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BACTERIOCINS OF SOME GROUPS OF GRAM-NEGATIVE BACTERIA

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The results of gram-negative bacteria bacteriocins research have been analyzed. These killer factors are characterized by powerful antimicrobial activity, narrowly directed spectrum of action and safety for the macroorganism. Bacteriocins, especially produced by gram-negative bacteria, are investigated very differentially and, in most cases, insufficiently, the available information is not systematized. The present article focused on bacteriocins, which are active against phytopathogenic bacteria and can be used in crop production as independent biocontrol agents, as well as on the killer factors of marine microorganisms, which application in aquaculture is allowed only with their producer-strains.

Keywords: bacteriocins, phytopathogenic bacteria, crop production, marine microorganisms, aquaculture.

Bacteriocins are considered to be one of the most widespread natural agents of bacterial defense [1, 2]. This large and quite diverse family of killer factors is characterized by storage stability and low toxicity to humans, but their narrow killer activity is limited to bacterium species closely related to producer strain [3, 4]. Bacteriocins are synthesized by the majority of gram-positive and gram-negative microorganisms [5]. However, in contrast to the killer factors of gram-positive bacteria, bacteriocins of gram-negative bacteria have not been sufficiently studied [6, 7]. Among the killer factors of gram-negative bacteria, the most carefully explored substances are colicins – bacteriocins of *Escherichia coli* [1, 8–10]. Thanks to a number of researches of foreign and Ukrainian scientists, bacteriocins of *Pseudomonas aeruginosa* (pyocins) [11–15] and *Pectobacterium carotovorum* (carotovoricins) are also well known [16–18]. At the same time, little is known about bacteriocins of phytopathogenic, marine, soil microorganisms, representatives of normal human and animal microflora and their closely related species [19, 20].

Today, bacteriocins attract attention as potential drugs, alternative to existing medicines for certain diseases [6, 21]. In most cases, these killer factors are active against closely related bacterial species. Considering insignificant amount of side effects, it creates the necessary prerequisites for their use as narrow-spectrum antimicrobials [22, 23]. However, not only purified or partially purified bacteriocin preparations can find application. It is known that most bacteria secrete killer factors for competitive advantage in a particular environmental niche [1, 24]. Therefore, bacteriocin-producing strains can become potential components of biological products for treatment and diseases prevention of plants, animals and even humans, since their use will contribute to elimination of pathogens from certain biotopes [22, 25]. Recent attempts to create genetically engineered bacteriocins, aimed at making these killer factors more usable, increase the possibility of widespread introduction of these substances in crop production, animal husbandry, medical practice, and food industry [3, 26].

Basic concept about bacteriocins

Bacteriocins are heterogeneous antibiotic-like substances, mainly of protein nature, synthesized by most bacteria and characterized by bactericidal action against representatives of phylogenetically close species of microorganisms [1, 8, 9]. This group of substances includes killer factors with different morphological and biochemical properties, molecular weight, activity spectrum and mechanisms of action: peptides, low molecular weight proteins, enzymes, phage-like structures,

that are synthesized by defective lysogenic bacteria [11, 19, 27]. These substances are joined in a single family according to common features: bacteriocins are the primary metabolites of protein nature, which are synthesized on the ribosomes, released at the exponential growth phase, active against a rather narrow range of related bacteria, except for their own producer strains [3, 19, 22]. Other bacteriocin-related groups of antimicrobials are antibiotics and bacteriophages [4, 28–32]. In contrast to bacteriocins, classical antibiotics are secondary metabolites of non-protein nature predominantly, not synthesized on the ribosomes, released at the stationary growth phase, have a broad spectrum of action, inhibit the growth of their own producer strains, and also affect eukaryotic cells [3, 13, 20, 33]. High molecular weight bacteriocins are morphologically similar to bacteriophages, but have slightly lower molecular weight, do not contain nucleic acids and therefore are not capable of self-replication in bacterial cells [6, 12]. When applied to a lawn of sensitive culture, high molecular weight bacteriocins form lysis zones, the intensity of which is reduced under dilution without the appearance of phage plaques, and these killer factors are not transferred from the formed zones to the fresh lawn of the indicator culture [16, 31].

According to producer strains, bacteriocins can be divided into two main groups: protein bacteriocins secreted mainly by Gracilicutes – gram-negative bacteria, and peptide bacteriocins of Firmicutes – gram-positive microorganisms [1, 34]. Bacteriocins of gram-negative bacteria can be divided into four substantially different groups: macromolecular (high molecular weight) bacteriocins, colicin-like (low-molecular-weight) bacteriocins, microcins, and unclassified bacteriocin-like substances [4, 10].

High molecular weight bacteriocins (tailocins) are protein particles with molecular weight of about 1–10 MDa. They resemble tails of bacteriophages, as they are composed of sheath, core, baseplate and tail fibres [35, 36]. Macromolecular killer factors affect sensitive cells by disrupting cell wall continuity [11]. Low molecular weight proteins of 20–100 kDa are referred to colicin-like bacteriocins [6, 11, 18, 37, 38]. These killer factors are synthesized as two proteins, which subsequently function together as a protein subunit. One protein is actually bacteriocin, while the other is immunity protein [1]. Microcins are a group of simple proteins synthesized by *Enterobacteriaceae* family members, however, they have much lower molecular weight – less than 10 kDa [39–41]. Most representatives of this killer factor group undergo posttranslational modification during maturation [42, 43]. A number of substances isolated from gram-negative bacteria can be classified as bacteriocins, however, due to significant differences in structure, they do not belong to any groups described [14, 44]. An example of such substances can be bacteriocin-like inhibitory substances (BLIS) synthesized by marine gram-negative microorganisms, only a few of them are characterized in detail [19]. Other representatives are synthesized by *Agrobacterium tumefaciens* and *Rhizobium leguminosarum* tripholitoxins or RTX-type (from repeats in toxin) toxins of *R. leguminosarum*. Genome analyses of gram-negative bacteria revealed that some members of this group may also produce peptide bacteriocins, referred to as "gram-positive-like" peptide bacteriocins [20]. It should be noted that the classification of bacteriocins, especially synthesized by gram-negative bacteria, is currently at the stage of evidence accumulation [1, 4]. Therefore, the search and study of new substances in this group will help to form a clearer conception of the prevalence and importance of bacteriocins in the processes of inter-bacterial interaction.

Bacteriocins of phytopathogenic bacteria

According to data of the Food and Agriculture Organization of the United Nations (FAO), bacterial diseases cause the loss of about 30 % of agriculture crop. Bacterial diseases of plants create great difficulties for crop production and horticulture [45–47]. The rhizosphere and plant-associated biotopes are densely populated by a large number of microbial species [48]. The ability of phytopathogenic microorganisms to survive at this bacterial diversity, as well as to interact with the host plant, is important factor for their ecological adaptation [20]. Under competition for nutrients, bacteria use different survival strategies. One of them is the synthesis of substances with antimicrobial properties, in particular bacteriocins, active against closely related bacteria that inhabit a certain ecological niche [10, 49]. By affecting the survival of microbial cells and, even their virulence, bacteriocins are able to regulate the abundance of bacterial population [9]. It should be noted that the application of narrowly specific killer factors can be an effective strategy for bacterial diseases control, including pathogens of crops, against which even chemical pesticides are ineffective [2, 20, 50].

Potential use of bacteriocins for the regulation of phytopathogenic bacteria has been suggested by a number of researchers [46, 51, 52]. Other authors consider the application of bacteriocin-producing strains, in particular gram-negative bacteria, in the composition of biopreparations for crop production [53]. The application of these biological means for plant disease control will limit the use of chemical pesticides, and therefore will lead to quality improvement of crop production, agricultural soils and ecological state of the environment [3, 47].

It is known that most plant pathogens belong to gram-negative species and almost all bacteriocins synthesized by these bacteria are proteins [47, 51]. Gram-positive species dominate among microorganisms found in soil and rhizosphere of plants, as well as among saprophytic bacteria. For these bacteria, many peptide bacteriocins, especially of the first class (lantibiotics), have been identified and characterized [9, 22]. Instead, bacteriocins of phytopathogenic bacteria have been investigated selectively [2]. Information on bacteriocins synthesized by most of these microorganisms is missing. For some species, only initial studies were conducted, denoting the presence of certain substances with antimicrobial activity and their basic characteristics. Also little is known about the structure, killer activity, regulatory systems, and killer activity spectrum of the detected bacteriocins [20].

Pseudomonas syringae strains are characterized by high injuriousness and a high frequency of isolation (50–80 % – on leguminous plants and up to 90 % – on cereal crop) among the plant pathogens. The inherent trait of these microorganisms is high resistance (90–100 %) to most commercial chemical and biological pesticide preparations [45, 54]. Therefore, it is relevant to search new means for plant protection against diseases caused by these microorganisms [50, 55]. So, in Lavermicocca et al. research [46] from *Pseudomonas syringae* pv. *ciccaronei* were isolated and purified bacteriocins, potentially containing three proteins with a molecular weight from 45 to 76 kDa. The authors showed that these bacteriocins inhibit the reproduction of *Pseudomonas syringae* subsp. *savastanoi* – causative agent of the olive knot disease both in the laboratory and in the field experiments [51]. The results of the genome sequence of a phytopathogenic bacterium *Pseudomonas syringae* pv. *syringae* revealed the presence of S-type pyocins [56], also found in human opportunistic pathogen *Pseudomonas aeruginosa* [11, 57]. These killer factors with a molecular weight of 65–80 kDa belong to colicin-like bacteriocins [6, 38]. S-type pyocins consist of two components: a large component with killer activity and small component (immunity protein) [11]. They mainly inhibit the activity of other pseudomonas species [38, 58]. It was found that under plant treatment with bacteriocins, killer factors prevent the spread of phytopathogenic microorganisms by influencing their epiphytic phase [59]. Antimicrobial activity against a significant number of *Pseudomonas* species, in particular to a number of *Pseudomonas syringae* pathovars, is characterized for putidacins – *P. putida* bacteriocins. So, for the rhizospheric isolate *Pseudomonas* sp. BW11M1 the production of lectin-like putidacins (LlpA) with a molecular weight of 30 kDa was revealed. This bacteriocin contains sites that are similar to the mannose-binding domains of lectins from monocotyledonous plants [20]. Recently, two lectin-like bacteriocins with similar inhibitory spectrum of action were also identified in widely used biocontrol strain *Pseudomonas fluorescens* Pf-5 [60]. The biological properties of some pseudomonad strains, in particular *P. fluorescens* and *P. putida*, which are not only antagonistic to phytopathogenic microorganisms but also characterized by growth-stimulating activity, have been described in the literature [61, 62]. The advantage of these *P. fluorescens* and *P. putida* cultures is also the ability to induce the development of systemic resistance in plants. The given examples indicate the urgency of development of biopreparations based on fluorescent pseudomonad species; however, their number remains low [51, 63].

Bacterial burn of fruit trees caused by phytopathogenic bacteria *Erwinia amylovora* is a particularly dangerous, quarantine disease [64]. It brings on necrotic lesions of *Rosales* plants and creates great difficulties leading to significant crop losses of fruit trees [3]. For biological control of bacterial burns of fruit trees Kearns and Mahanty proposed to use *E. herbicola* bacteriocins [65].

Also the influence of seracin P – high molecular weight bacteriocin of phage-tail type isolated from *Serratia plymithicum* – on the causative agent of this disease is investigated [20, 64]. Another bacteriocin-producing strain *Serratia entomophila* is the basis of biopreparation (biopesticide) Invade, used against New Zealand larvae *Costelytra zealandica* [66]. Similar killer factors have also been found in *Rhizobium* strains. Quite

possible, that tailocins described in earlier studies of *Pseudomonas syringae* also belong to this type [20]. It was shown that *Pseudomonas aeruginosa* bacteriocins are also characterized by high activity against phytopathogenic bacteria. Moreover, they affect not only *P. syringae* strains but also *P. savastanoi* [50, 55].

Other representatives of *Erwinia* genus and microorganisms currently reclassified into the new *Pectobacterium* genus also cause significant losses to the national economy. *P. carotovorum* and *P. chrysanthemi* should be especially noted as they cause maceration and necrosis of plant tissues, entailing lesions of potatoes, cereals and many other cultivated plants in temperate, tropical and subtropical latitudes [67]. F.I. Tovkach and his scientific school have made a significant contribution to the study of *P. carotovorum* bacteriocins. For example, it was shown that the lysogenic system of the phytopathogenic bacterium *P. carotovorum* is a unique model for research, since the simultaneous production of two bacteriocin types – macromolecular and colicin-like carotovoricins – is possible under the induction of this system [16, 68]. A similar multiplicity of killer factor synthesis was observed only for a small number of bacterial species. It was also determined that killer particles were produced in different percentages depending on type of inducing factors [69]. And the secretion of carotovorcins has a wave-like character [70]. In F.I. Tovkach's study it was shown that *P. carotovorum* bacteriocins are capable of affecting a number of *Enterobacteriaceae* family microorganisms that can cause disease in animals and humans [16]. Pectobacteria, in turn, don't possess pathogenic properties against mammals, and substances produced by these bacteria are less toxic under parenteral dosing than similar substances of other producers.

Recently it was shown that *Pectobacterium carotovorum* (formerly known as *Erwinia carotovora* ssp. *carotovora*) produce small colicin and pyocin-like antimicrobial proteins with a molecular weight of about 55 kDa, designated as S1 carocins. This protein inhibits other strains of the same species, probably due to DNase activity [71].

Carotovoricin-producing strains can be also referred to means of bacterial plant diseases control. Thus, for treatment and spread limitation of Chinese cabbage soft rot caused by *P. carotovorum* subsp. *carotovorum* pathogenic strains, avirulent bacteriocin-producing mutant strains of the same species can be used [72].

Gram-negative phytopathogenic bacteria can also produce bacteriocins, which possess antibacterial activity by self-assembly into cytotoxic phage-like killer particles (high molecular weight bacteriocins). Carotovoricins produced by *Pectobacterium carotovorum* are the most studied representatives of this group [73]. It was shown that these bacteriocins are also characterized by lytic activity against closely related microorganisms [69]. Moreover, the killer spectrum of these bacteriocins is determined by the structure of tail fibres [74].

Tailocins have also been detected in *Ralstonia solanacearum* (previously *Pseudomonas solanacearum*). These bacteriocins inhibit growth of phytopathogenic microorganisms not only in laboratory experiments but also on plant objects in field conditions [20]. It was shown that plant immersion in a suspension of a bacteriocin-producing avirulent strain of these bacteria prevents the development of bacterial tobacco fading. Also tomatoes treatment by bacteriocin-producers reduces the percentage of their loss from withering. Similar activity was also observed for high molecular weight bacteriocins synthesized by *Rhizobium lupini* 16-3 that inhibits *Pseudomonas syringae* [20].

Several representatives of phytopathogenic microorganisms can produce protein bacteriocins, which are different from the antimicrobial agents described above. These bacteriocins are not sufficiently characterized to be referred to certain group or for classification, but they possess high activity against plant pathogens. Thus, heterodimeric bacteriocins called glycinecins were isolated from *Xanthomonas campestris* pv. *glycines* – the causative agent of soya bacterial pustules [3]. These substances are heterodimers of two polypeptides. Genetic determinants of this killer factor are localized in two separate genes glyA and glyB, which synthesize 39 and 14 kDa subunits respectively [75]. Glycinesin does not have substantial similarity to sequences of other currently known protein bacteriocins and acts by increasing the permeability of target cell membranes [76]. The spectrum of these bacteriocins activity mainly includes other pathovars of *X. campestris*, and *X. oryzae* pv. *oryzae*, which causes bacterial burns in rice [75]. The results of other investigations revealed that glycinesin A is capable of affecting most phytopathogenic bacteria of *Xanthomonas* genus [3]. The plant treatment by nonpathogenic bacteriocin-producing *X. campestris*

pv *oryzae* strains substantially reduced the spread and development of the most dangerous leaf form of bacterial burns, which provokes watery spots, stripes and yellowing of rice leaves [3, 77]. Hert et al. showed in laboratory and field experiments that uncharacterized proteins production helped *Xanthomonas perforans* to inhibit tomato pathogen *Xanthomonas euvesicatoria* [78]. Colicin-like proteins were also found in the genomes of both sequenced strains *Xanthomonas oryzae* pv. *oryzae* and *Xylella fastidiosa* [20]. Oresnik et al. revealed that *Rhizobium leguminosarum* strains produced RTX-type toxins with molecular weight about 100 kDa. These proteins provide a competitive advantage of producing strains against closely related phytopathogenic microorganisms in the process of plant nodules occupancy [79].

The research of phytopathogenic microorganisms' bacteriocins is obviously important. The agriculture needs environmentally safe and effective methods for plant disease control [60]. Under such study, new narrow-spectrum antimicrobial compounds can be revealed, capable of satisfying these requirements [20, 25]. If economically substantiated production is created or approaches to their synthesis are developed, bacteriocins can be used in the semipurified state. On the other hand, killer factors must be obtained from avirulent producing strains. After all, there is an alternative to create bacteriocin-producing transgenic crops [7, 80]. Thus, several approaches for bacteriocin application in crop production are considered, which can provide the creation of new means for plant diseases control [2]. The study of bacteriocins of marine microorganisms is at similar stage. However, the prospects for their use have other peculiarities.

Bacteriocins of marine microorganisms

According to the FAO report, the average consumption of aquaculture products comparatively to the total fish consumption per person increased from 14 % in 1986 to 47 % in 2006 [19]. It is expected that this index will grow up to 50 % in following years [81]. However, the development and intensification of water industry can lead not only to a rise of the density of aquaculture population, but also to an increase in the infectious lesion of its representatives by pathogenic microorganisms [82]. Significant economic losses in fish production are due to diseases provoked by a limited range of microorganisms. *Vibrio* strains are one of the most widespread pathogens that cause high mortality of fish larvae culture [83]. Climate change is also important, as many microorganisms at higher temperature have higher virulence and transmission [84]. At the same time, the preventive use of antibiotics is harmful [85]. Vaccines, antimicrobials and probiotic cultures were proposed for pathogen growth inhibition [82]. Bacteriocinogenic strains are considered as a great alternative to antibiotics, since bacteriocins can substitute antibiotics and their producing bacteria – potential probiotics [7, 22].

Living organisms, present in the upper layer of the sea surface, are capable for production of a considerable number of antibacterial compounds [86]. Many papers described antimicrobial substances synthesized by marine microorganisms isolated from sponges, corals, algae and shellfish [87, 88]. However, only a few studies examined the ability of bacteria associated with marine animals to synthesize bacteriocins [19]. There are several cases when substances previously considered being produced by higher organisms were synthesized by microorganisms. An example of such substance is patellamide – a peptide with bioactive properties, synthesized by *Prochloron didemni* [89]. The microorganisms associated with marine animals include bacteria of *Vibrio*, *Pseudoalteromonas*, *Aeromonas*, *Alteromonas* genera, and also *Cytophaga–Flavobacterium–Bacteroides* group [87]. There is a set of reports concerning antibacterial peptides or proteins which were synthesized by marine bacteria and identified by combining sequencing methods and detection of structural organization. Wilson et al. isolated 8 strains of marine bacteria able to produce antibacterial substances from a wide variety of marine invertebrates (oysters, shellfish, sponges, tunics, sea urchins, algae) [87]. The loss of activity of these substances after proteolytic cleavage suggests their protein nature [19].

However the application of purified bacteriocins in aquaculture is insufficiently profitable. Selection of associated with marine animals bacteriocinogenic and nonpathogenic bacteria for their use as probiotics is more advantageous [19]. The definition of term "probiotic" was formulated by Fuller in 1989, then partially modified by Salminen in 1999, and now WHO interprets that "Probiotics are live microorganisms, which in adequate quantities help to improve the health of the host" [90]. It should be noted that pathogenic bacteria in the aquatic environment reproduce independently of the host. They can reach a

high concentration in water and become able to permanently affect the intestinal microbiota of aquatic organisms through their ingestion [91]. Therefore, probiotic strains for aquaculture must be significantly different from those used in animal husbandry or medicine, since they have to influence not only host, but also environment [92].

In view of the above, a strategy for the selection of bacteriocin-producing strains for aquaculture was developed. The first and most important step is the *in vitro* screening of bacteria associated with aquatic organisms for the presence of antagonistic activity against pathogens [93]. Probiotic bacteria must be isolated from microbiota of aquatic vertebrates or invertebrates. It will improve their establishment and allow maintain the productivity under possible difference of temperature and salinity from optimal parameters for these microorganisms [94]. The second stage of selection involves determination of nature of inhibitory substance, its mechanism of action and genetic aspects of localization. The third step is the test of *in vivo* safety of bacteriocin use for both the host and the environment. Also at this stage the profitability of selected substances application under appropriate growing conditions is evaluated [93].

It is important that dominant members of the normal microbiota of gastrointestinal tract of endothermic animals in the early stages of their life are gram-positive bacteria. The gastrointestinal microbiota of healthy fish usually contains lactic acid bacteria belonging to *Streptococcus, Lactobacillus, Carnobacterium, Leuconostoc* genera [19]. Most of the probiotics used in aquaculture are widely researched and belong to lactic acid bacteria and members of the genus *Bacillus* [95–97]. Among gram-negative bacteria, the representatives of *Aeromonas, Pseudomonas, Pseudoalteromonas, Roseobacter* and *Vibrio* genera have considerable potential [93, 98].

Bacteriocin-like substances of *Vibrio* sp. Bacteria of *Vibrio* genus are widespread in the marine environment and, in most cases, isolated from fish and shellfish [99]. Some types of vibrios can cause diseases of marine organisms and humans, while others are non-pathogenic. For some *Vibrio* strains the ability to secrete bacteriocin-like inhibitory substances (in abbreviated form – BLIS) was described.

Zai et al. [100] isolated and identified 50 *Vibrio* strains from gills and viscera of healthy and infected catfish. For these strains BLIS was found out and called vibriocin AVP10 [19].

Fresh and frozen seafood was analyzed by Carraturo et al. [101]. These researchers isolated 3 non-pathogenic for human *Vibrio* species (*V. mediterranei* 1, *V. mediterranei* 4 and *V. fluvialis*), which on a dense agar medium exhibited antagonistic activity against pathogenic strains of the same genus – *V. parahaemolyticus* and *V. mediterranei*. For BLIS of *V. mediterranei* 1, the step-by-step partial purification was described. The albuminous nature of the isolated substances was confirmed by enzymatic cleavage by proteinase K. Carraturo et al. [101] used gel chromatography to purified the fraction with antimicrobial activity. Determined by SDS-PAGE the molecular weight of purified BLIS was 63–65 kDa and corresponded to a mixture of unrelated polypeptides, among which bacteriocins were detected [19].

V. harveyi strains are pathogenic for many invertebrates and vertebrates marine animals [102, 103]. McCall and Sizemore [19] firstly informed of bacteriocin production by strain *Beneckea harveyi* (*V. harveyi*), which was designated as harveyicin SY. This bacteriocin was characterized by a molecular weight of about 24 kDa and caused lysis of two strains *V. harveyi* KN96 and BBP8. Harveicin proved to be sensitive to proteolytic enzymes, and its genetic determinants were obviously plasmid-associated [19]. During screening of *V. harveyi* culture collection, Prasad *et al*. [102] discovered ability of strain VIB 571 to produce BLIS. This culture was pathogenic for rainbow trout (*Oncorhynchus mykiss*) and Atlantic salmon (*Salmo salar*) [103]. Inter-strain and inter-species inhibition due to bacteriocin-like inhibitors of *V. harveyi* VIB 571 was detected against 4 isolates of the same species and also against *V. fischeri*, *V. gazogenes,* and *V. parahaemolyticus*. The impure BLIS was obtained by ammonium sulfate precipitation from 72-hour culture previously purified from cells. It was inactivated by lipase, proteinase K, pepsin, trypsin, pronase E and SDS. Incubation of bacteriocins for 10 min at 60° C and above caused loss of their activity. On the other hand, the change in pH did not affect the antibacterial activity. By means of ion exchange chromatography, gel filtration, SDS-PAGE and two-dimensional gel electrophoresis it was detected one major peak represented by a protein with a pI of about ~ 5.4 and molecular weight of about 32 kDa. The sequence of the N-terminus of this protein was established: D-E-Y-I-S-X-N-K-X-S-S-A-D-I, where at position

'X' there could be either cysteine or a modified amino acid residue [19].

Shehane and Sizemore obtained other vibriocins [104]. The purpose of these researches was to identify bacteriocins, active against *V. vulnificus*, isolated from seafood. They tested antimicrobial activity of plasmid-containing strains sampled from the mouth near the port of Wilmington (North Carolina, USA). Strains whose antimicrobial activity was due to lytic bacteriophages or low molecular weight nonspecific molecules were rejected immediately. As a result, three producers of bacteriocins were revealed among *V. vulnificus* and their inhibition spectrum was determined. Thus, the first selected strain IW1 inhibited a small number of *V. vulnificus* cultures. The second isolate BC1 – several strains of *V. vulnificus*, as well as *V. cholerae* and *V. parahaemolyticus*. Instead, a third producer BC2 excreted substances that inhibited the growth of all used strains of *Vibrio* spp, *Plesiomonas shigelloides,* and *E. coli*. It was found that the loss of bacteriocinogenic plasmid by these isolates caused the loss of inhibitory activity. By means of gel chromatography, the molecular weight of bacteriocins from strain IW1 was found to be 9.0 kDa, for strain BC1 it was 7.5 kDa, and for BC2 – 1.35 kDa. It should be noted that isolated bacteriocins differed significantly in the sensitivity to temperature. Thus, the substances of strain IW1 were thermolabile. The bacteriocins of BC1 isolate were characterized by moderate stability as they lost activity only after treatment with extreme temperatures. Instead, *V. vulnificus* BC2 substances were highly stable and retained activity after autoclaving, freezing, and exposure to extreme pH values [104]. In consideration of the broad activity spectrum of obtained bacteriocins, the authors have suggested that these substances can be used as control agents for *V. vulnificus* to remove it from seafood [19].

Vibrio sp. NM 10 was isolated from *Leiognathus nuchalis* fish caught in coastal areas near Enosima Island (Kanagawa, Japan). This strain was characterized by high activity against *Pasteurella piscicida* K-III, and was also able to inhibit growth of *E. coli* IAM 1264, *V. vulnificus* RIMD 2219009 and *Enterococcus seriolicida* YT-3 [105]. Antibacterial substance synthesized by *Vibrio* sp. NM 10 proved to be a protein thermolabile compound with a molecular weight below 5 kDa, which indicates its belonging to bacteriocins or bacteriocin-like substances [19, 105].

Bacteriocin-like substances of *Aeromonas* sp. The production of bacteriocin-like substances in *Aeromonas hydrophila* was studied by Moro et al. and Messi et al. [106]. All investigated *A. hydrophila* strains possessed the inhibitory activity against several *Staphylococcus aureus* strains. Messi et al. revealed an additional inhibitory effect of BLIS against *Listeria* sp, *Streptococcus agalactiae* and *Lactobacillus* sp. However, the obtained substances did not influence on all tested gram-negative strains, including closely related species – *Aeromonas sobria* ATCC 43979 and *A. caviae* ATCC 13137. It should be noted that such an inhibitory spectrum is incompatible with the determination of bacteriocins, which allows belonging of the test substances to another class of antimicrobial compounds [19].

Bacteriocin-like substances of *Pseudoalteromonas* sp. In Longeon et al. [107] paper the bacteria isolated from different ecosystems on the UK coast were discussed. The main focus of this work was on the study of nature and biological properties of antimicrobial substances of *Pseudoalteromonas* X-153. This priority was caused by the high antagonistic activity of this strain against a wide range of microorganisms. As a result of conducted research the main active substance was obtained and purified – protein P-153. This protein possessed antibacterial activity and, according to gel chromatography data, had molecular weight of 87 kDa. Protein P-153 was active against both Gracilicutes (ichthyopathogenic *Vibrio*) and Firmicutes (*Staphylococcus epidermidis, Propionibacterium acnes* and *P. granulosum*) bacteria [107]. However, it should be noted that the described spectrum of activity is too broad and not compatible with the determination of bacteriocins [19].

Highly active bacteriocins have been isolated from marine strain BS107 identified as *Roseobacter* [108]. However, it was found that these microorganisms cannot be used for treatment, when the pathogen is present in a concentration sufficient to cause an outbreak of the disease. The authors demonstrated that under simultaneous inoculation of BS107 (even in concentration of 10^6 CFU/ml) and *Vibrio pectenicida* A496 (in concentration of 10^4 CFU/ml), the strain BS107 did not possess any probiotic activity. Therefore, it is important to note that bacteriocin-producing probiotics should be used for preventive purposes [36, 108].

Thus, killer factors of marine microorganisms are powerful antimicrobials, but their use is possible only due to application of producing strains [92, 95]. Getting into the environment, microorganisms, like most other bacteria, start to form a biofilm [109, 110]. In its composition the intensity of bacteriocin production and effectiveness of their action against competitive strains may differ significantly from that described for bacteria in planktonic form [111, 112]. We have shown that the synthesis of killer factors in the biofilm form depend on the environment and, apparently, the temperature of microorganism cultivation [113, 114]. Studies conducted on the classic model of *Pseudomonas aeruginosa* indicate that the process of biofilm formation goes through a series of stages characterized by certain structural features [115–117]. In this case, the transition to the biofilm form almost immediately causes the bacterial resistance to the action of antimicrobial substances [118]. The mentioned features should be taken into consideration and bacteriocins and their producing strains [119, 120] must be tested before their application to the environment.

Thus, the need for specific antimicrobial agents that can be used in crop production and aquaculture, and significant potential of bacteriocins promoted the intensive increase of work quantity in this area over the past decades. However, it should be noted that most of the studies are still at the initial stages. There is a hope that these investigations will be continued and find practical application in the form of biopreparations.

БАКТЕРІОЦИНИ ДЕЯКИХ ГРУП ГРАМНЕГАТИВНИХ БАКТЕРІЙ

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Резюме

Проаналізовано результати досліджень бактеріоцинів грамнегативних бактерій – кілерних факторів, які характеризуються потужною антимікробною активністю, вузько направленим спектром дії і безпечністю для макроорганізму. Бактеріоцини, особливо грамнегативних бактерій, досліджені вкрай неоднаково і, в більшості випадків, недостатньо, а наявна інформація не систематизована. В наведеній роботі зроблено акцент на бактеріоцинах, активних щодо фітопатогенних бактерій, які можуть бути використані в рослинництві як самостійні засоби впливу, а також на кілерних факторах морських мікроорганізмів, застосування яких у водному господарстві припустиме лише разом із їх мікроорганізмами-продуцентами.

Ключові слова: бактеріоцини, фітопатогенні бактерії, рослинництво, морські мікроорганізми, водне господарство.

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