenysin B, lichenysin C, lichenysin D, lichenysin G, [Val7] lichenysin G, [Ile4] lichenysin G and [Ile2,4] lichenysin G [131,162–164]. Lichenysin B producing strain JF-2 was re-identificated as Bacillus mojavensis strain JF-2 [158,165]

Differences of lichenysin types are due to the type and sequence of amino acids in the lactone ring [164].

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In 1999, a series of 9 lactone lipopeptide biosurfactants, representatives of the lichenisins group, was isolated from the strain *B. licheniformis* IM 1307. According to the authors, they were at least 10 times more active than surfactins [163].

Later, nine lipopeptides (surfactins and lichenisins) produced by *B. licheniformis* HSN221 were identified by chromatography and mass spectrometry. By varying the composition of the nutrient medium, the strain produced either surfactins or lichenisins. Types of lipopeptides from natural substrates were the same, which contained lichenysin C13, lichenysin C14 and lichenysin C15 as well. Lipopeptides from synthesized media were homologues of surfactin C13 and those of lichenysin C12. According to the structure of lichenysin A, the molecular masses of lichenysin C12, lichenysin C13, lichenysin C14, lichenysin C15 and lichenysin C16 are 992, 1006, 1020, 1034, and 1048, respectively [136].

Lichenysin showed toxic effects in pig ileum organoids and human epithelial $CaCO_2$ cells. The concentration of lichenysin needed to reduce cell viability by 50% (IC_{50}) was 16.6 μg/ml for Caco- 2 human intestinal epithelial cells and 16.8 μg/ml for pig ileum organoids. For surfactin, the IC_{50} value was 23.5 μg/ml for Caco2 cells while no toxicity was seen for the ileum organoids at the highest levels tested (>200 μg/ml). This indicates that lichenysin is more toxic to these cell types than surfactin [157].

B. licheniformis strain P40 produced antibacterial cyclic peptide (BLS) that contains fatty acids like surfactin and lichenisin but with lower molecular weight - 800 Da. It was resistant for up to 100 ^{0}C and pH ranging 3–10, lost its activity when treated with pronase E, but resistant to papain, trypsin, proteinase K and trichloroacetic acid. This peptide already demonstrated a wide action spectrum, presenting bactericidal activity to pathogenic and spoilage bacteria, such as *B. cereus*, *L. monocytogenes*, *E. carotovora, Streptococcus spp., but Staphylococcus aureus* and *Escherichia coli* were resistant to action of this substance. The precipitation at low saturation of ammonium sulfate and elution at void volume of gel filtration indicate that the BLS was secreted in the form of large aggregates [29,166].

2.2.3. Licheniformin

The physical properties and chemical structure of the licheniformin lipopeptide produced by *B. licheniformis* MS3 were studied [167]. The molecular weight of licheniformin corresponds to 1438 Da. This lipopeptide has a lactone ring consisting of four amino acid residues (Asp, Ser, Gly and Tyr), which is additionally linked by an amide bond to the remaining amino acids (Gly, Ala and Val). So, its peptide ring is not directly linked to the fatty acid moiety [167]. The structure of licheniformin is similar to the lipopeptide biosurfactant (Kurstakin) produced by *Bacillus thuringiensis* with antifungal activity against *Stachybotrys charatum* [168].

2.2.4. Fengycins

Fengycin family consists of a β-hydroxy fatty acid connected to the N-terminus of a decapeptide. The C-terminal residue of the peptidic moiety is linked to the tyrosine residue at position 3, forming the branching point of the acyl-peptide and the eight-membered cyclic lactone. The length of the β-hydroxy fatty acid tail is variable and links the amino group of its N-terminal amino acid Glu [139]. Fengycins exhibit antibacterial activity against both Gram-positive and Gram-negative microorganisms. In addition, this substance have been shown to be active against filamentous fungi [169–171]. Being a surfactant, fengycins interact with biological membranes and form pores in them, which leads to a change in the permeability of the membrane [172]. Its action is associated with a modification of the alignment of the phospholipid acyl chain and a global decrease in the cooperativity of the lipid-lipid and lipid-phengycin interaction in the bilayer membrane [173]. This effect may be related to the ability of fengycins to change the hydrophobicity of the bacterial surface, influence the development of biofilms and flagella, and prevent the attachment of bacterial cells to various surfaces, including plastic, glass, and tissues [146,174,175].

B. licheniformis B6 produced lipopeptides (LP) that manifested antibacterial activity against clinical pathogenic strains *Staphylococcus aureus*, *Escherichia coli*, *Klebsiella* sp. In presence of LP biofilm

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structures were destabilized, these strains turning into weak biofilm formers. Kurstakin and iturin were identifed by MALDI TOF. Mass spectra revealed mass peaks assigned to fengycins and bacitracins ranging from m/z 850 to m/z 1200 Da, assigned to the isoforms of kurstakins, surfactins, and iturins ranging from m/z 1300 and m/z 1650 Da. Interestingly, surfactin was detected, rather than lichenysin, the expected lipopeptide in *B. licheniformis* species. Signals of bacitracin and fengycins were also found, the latter with a higher number of homologues and relative intensity than the other lipopeptides. These results show that the lipopeptides synthesized by *B. licheniformis* B6 have both potential antibacterial and antibioflm activity against pathogenic bacteria of health importance [148].

Lipopeptide biosurfactants produced by the *B. licheniformis* V9T14 strain showed an anti-adhesion activity against biofilm formation of human pathogenic bacterial strains. It was found the presence of two main fengycin isoforms, with the protonated molecules at m/z 1478 and 1506 corresponding to C_{17} fengycin A and C_{17} fengycin B, respectively. Other homologues (C_{14} to C_{16}) were revealed and confirmed as belonging to fengycin A or B [139]. In previous study was reported that biosurfactants produced V9T14 inhibit *E.coli* and V19T21 strain inhibit *S. aureus* biofilm formation [147] Moreover, the V9T14 biosurfactant was able to increase the biofilm eradication efficacy of different antibiotics against an uropathogenic *Escherichia coli* strain [176].

2.3. Others lipopeptides

B. licheniformis strain M104 grown on whey produced a lipopeptide biosurfactant with activity against Gram-positive (*Bacillus subtilis*, *Bacillus thuringiensis*, *Bacillus cereus*, *Staphylococcus aureus*) and Gram- negative bacteria (*Pseudomonas aeruginosa*, *Escherichia coli*, *Salmonella typhimurium*, *Proteous vulgaris*), as well as yeast (*Candida albicans*). *Listeria monocytogenes* and *Klebsiella pneumoniae* were resistant to action of this biosurfactant [132]. Its chemical structure is not established.

B. licheniformis 603, isolated from a mixture of drilling fluid and subsurface thermal water, produced a cyclic lipopeptide with growth-inhibiting activity against *Corynebacterium variabilis* and *Acinetobacter sp.* Also, this lipopeptide prevents adhesion of bacterial cells to a glass surface. This compound is a heptapeptide containing L-Asp, L-Leu, L-Leu, L-Val, L-Val, L-Glu, L-Leu, N-acylated to the N-terminal amino acid, L-Asp, by a 3-hydroxy fatty acid, the 3-OH group of which is esterified by the C-terminal amino acid, L-Leu [177].

CB-1 is a unique chitin-binding antifungal including peptides and fatty acids. It considered to be an aggregation product of 4 peptides of 1035, 1504, 4018, and 5024 Da, by gel filtration column chromatography the molecular mass was estimated as 42 kDa. It shows inhibitory activity against some phytopathogenic fungi, including *Pyricularia oryzae* and *Rhizoctonia solani,* and less activity against bacteria and yeast [178].

A lipopeptide surfactant from the marine sponge-associated *Bacillus licheniformis* NIOT-AMKV06 shows antimicrobial activity against life-threatening clinical pathogens, such as *Enterococcus faecalis, Bacillus subtilis, Salmonella typhi*, *Vibrio cholera, Klebsiella pneumoniae* and some other bacteria [179].

Thus, the surfactants synthesized by *B. licheniformis* have the potential to inhibit the growth and biofilm formation of human and animal pathogenic bacteria, mainly Gram-positive ones, like *Staphylococcus aureus*, *Listeria monocytogenes*, and *B. cereus*, and some Gram-negative bacteria, including *Escherichia coli*, *Salmonella Typhimurium*, and *Aeromonas sp.* [29,31,171,180,181]. However, due to their toxicity for animal and human cells their application in medical and veterinary practice is limited by topical usage and in form of disinfectants.

Many antimicrobial and antifungal peptides and proteins produced by *B. licheniformis* are resistant to action of proteinases. Perhaps this stability of these proteins can be explained by the presence of a cyclic peptide structure of these bacteriocins containing unusual amino acids [182].

3. Exopolysaccharides

Exopolysaccharides (EPS) are high molecular weight compounds and composed of repeated units of sugar moieties, attached to a carrier lipid, and can be associated with proteins, lipids, organic and inorganic compounds (acetate, glycerol, pyruvate, sulfate, carbo xylate, succinate and

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phosphates), metal ions, and DNA [183]. In some cases, EPS demonstrated antimicrobial activity against bacterial pathogens, both Gram-positive and Gram-negative. Their antagonistic action was revealed in relation to bacteria, viruses, fungi. EPS also inhibit the formation of biofilms by pathogenic bacteria and prevent their colonization on various surfaces [184].

B. licheniformis can synthesize EPSs of various biological activities (Table 1), including antibacterial and antioxidant effects [185]. A typical example is levan (fructan) - the fructose polymers linked by the β-2,6-fructofuranosidic bond. Levan is synthesized by an enzyme, levansucrase. It has antioxidant activity and antibacterial activity against *Staphylococcus aureus*, *E. coli*, and *Pseudomonas aeruginosa* [186]. *B. licheniformis* RN and *B. licheniformis* SVD1 produced levans which have a high potential as substances with antibacterial, antibiofilm, antiviral and anticarcinogenic effects [187,188].

B. licheniformis 24 produced EPSs consisting of galactose, glucose and mannose with antioxidant activity. Also this EPS possessed antibacterial activity against *Vibrio cholera* [185].

B. licheniformis Dahb1produced EPS with antioxidant and the antibiofilm/antibacterial activity against Gram-negative (*Pseudomonas aeruginosa* and *Proteus vulgaris*) and Gram-positive species (*Bacillus subtilis* and *Bacillus pumilus*) as well as the fungus *Candida albicans*. The content of carbohydrates, proteins, and uronic acid in EPS was 680.43, 386.15, and 56.72/mg, respectively. The hemolytic assay showed low cytotoxicity of this EPS at 5 mg/ml [189].

B. licheniformis T14 produced EPS-T14 (molecular weight of 1000 kDa) with antibiofilm activity. It contained fructose and fucose as major monosaccharides. EPS-T14 reduced biofilm formation of both Gram-negative and Grampositive bacteria (multiresistant clinical strains of *Escherichia coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, and *Staphylococcus aureus)* [190].

Exopolysaccharide (1800 kDa) purified from the culture supernatant of sponge-associated B. licheniformis is able to inhibit biofilm formation of *E. coli* and *Pseudomonas fluorescens* but not able to reduce the growth of these bacteria. This EPS is composed of a-D-galactopyranosyl-(1→2)-glycerol-phosphate monomeric units [191].

Antimicrobial substances of *B. licheniformis* active against mycobacteria

Most of the antibacterial components produced by different strains of *B. licheniformis*, active against only Gram-positive microorganisms. Some are also active against Gram-negative microorganisms. Few substances have been reported to be active against mycobacteria (Table 2), whose cell wall is very different from Gram-positive and Gram-negative bacteria and functions as an effective permeability barrier [192].

Mycobacterium tuberculosis causes a respiratory tract infection known as tuberculosis. On average, 10 million people worldwide are infected with this disease each year, and the mortality rate is between 11 and 15%. Cases of multidrug and extensive drug resistance in *M. tuberculosis* place a huge burden on efforts to control the spread of *M. tuberculosis*, especially in developing countries[193]. In addition to drug resistance, the causative agent of tuberculosis (*Mycobacterium tuberculosis* (Mtb) is able to persist asymptomatically in the host organism for many years, causing latent forms of tuberculosis. In this dormant state, Mtb cells are also resistant to known antibiotics [2,3]. Due to its unique metabolic plasticity, the mycobacterium survives under the stressful conditions of the host organism and under antibiotic therapy. In these cases, mycobacteria can gradually move into a state of reduced metabolic activity - dormancy associated with ineffective treatment of latent tuberculosis infection [194].

In recent years, it has been demonstrated that infection with COVID-19 often results in the transition of latent tuberculosis to an active form, which in a significant percentage of cases turns out to be drug-resistant [195]. According to WHO, today every forth inhabitant of the planet is an asymptomatic carrier of tuberculosis, thus, there is a permanent reservoir of tuberculosis infection, from which a pandemic can develop at any moment. Lockdowns and restrictions imposed during COVID-19 could lead to an additional 1.4 million TB deaths between 2020 and 2025, according to the WHO [193]. The search for new substances capable of killing mycobacteria is an important task for medical microbiology and chemistry.

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The unique structure of the mycobacterial cell wall and the characteristic slow growth of *M. tuberculosis* may presumably interfere with the action of lantibiotics. Lantibiotics can bind to lipid II of mycobacteria, making them potential candidates for anti-tuberculosis drugs. The structure of lipid II of mycobacteria is modified in comparison with other bacteria. There are modifications of both N-acetylmuramic acid (MurNAc) and the side chain of the peptide [196].

Nevertheless, nisin produced by lactococci has been shown to have activity against mycobacteria *M. smegmatis* and *M. bovis* with intracellular ATP leakage and proton motive force dissipation. Nisin and lacticin are also active against clinical isolates of mycobacteria *in vitro*, including *M. tuberculosis* [197,198]. *B. licheniformis* MCC 2512^T produced a natural variant of nisin – subtilin [54,55] which is active against *M. tuberculosis* [199].

1946, it was demonstrated that *B. licheniformis* produced several antibacterial substances that inhibit the growth of mycobacteria, including the causative agent of tuberculosis *M. tuberculosis* [200]. One of these substances was named licheniformin. Later, it was found, however, to be toxic, causing damage to the kidneys after prolonged administration [75]. In the following study it has been revealed that *B. licheniformis* produced three similar components, designated as licheniformins A, B and C. They are peptides with very similar molecular weights and amino acid compositions, possessing both antibacterial activity and toxicity, although to somewhat different degrees. All three peptides have similar molecular weight (3800-4800), optical rotation and elemental composition. Purified licheniformin C was less active against mycobacteria than the original crude preparation and caused more pronounced kidney damage. Licheniformin B was slightly more active *in vitro* than the parent substance, but also caused extensive renal damage. Licheniformin A was much less toxic than either of the other fractions, but still caused little kidney damage and was less effective than streptomycin in controlling tuberculosis in mice. Licheniformins A and B are more active against *Mycobacterium phlei* than licheniformins C and less toxic to mice than licheniformins C [37].

Different species of laboratory animals are not equally susceptible to the nephrotoxic action of licheniformin A5. Compared with the mouse, the rabbit is resistant and the rat relatively sensitive [201]. Nevertheless, the nephrotoxicity obviously, suspended further work with these compounds despite their high effectivity as antiTB substances *in vitro*. In addition to inhibiting the growth of mycobacteria, licheniformins showed efficacy against *Staphylococcus aureus* and *Escherichia coli* [75].

Bacitracin at concentrations of 6.5-13.0 μg/ml inhibited the growth of *Mycobacterium smegmatis*. For inhibition of *M. tuberculosis* BCG the concentration of bacitracin was 10 times higher. The main target of bacitracin action on mycobacteria presumably is the membrane system. Bacitracin caused marked alterations in mycobacterial membranous structures. Bacitracin is highly bactericidal to mycobacteria during the middle or late exponential growth phase [202].

The strain *B. licheniformis var. mesentericus* produced proticin that is especially active against a number of Gram-positive and Gram-negative bacteria including mycobacteria (*Mycobacterium tuberculosis)*. Median lethal dose of proticin for mice was >150 mg/kg intravenously and 1,000 mg/kg subcutaneously [38]. Proticin is a phosphorus-containing, strongly unsaturated amorphous compound with a conjugated triene with molecular weight 560.666 Da. On the basis of this derivative and of several degradation products the molecular formula of proticin was found to be $C_{31}H_{44}O_7PNa$. The functional groups of proticin include one OH capable of acetylation, one lactone group, and one monoester of phosphoric acid as enol ester. Proticin contains a conjugated triene [203].

Peptide A12-C from *B. licheniformnis* A12 has a antimycobacterial effect in relation to *Mycobacterium phlei* [46].

According to our unpublished observation, a laboratory strain of *B. licheniformis* LBSM secretes anti *M. tuberculosis* 14 kDa substance(s) which inhibited growth of multiply cells and destroyed dormant *M. tuberculosis* forms. This substance is resistant to proteinase action.

Although these bacteriocins have a potential, *in vivo* studies are still required, and an appropriate delivery system still needs to be developed to reach *M. tuberculosis* residing within tissues. For example, in the context of *M. tuberculosis*-infected macrophages in the distal lung, promising results have been reported for the *in vivo* efficacy of class IIa bacteriocins complexed with

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phosphatidylcholine-cardiolipin liposomes. As a complex with liposomes bacteriocins inhibited intracellular growth of *M. tuberculosis* and to prolong survival of mice in an acute TB model. [204].

Prospects for using natural substance in the treatment of tuberculosis

Natural producers of antimicrobial compounds are attractive starting points for finding new and better anti-tuberculosis drugs because they are surprisingly rich in chemical diversity and have tremendous antimicrobial activity. Natural drugs have a diverse molecular structure and have high screening performance with high throughput and high ability to approach their site of action in target cells [205]. Traditionally, natural products have been the prototype of various drugs that are currently actively used in medicine. These include pyrans, flavones, chalcones, coumarins, pyrimidones, and oxzolidines, which are used as anti-cancer, anti-inflammatory, antimicrobial, antiviral, and anti-tuberculosis medicines [205].

Above mentioned examples demonstrated that bacteriocins of different bacteria exhibited stronger *in vitro* antimycobacterial activity than equal concentrations of rifampicin - a widely used anti-TB antibiotic. They can be considered as an alternative for the development of means to combat antibiotic-resistant strains of mycobacteria that cause tuberculosis.

It is known that antimicrobial peptides are capable of disrupting the normal function of the mycobacterial cell wall in various manners and then interacting with different intracellular targets (including nucleic acids and enzymes) [206]. Importantly, the likelihood of developing resistance to antimicrobial natural peptides is rather low. This is due, firstly, to a non-specific mode of action, as well as to the fact that the same molecule has different mechanisms of destruction. In addition, mutations that make bacteria resistant to bacteriocins are energy-intensive and harmful [207]. Usually these peptides have a positive charge and can interact with a negatively charged mycobacterial cell wall [208]. As a result of this interaction, peptides enter the cytoplasm, where they can interact with intracellular targets. Due to their amphipathic nature, antimicrobial peptides can be active in both aqueous and lipid environments [209]. The interaction of bacteriocins with the mammalian cell membrane is weaker than with the bacterial membrane. This is due to the different composition and structure of lipids. Mammalian phospholipids are mostly zwitterionic, resulting in a neutral charge, while bacterial membranes have a negatively charged outer surface [210]. In mammalian membranes, zwitterionic phospholipids are found in the outer leaflet, while negatively charged phospholipids are found closer to the cytoplasm in the inner leaflet. The interaction of antimicrobial peptides and mammalian cell membranes is possible due to hydrophobic contacts, which are weaker than electrostatic interactions between bacteriocins and bacterial membranes. The presence of cholesterol, which stabilizes the phospholipid bilayer of mammalian membranes, reduces the activity of antimicrobial peptides [211]. Thus, due to structural differences between mammalian and bacterial membranes, peptides act selectively on bacterial cells rather than mammalian cells, which makes them a potential therapeutic agent against pathogenic bacteria [212]. In summary, natural bacteriocins possess evident advantages in comparison with traditional antibiotics.

Many antibacterial peptides are resistant to proteases, which makes them suitable for intravenous or *per os* administration. Nevertheless, medical application of some bacteriocins with promising *in vitro* antimycobacterial activity is limited by their toxicity for animals and humans.

In this connection, the recent technological advances allow to produced new antimicrobials through structural modification of natural peptides to overpower resistance to antibiotics [213].

To enhance the antimycobacterial activity of natural bacteriocins, as well as to reduce their toxicity, biotechnological approaches are used. Thus, it was demonstrated that biotechnological derivatives of nisin have enhanced activity against mycobacteria than the prototypical substance [214]. We might expect that similar approach could be used to return in medical studies and eventually in application very efficient *in vitro* licheniformins (see above) discovered in last century. The generation of mycobacterial species specific bacteriocins would be an exciting step forward in the development of novel anti-mycobacterial drugs.

Since many bacteriocins are synthesized on ribosomes, and therefore there are genes encoding a structural (though as yet inactive) peptide, it was recognized that bacteriocins are probably more

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convenient for bioengineering than classical antibiotics, since the latter are usually generated from small building blocks through multienzyme complexes and are not ribosomal in nature. Various strategies have been developed to modify the properties of natural bacteriocins [215,216].

Natural biosurfactants with antimicrobial, antibiofilm and antiviral properties may be applied for the production of disinfectants, handwashing and cleaning products active against mycobacterial contaminations as well. They exhibit higher biodegradability, lower toxicity and better environmental compatibility compared to synthetic surfactants [217].

Perhaps the synergistic effect of natural bacteriocins and traditional antibiotics will allow more successful treatment of patients with lower side effects [218].

4. Conclusions

B. licheniformis is a perspective organism in bacterial world which is armed by a very effective for bacterial antagonism system based on the production of antimicrobial peptides of different structures targeting many bacterial and fungal representatives including pathogenic for animal and humans. Some of them are currently in use in medical and veterinary practice. However, many of them were studied *in vitro* only and awaiting for *in vivo* experiments.

Studying the relationship between peptide structure, function, toxicity, and molecular mechanism of action can provide a more complete understanding of peptides and the development of strategies to modify them. This information will be useful in developing new molecules with desired properties.

From the other hand, particular strain of *B. licheniformis* could be used as a natural vehicle for antibiotic substance in form of true probiotic cultures strains to combat various types of pathogens including mycobacteria. Moreover, current technologies allow to construct *B. licheniformis* strains producing multiply antibacterial peptides or proteins or their combinations directed against particular pathogen. In this case, antibacterials would be continuously produced for long time until extinction of the producer strain from the intestinal tract. In addition, intestinal localization of multiplying *B. licheniformis* will protect secreted active substances from aggressive action of stomach environment. However, more studies are needed for the exploration and development of perspective capability of *B. licheniformis* to synthesize and produce a bouquet of different antibacterial compounds for application in medicine and veterinary.

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