

## **BACTERIOCINS IN POULTRY NUTRITION – A REVIEW\***

Damian Józefiak<sup>1\*</sup>, Anna Sip<sup>2</sup>

<sup>1</sup>Department of Animal Nutrition and Feed Management, Poznań University of Life Sciences,  
Wołyńska 33, 60-637 Poznań, Poland

<sup>2</sup>Department of Biotechnology and Food Microbiology, Poznań University of Life Sciences,  
Wojska Polskiego 48, 60-627 Poznań, Poland

\*Corresponding author: damjo@up.poznan.pl

### **Abstract**

**In recent years, a number of studies have shown a close relationship between broiler performance, health and the gastrointestinal microbiota. However, taking the complexity and biodiversity of the micro-ecosystem into consideration, a manipulation of the microbiota in a way that is profitable both for the host bird and for the farmer seems a difficult goal to achieve. Bacteriocins are extracellular proteinaceous compounds, synthesized by many bacterial species. Due to their different bacteriostatic effects, they have been used in human nutrition for decades. However, limited information is available regarding their effects in poultry, even though that similar mode of action as in other animals is possible. Therefore, the aim of the present review is to discuss present bacteriocin classification, mode of action and their potential role in poultry nutrition.**

**Key words: poultry, feed additives, microbiota, bacteriocins**

The impact of broiler chicken gut microbiota on birds' performance as well as poultry products (i.e. meat, eggs) have been investigated in recent years quite thoroughly. There are many reasons why researchers all over the world have started to explore this unique micro-ecosystem but probably one of the main aspects is the ban on feed antibiotics. Thus on the market today there are plenty of products, which are claimed to be potential alternatives of antibiotics. Moreover, in the available literature, there are even more papers showing different aspects of probiotics, prebiotics, organic acids, phytobiotics, etc. (Alloui et al., 2013; Dahiya et al., 2006; Totton et al., 2012). However, the problem is that poultry gastrointestinal tract (GIT) microbiota is still a relatively poorly described ecosystem. Thus, even knowing the mode of action of those substances, in most cases we have no idea which populations are targeted and if this is somehow beneficial for the host bird. This is why, very often, the only parameters which are then taken into consideration, are feed conversion and body weight gain of the birds. Apart from birds' performance, the important aspect of poultry production is the effect of the diet on carcass quality, not only in terms of

---

\*Consortium research project carried out in a frame of EU Structural Funds, WNP-POIG.01.03.01-30-179/09.

its composition and post-slaughter values but also microbiological contamination, which is strictly combined with processing but also feeding strategies. Thus, it seems that in coming years, it will become more important to develop substances which can have complex effects on poultry production, improving the quality of the meat at different stages, from farm to fork. In this aspect, bacteriocins can have some beneficial features, as they can modulate different populations of poultry GIT microbiota but also can be used as preservatives in raw meat as well as in ready-to-eat products. Therefore, the aim of the present review is to discuss present bacteriocin classification, mode of action and their potential role in poultry nutrition.

### **Classification of bacteriocins**

In contrast to antibiotics, so far there has been no information about negative effects of bacteriocins on animals or humans. It should be remembered that they have been used as antimicrobial agents in human nutrition for decades (Cleveland et al., 2001; Joerger, 2003; Leisner et al., 2007); however, only limited information is available regarding their effects in poultry nutrition and/or microbiology. Bacteriocins are described as extracellular proteinaceous compounds which are synthesized by many bacterial species. These compounds are diversified with respect to their chemical properties, genetic determinants, mode of action, scope of antimicrobial activity, structure (especially secondary), and mechanism of post-translational modification and secretion (Jack et al., 1995; Klaenhammer, 1993).

The general characteristics of bacteriocins are presented in Table 1. Bacteriocins have molecular weights of a few to several dozen kDa, hydrophobic or amphipathic character, and are positively charged. They are stable in many organic solvents, polymers and detergent solutions. Bacteriocins are synthesized by ribosomes in an inactive precursory form. Bacteriocin-encoding genes are localized on plasmids, chromosomes and transposons. Bacteriocin producing strains protect themselves against the toxicity of their own bacteriocins by the expression of specific immunity proteins, which are generally encoded in the bacteriocin operon. Bacteriocin production is frequently regulated by a three-component signal transduction system consisting of an induction factor (IF), histidine protein kinase (HPK) and a response regulator (RR) (Diep et al., 2007; Jack et al., 1995; Klaenhammer, 1993). Due to their proteinaceous nature, bacteriocins are sensitive to proteolytic enzymes. Most of them get inactivated after being treated with pepsin, trypsin, proteinase K and pronase E. Bacteriocins composed of protein-carbohydrate complexes are also sensitive to the action of amylolytic enzymes; and those composed of proteins and lipids are sensitive to lipases. Most bacteriocins are stable at pH in the range of 3.0 to 9.0, and are extremely heat-resistant. At 1 atmosphere pressure they remain stable after exposure to 121°C for a few minutes (Montville et al., 1995). Their resistance to high temperature depends on the degree of the purity of bacteriocin preparations, pH and ionic strength and increases with higher acidity and lower degree of purification.

Many Gram-positive and Gram-negative bacteria have the ability to synthesize bacteriocins (Table 2). Lactic acid bacteria (LAB) are particularly interesting. LABs are commonly used in food and feed industry and have GRAS status (generally regarded as safe, 21 CFR 184.1538). Bacteriocins produced by LAB have been or-

ganized into four classes: I, II, III and IV (Diep et al., 2007; Hechard and Sahl, 2002; Klaenhammer, 1993; Nes et al., 1996). Class I includes lantibiotics, i.e. thermostable, membrane-active peptides, of molecular mass under 5 kDa, containing lanthionine in their structure. Class II comprises non-lantibiotics, thermostable and also membrane-active peptides, of molecular mass under 13 kDa. The characteristic trait of class II bacteriocins is a Gly-Gly sequence present in the precursory peptide, which is recognized by site-specific proteases cutting off a leader peptide from an active bacteriocin. Class II comprises 4 sub-classes: IIa – pediocin-like bacteriocins, also known as cystibiotics, IIb – dipeptide bacteriocins, IIc – sec-dependent bacteriocins, and IId – bacteriocins differing from all the other bacteriocins class II. Class III consists of thermolabile bacteriocins of molecular mass above 30 kDa having no membrane damaging properties. Bacteriocins which form protein-lipid or protein-carbohydrate complexes have been included into class IV.

Table 1. General characteristics of bacteriocins (Cleveland et al., 2001; Deegan et al., 2006; Galvez et al., 2007 a; Jack et al., 1995)

Origin	– natural, extracellular metabolites of many Gram-positive and Gram-negative bacterial strains
Effect on human organism	– safe for human organism; non-cytotoxic, non-carcinogenic, non-allergic, inactivated by digestive proteases
Spectrum of activity	– usually narrow; most bacteriocins are effective against closely-related bacteria – some bacteriocins produced mainly by LAB have broad spectrum of antimicrobial activity and act also on many food-borne pathogenic and spoilage microorganisms – range of antimicrobial activity of individual bacteriocins is different
Mode of action	– bactericidal – bacteriostatic – fungicidal – some bacteriocins; weakly documented
Mechanism of action	– membrane permeabilization – inhibition of DNA, RNA and protein biosynthesis – cell lysis
Chemical structure	– simple proteins – glycoproteins – lipoproteins
Molecular weight	– from a few to a dozen kDa; usually under 10 kDa
Number of amino acids in molecule	– from 19 to 80; usually about 40
Character	– hydrophobic – amphiphilic
pI	– from 8.1 to 10.0
Localization of bacteriocin-encoding genes	– plasmids – chromosome – transposons (both plasmids and chromosome)
Sensitivity to enzymes	– all bacteriocins are sensitive to proteolytic enzymes (pepsin, trypsin and pronase) – bacteriocins with complex structure, are also sensitive to amylolytic and/or lipolytic enzymes
Sensitivity to temperature	– heat-stable compounds; most of bacteriocins endure heating at 100–121°C for 15–30 min
Sensitivity to pH	– most bacteriocins are stable at pH range of 3.0 to 9.0

Table 2. Bacteriocin-producing bacteria (Jack et al., 1995; Montville et al., 1995)

Gram-positive	Gram-negative
<i>Bacillus</i>	<i>Actinobacillus</i>
<i>Bifidobacterium</i>	<i>Acetobacter</i>
<i>Brevibacterium</i>	<i>Bacterioides</i>
<i>Carnobacterium</i>	<i>Brucella</i>
<i>Clostridium</i>	<i>Caulobacter</i>
<i>Corynebacterium</i>	<i>Citrobacter</i>
<i>Enterococcus</i>	<i>Enterobacter</i>
<i>Lactobacillus</i>	<i>Erwinia</i>
<i>Lactococcus</i>	<i>Escherichia</i>
<i>Leuconostoc</i>	<i>Haemophilus</i>
<i>Listeria</i>	<i>Halobacterium</i>
<i>Micrococcus</i>	<i>Klebsiella</i>
<i>Mycobacterium</i>	<i>Niesseria</i>
<i>Pediococcus</i>	<i>Pasteurella</i>
<i>Propionibacterium</i>	<i>Proteus</i>
<i>Sarcina</i>	<i>Pseudomonas</i>
<i>Staphylococcus</i>	<i>Salmonella</i>
<i>Streptococcus</i>	<i>Serratia</i>
<i>Weissella</i>	<i>Shigella</i>
	<i>Yersinia</i>
	<i>Vibrio</i>

### Mode of action

Bacteriocins have either bactericidal or bacteriostatic activity. They usually bind to specific receptors located on the surface of microorganisms. These receptors facilitate the transport of bacteriocins and other compounds through cell membranes. So far the structure and properties of these receptors have not been clarified. Bacteriocins may trigger: 1. Cell membrane poration, entailing dissipation of transmembrane potential ( $\Delta\Psi$ ) and induction of  $K^+$  ions, ATP and amino acids leakage from affected cells, 2. Cell lysis, 3. Disruption or inhibition of DNA, RNA and protein synthesis (act like DNAses or RNAses) (Diep et al., 2007). Some bacteriocins have been suggested to exert their antagonistic activity on moulds as well (Adebayo and Aderiyi, 2011) but this activity is hardly documented in the literature. Irrespective of the type and producing strain, bacteriocins are highly specific in their action. In many cases, their specificity is comparable to the specificity of antibiotics. However, the scope of antimicrobial activity is much narrower than that of antibiotics; since bacteriocins usually are antagonistic against a few bacterial groups, usually closely related to the bacteriocin-producers themselves. Still, some bacteriocins are characterized by a wider scope of action also targeting non-related microorganisms, among those human or animal pathogens (Klaenhammer, 1993; Marugg, 1991). Moreover, some bacteriocins toxic to food-borne pathogens are often inactive towards microorganisms beneficial for human and animal organisms, i.e. probiotics (Cleveland et al., 2001; Galvez et al., 2007 b; Galvez et al., 2008). This feature is reverse to the scope of activity of most antibiotics. Furthermore, the use of bacteriocins helps avoiding bacterial resistance and does not disturb the natural equilibrium of the intestinal ecosystem. In contrast to antibiotics, these peptides are fully safe for human and animal consumption, since they are digested into simple, non-harmful and well-metabolized

compounds. Further, bacteriocins are also considered to be non-cytotoxic and non-carcinogenic (Cleveland et al., 2001).

LAB bacteriocins are frequently applied in food preservation, as they inhibit growth of undesired microorganisms and improve sensory properties of food (O'Sullivan et al., 2002; Schillinger et al., 1996). Furthermore, bacteriocins meet majority of requirements for good food additives (Barnby-Smith, 1992). Thus, it should be stated that bacteriocins are: 1) safe while digested into simple, non-harmful and well-metabolized compounds, 2) non-cytotoxic and non-carcinogenic, 3) heat-stable, stable during processing and storage, 4) do not confer undesirable taste and flavour to foods, 4) active against important food-borne pathogens and spoilage agents, 5) effective at low concentration, 6) received the GRAS status (generally recognized as safe) and were accepted by FDA as food additives. However, out of a number of different bacteriocins described in literature, only two, i.e. nisin and pediocin AcH (PA-1), received the GRAS status and were accepted by the FDA as food additives (Galvez et al., 2007 a). The commercial preparations of these bacteriocins are: Nisaplin® or Novasin™, which are preparations of nisin with activity against *Clostridium*, *Bacillus*, *Staphylococcus*, *Listeria*, *Micrococcus*, *Corynebacterium*, *Mycobacterium*, *Lactococcus* and ALTA™ 2341, a pediocin preparation active against *Listeria*. Preparations of other bacteriocins have already been analysed for practical application. LAB bacteriocins do not only have great potential for applications in the food industry, but can also be used as components of cosmetics, e.g. soaps, creams, tonic agents, deodorants and many others. Some authors suggest that bacteriocins may also be used therapeutically, e.g. to combat intestinal infections, especially those triggered by antibiotic-resistant microorganisms (Le Blay et al., 2007), and in the treatment and prophylaxis of tuberculosis (Richard et al., 2006). A number of positive effects in human nutrition, food industry applications, as well as studies on bacteriocins fighting pathogenic microflora suggest that bacteriocins might need more attention also in animal nutrition.

### **Bacteriocins in poultry gastrointestinal tract**

From the first days of life, microorganisms successively colonize the digestive tract of the chicken. Naturally occurring succession of intestinal bacteria leads to establishing the climax community, and by means of competitive exclusion inhibits the pathogens from entering the intestines. However, this process may be disturbed in intensive production conditions as birds have little chance to acquire properly balanced intestinal microflora (Józefiak et al., 2004; Rehman et al., 2007). Probably many of the bacterial species classified by now in broiler digestive tract produce bacteriocins, though the majority has not been investigated yet. Their activity is considered to be an important tool of native bacteria GIT colonization. Additionally, many strains used as dietary probiotics are also capable of bacteriocin production (Stern et al., 2006). Thus, the use of pure bacteriocins as feed additive could be a useful boost of intestinal bacteriocin concentrations or may improve the efficacy of bacteriocin producing bacteria present in the GIT.

Until now, presence of various bacteriocin-producing strains in poultry GIT has been reported by several authors (Bordignon et al., 2011; Musikasang et al., 2012;

Robyn et al., 2012; Shin et al., 2008). Unfortunately, in many cases authors do not define diet composition, i.e. the presence of other antimicrobial agents. It should be stressed that these factors may interfere with the number and composition of bacteria inhabiting the poultry GIT and, consequently, with the concentration and activity of bacteriocins they produce. Lactic acid bacteria seem to be predominant populations in upper parts of the chicken GIT. Stern et al. (2006) isolated bacteriocin producing *Lactobacillus salivarius* NRRL B-30514 that significantly reduced the chicken caeca colonization by four isolates of *Campylobacter jejuni*. These bacteria colonize chicken digestive tract without doing any visible harm to the birds (Stern et al., 2005; Stern et al., 2006). However, both live chickens and carcasses contaminated with fecal material create a serious health hazard to consumers and to the staff working on poultry farms. In 2005 Svetoch et al. described class IIA bacteriocins produced by *Bacillus circulans* and *Paenibacillus polymyxa* as being toxic to *Campylobacter jejuni* *in vitro*. These results were then confirmed in several *in vivo* experiments which demonstrated that infection of 1-day-old chicks with *C. jejuni* can be suppressed by feed supplemented with bacteriocins (Stern et al., 2005). Portrait et al. (2000) isolated *Fusobacterium mortiferum*, (FM1025) from poultry caeca, showing “*in vitro*” activity against *Salmonella enteritidis*, *Salmonella wien*, *Shigella exneri*, *Pseudomonas aeruginosa* and *Pseudomonas stutzeri*. Audisio et al. (1999) characterized a bacteriocin producing strain of *Enterococcus faecium* J96 isolated from the GIT of free-range chickens, and showing activity against *Salmonella pullorum*. Shin et al. (2008) isolated 291 bacterial strains from the chicken and three of them, namely: *Enterococcus faecium* SH 528, *Enterococcus faecium* SH 632 and *Pediococcus pentosaceus* SH 740, exhibited antagonistic activities against *Listeria monocytogenes* and/or *Clostridium perfringens*. They also showed inhibitory activities against Gram-negative bacteria such as *E. coli* and *Salmonella enterica* serovar *Typhimurium*. As *Campylobacter* infections in humans are usually linked to the consumption of fresh poultry products, the reduction of the frequency and load of this microorganism in the food chain is very important. In this aspect, bacteriocins could be a very effective tool for improving food safety.

There is evidence that bacteriocin producing strains are also present in faeces. In broiler chicken excreta, Nazef et al. (2008) identified *Enterococcus faecalis* 37, exhibiting activity against *Listeria innocua* F. *Lactobacillus reuteri* S42, another strain isolated from chicken faeces, was found to be toxic to *Campylobacter jejuni*. Further studies of these authors showed that *Enterococcus faecalis* S37 produced enterococin S37 – a bacteriocin with molecular weight from 4 to 5kDa, active against *Listeria monocytogenes* EGDe, *L. innocua* F, *Enterococcus faecalis* JH2-2, and *Lactobacillus brevis* F145 (Belguesmia et al., 2011).

Bacteriocins isolated from poultry GIT have a relatively broad spectrum of antimicrobial efficacy including Gram-positive and Gram-negative bacteria. Our knowledge regarding bacterial biodiversity and poultry digestive system colonization is still rather limited and usually focuses on the host pathogenic microflora like *Clostridium perfringens*, or on zoonotic bacteria, like *Campylobacter jejuni* or *Salmonella* sp. There are probably still many more antibacterial peptides to define and explore in poultry intestinal tract.

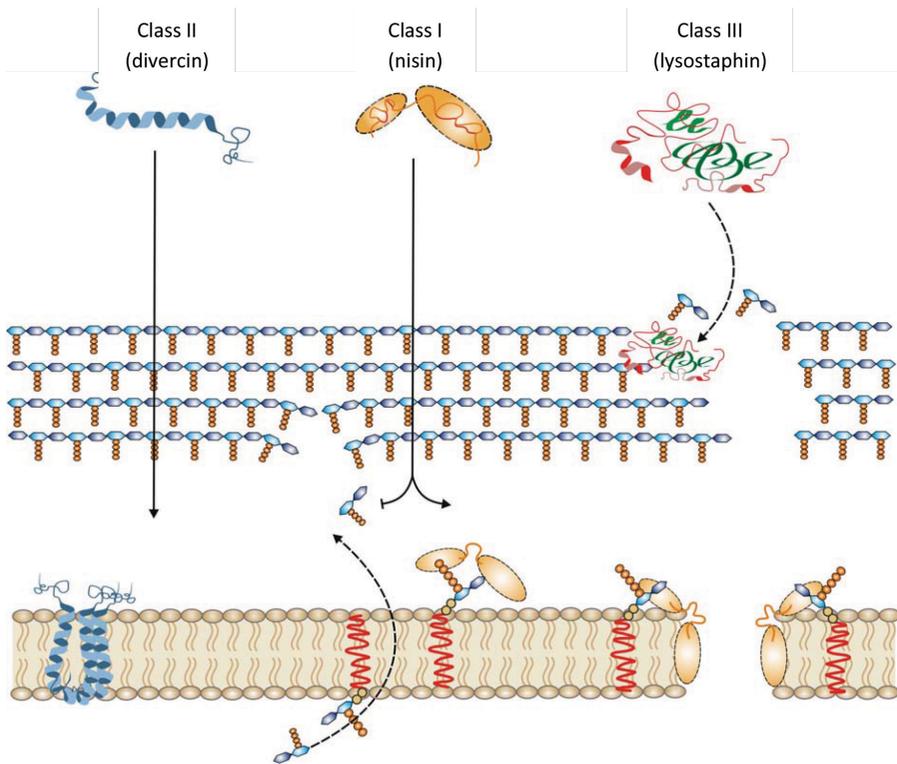


Figure 1. Bacteriocins: mode of action (Cotter et al., 2005) modified after. In general, the class II peptides have an amphiphilic helical structure, which allows them to insert into the membrane of the target cell, leading to epolarization and death. Some members of the class I (lantibiotic bacteriocins), such as nisin, have been shown to have a dual mode of action. They can bind to lipid II, the main transporter of peptidoglycan subunits from the cytoplasm to the cell wall and, therefore, prevent correct cell wall synthesis, leading to cell death. Furthermore, they can use lipid II as a docking molecule to initiate a process of membrane insertion and pore formation that leads to rapid cell death. A two-peptide lantibiotic, such as lactacin 3147, can have these dual activities distributed across two peptides, whereas mersacidin has only the lipid-II-binding activity, but does not form pores. Large bacteriolytic proteins, formerly class III bacteriocins called bacteriolysins, such as lysostaphin, can function directly on the cell wall of Gram-positive targets, leading to death and lysis of the target cell.

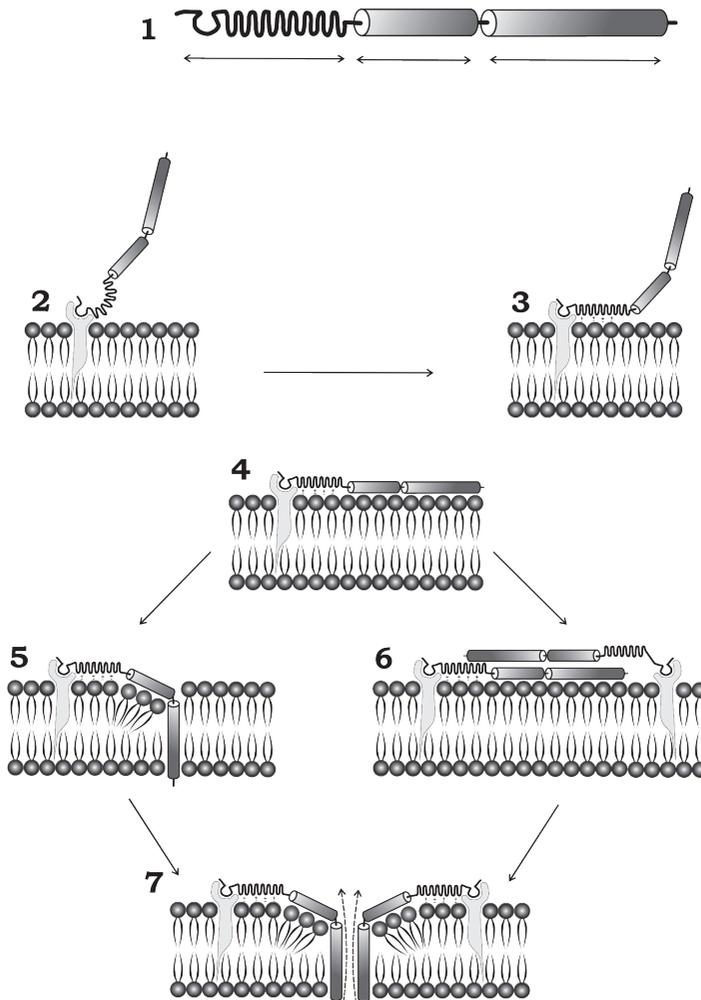
### Bacteriocins as feed additives

Since the beginning of modern animal nutrition, scientists and poultry producers have been trying to optimize the composition of the feed to improve its nutritive value and to meet the nutrient requirements of poultry, thus taking full advantage of their genetic potential. In recent years, research results have indicated that more

attention should be paid to the microbial populations in the gastrointestinal tract (GIT) that, on the one hand, compete with the host for available nutrients, which may affect animal growth negatively. On the other hand, certain representatives of the indigenous microbiota are supposed to support animal performance and health of poultry (Choct, 2009). For instance, research on necrotic enteritis (NE) in poultry indicates that this economically important disease has multifactorial background where *Clostridium perfringens* and *Eimeria* sp. play an important role (Kaldhusdal et al., 1995; Kaldhusdal and Hofshagen, 1992; Van Immerseel et al., 2009). Many diseases associated with intestinal bacteria are only present in their subclinical forms and can only be registered as poorer performance and are hard to estimate under commercial practical conditions, e.g. small intestinal overgrowth or “dysbacteriosis” (Gholamiandehkordi et al., 2007; Van Immerseel et al., 2009; Williams, 2005; Wilson et al., 2005). Apart from effects on performance and well-being of the host, some bacterial populations from the chicken intestine cause disease in humans. Poultry species are considered to be an important reservoir of zoonotic bacteria like *Campylobacter jejuni* or *Salmonella* sp. In many countries where hygiene standards are low, human infections caused by consumption of contaminated poultry meat are quite common. Thus, gut health in modern poultry production is a very complex area, and is a key point in food chain quality and safety.

The number of feed additives, which are suggested to “balance and optimize” GIT microflora, is enormous (Dahiya et al., 2006; Józefiak et al., 2010 a). Many preparations, including prebiotics, probiotics, enzymes, essential oils, organic acids have been proposed so far to control the intestinal populations of *Clostridium perfringens*, *Campylobacter jejuni* or *Salmonella* sp. However, according to papers published by different authors so far, less than 15% of the bacteria inhabiting broiler chicken GIT have been classified yet (Johansen et al., 2007; Józefiak et al., 2010 a; Józefiak et al., 2004; Lu et al., 2008; Rehman et al., 2007). Thus, the search for new antibacterial agents as well as more work to describe chicken GIT micro-ecosystem seem to be very important in the coming decades.

At the present time, our knowledge concerning pure bacteriocins used as feed supplements in poultry diets can be described as inadequate, to say the least. However, in recent years more research has been done on the topic, showing some positive effects of dietary bacteriocin supplementation. Additionally, several reports suggest that bacteriocins may not only be beneficial as feed preservatives but also as animal performance enhancers. Bacteriocins are expected to be stable during feed production (especially pelleting) as most of them are resistant to high temperature and pressure. As previously discussed, bacteriocins are easily digested, which may limit their use as feed additives. Encapsulation of many feed additives (i.e. essential oils, organic acids, etc.) to avoid digestion/absorption in the upper segments of the GIT is today a common practice. However, there is limited information on usage of this technique to improve efficacy of bacteriocins. On the other hand, instead of adding pure bacteriocins to feed, which is rather costly, it might be easier to administer probiotic bacteria, producing bacteriocins *in situ*, i.e. in the gastrointestinal tract (De Vuyst and Leroy, 2007).



1. The hydrophilic N-terminal domain, forming characteristic hairpin structures; the hydrophobic C-terminal fragment folded into an alpha-helix conformation.
2. Docking of a bacteriocin molecule to the plasma membrane of a sensitive cell; docking of the N-terminal fragment of a bacteriocin molecule to the receptor proteins localized on the plasma membrane surface.
3. Electrostatic interactions between a bacteriocin molecule and hydrophilic “heads” of phospholipids, leading to binding of the peptide with the targeted cell surface.
4. Stabilization of a bacteriocin molecule on the cell surface driven by hydrophobic interaction.
5. Reorientation of the hydrophobic fragment of a bacteriocin molecule and penetration of the plasma membrane.
6. Aggregation of greater number of bacteriocin molecules on the cell surface.
7. Assembly of a poration complex.

Figure 2. Formation of lethal pores in the cytoplasmic membrane of target cells by bacteriocins (Ennahar et al., 2000) modified after

Application of bacteriocins is supposed to modify ileal and caecal microbiota populations, which are more dense and diverse when compared to crop or gizzard ecosystems (Józefiak et al., 2011 a). Another question, which needs to be addressed, is dosage, timing, and possible synergistic/antagonistic reactions of mixed bacteriocin preparations. As already mentioned, there are few reports describing the effect of pure bacteriocin preparations in poultry. Some authors try to explain positive effects of probiotic cultures administered in feed, by their ability to produce bacteriocins (Nava et al., 2005). Ogunbanwo et al. (2004) challenged birds with *E. coli* 02:KH6 and treated them with a bacteriocin producing strain of *Lactobacillus plantarum* F1 administered via the drinking water. The performance of the birds was similar to the non-supplemented control group; however, in the group of infected birds, *E. coli* 02:KH6 was re-isolated in 60% of the birds, while in the bacteriocin-supplemented group – it was found only in 12% of the birds. In an earlier work, Laukova et al. (2003) reported on the enterocin A producing *E. faecium* strain, which significantly reduced the infection with *Salmonella* in gnotobiotic Japanese quails, when given in the drinking water. However, it was found that the effect was present only when the bacteriocin was used in therapeutic doses. When administered prophylactically in lower doses, it merely reduced the pathogen counts in faeces, and not in the contents of caecum or ileum (Laukova et al., 2003). A more recent study of Grilli et al. (2009) focused on the application of pediocin A produced by *Pediococcus pentosaceus* FBB61. The performance trial showed positive effects of this peptide, which improved growth and feed conversion ratio of broiler chickens challenged with *Clostridium perfringes*. The *in vitro* study of Line et al. (2008) revealed that enterocin E-760 inhibited growth of the following bacteria: *Salmonella enterica* serovar Enteritidis, *S. enterica* serovar Choleraesuis, *S. enterica* serovar Typhimurium, *S. enterica* serovar Gallinarum, *Escherichia coli* O157:H7, *Yersinia enterocolitica*, *Citrobacter freundii*, *Klebsiella epolariz*, *Pseudomonas aeruginosa*, *Proteus mirabilis*, *Morganella morganii*, *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Listeria monocytogenes*, *Campylobacter jejuni*. When broiler chickens were challenged with *C. jejuni*, this bacteriocin significantly reduced colonization of *Campylobacter* sp. The work of Cole et al. (2006) showed a significant reduction of *Campylobacter jejuni* in turkey poultlets after dietary addition of bacteriocin B602 from *Paenibacillus polymyxa* (NRRL B-30509), and bacteriocin OR7 from *Lactobacillus salivarius* (NRRL B-35014). Moreover, these bacteriocins were shown to reduce duodenal crypt depth and the number of goblet cells, thus modifying *Campylobacter* colonization site. Cole et al. (2006) were probably the first researchers to notice that pathogens might be eliminated by means of physical or functional alteration in the GIT.

Our recent work on *Carnobacterium divergens*, which is a divercin AS7 bacteriocin producer shows its efficacy in broiler nutrition and GIT microbiology (Józefiak et al., 2010 b; Józefiak et al., 2011 a; Józefiak et al., 2011 b; Józefiak et al., 2012). The *Carnobacterium* genus comprises nine species of which *Carnobacterium divergens* and *Carnobacterium maltaromaticum* have been widely studied due to their role in the inhibition of many pathogenic and food spoilage bacteria such as *Clostridium*, *Listeria*, *Bacillus*, *Brochothrix*, *Staphylococcus* and *Micrococcus*. They are also ac-

tive against some other LAB belonging to *Lactobacillus*, *Leuconostoc*, *Carnobacterium*, *Pediococcus* and *Enterococcus* genera (Leisner et al., 2007; Sip et al., 1998). *Carnobacterium divergens* produces the subclass IIa bacteriocins consisting of 30 to 60 amino acids (Leisner et al., 2007). So far, three major bacteriocins produced by this species have been described. Divercin V41 showed antilisterial activity in mice challenged intravenously with *L. monocytogenes* (Rihakova et al., 2009). The third described *Carnobacterium* that produced bacteriocin is divergicin M35, also exhibiting strong antilisterial activity (Tahiri et al., 2004). Reduction of zoonotic bacteria as well as those impairing broiler performance could be an important future of the bacteriocins. Until now, very limited research has been done in this area, though some preliminary data may suggest promising effects. Divercin AS7 was observed to reduce microbial populations isolated from broiler gastrointestinal tract, and to improve the apparent metabolizable energy level ( $AME_N$ ) in broiler chickens (Józefiak et al., 2010 b). Moreover, the observations from our last study illustrate a complex response pattern of *C. perfringens* challenge and divercin AS7 supplementation. The bacteriocin mode of action seems to depend not only on microbiota composition and GIT health status but also on the physical form of the applied compound; the effects obtained with divercin lyophilized on a microcrystalline cellulose carrier were thus different from those observed earlier with liquid divercin preparations. However, it can be concluded that divercin AS7 may reduce the negative effects related to a *C. perfringens* challenge by protecting broiler performance, improving AMEn content of the feed, and maintaining histomorphology of the GIT.

In conclusion it should be stated that bacteriocins are characterized by many features, which are interesting for poultry nutritionist and microbiologist. Moreover, today our knowledge of broiler chicken GIT microbiota is limited and much research should be conducted in this area to explore and define this unique microecosystem. Without this essential knowledge it is almost impossible to control bird performance and health even with well defined and purified bacteriocins.

## References

- Adebayo C.O., Aderiye B.I. (2011). Suspected mode of antimycotic action of brevicin SG1 against *Candida albicans* and *Penicillium citrinum*. *Food Control*, 22: 1814–1820.
- Alloui M.N., Szczyrek W., Świątkiewicz S. (2013). The usefulness of prebiotics and probiotics in modern poultry nutrition: a review. *Ann. Anim. Sci.*, 13: 17–32.
- Audisio M.C., Oliver G., Apella M.C. (1999). Antagonistic effect of *Enterococcus faecium* J96 against human and poultry pathogenic *Salmonella* spp. *J. Food Prot.*, 62: 751–755.
- Barnby-Smith F.M. (1992). Bacteriocins: applications in food preservation. *Trends Food Sci. Technol.*, 3: 133–137.
- Belguesmia Y., Madi A., Sperandio D., Merieau A., Feuilloley M., Prevost H., Drider D., Connil N. (2011). Growing insights into the safety of bacteriocins: the case of enterocin S37. *Res. Microbiol.*, 162: 159–163.
- Bordignon S.E., Miyaoka M.F., Spier M.R., Rubel R., Soccol V.T., Soccol C.R. (2011). Production biomolecule with inhibitory activity against Gram-negative bacteria isolated from faeces of broilers and swine. *Braz. Arch. Biol. Technol.*, 54: 723–731.
- Choct M. (2009). Managing gut health through nutrition. *Br. Poultry Sci.*, 50: 9–15.

- Cleveland J., Montville T.J., Nes I.F., Chikindas M.L. (2001). Bacteriocins: safe, natural antimicrobials for food preservation. *Int. J. Food Microbiol.*, 71: 1–20.
- Cole K., Farnell M., Donoghue A., Stern N., Svetoch E., Eruslanov B., Volodina L., Kovalev Y., Perelygin V., Mitsevich E. (2006). Bacteriocins reduce *Campylobacter* colonization and alter gut morphology in turkey poults. *Poultry Sci.*, 85, p. 1570.
- Cotter P.D., Hill C., Ross R.P. (2005). Bacteriocins: Developing innate immunity for food. *Nature Reviews Microbiology*, 3: 777–788.
- Dahiya J., Wilkie D., Van Kessel A., Drew M. (2006). Potential strategies for controlling necrotic enteritis in broiler chickens in post-antibiotic era. *Anim. Feed Sci. Technol.*, 129: 60–88.
- De Vuyst L., Leroy F. (2007). Bacteriocins from lactic acid bacteria: production, purification, and food applications. *J. Mol. Microbiol. Biotechnol.*, 13: 194–199.
- Deegan L.H., Cotter P.D., Hill C., Ross P. (2006). Bacteriocins: Biological tools for bio-preservation and shelf-life extension. *Int. Dairy J.*, 16: 1058–1071.
- Diep D.B., Skaugen M., Salehian Z., Holo H., Nes I.F. (2007). Common mechanisms of target cell recognition and immunity for class II bacteriocins. *Proc. Natl. Acad. Sci. USA*, 104: 2384–2389.
- Ennahar S., Sashihara T., Sonomoto K., Ishizaki A. (2000). Class IIa bacteriocins: biosynthesis, structure and activity. *FEMS Microbiol. Rev.*, 24: 85–106.
- Galvez A., Abriouel H., Lopez R.L., Ben Omar N. (2007 a). Bacteriocin-based strategies for food biopreservation. *Int. J. Food Microbiol.*, 120: 51–70.
- Galvez A., Abriouel H., Lopez R.L., Omar N.B. (2007 b). Bacteriocin-based strategies for food biopreservation. *Int. J. Food Microbiol.*, 120: 51–70.
- Galvez A., Lopez R.L., Abriouel H., Valdivia E., Ben Omar N. (2008). Application of bacteriocins in the control of foodborne pathogenic and spoilage bacteria. *Crit. Rev. Biotechnol.*, 28: 125–152.
- Gholamiandehkordi A., Timbermont L., Lanckriet A., Broeck W.V.D., Pedersen K., Dewulf J., Pasmans F., Haesebrouck F., Ducatelle R., Van Immerseel F. (2007). Quantification of gut lesions in a subclinical necrotic enteritis model. *Avian Pathol.*, 36: 375–382.
- Grilli E., Messina M.R., Catelli E., Morlacchini M., Piva A. (2009). Pediocin A improves growth performance of broilers challenged with *Clostridium perfringens*. *Poultry Sci.*, 88: 2152–2158.
- Hechard Y., Sahl H.D. (2002). Mode of action of modified and unmodified bacteriocins from Gram-positive bacteria. *Biochimie*, 84: 545–557.
- Jack R., Tagg J., Ray B. (1995). Bacteriocins of gram-positive bacteria. *Microbiol. Rev.*, 59: 171–200.
- Joerger R. (2003). Alternatives to antibiotics: bacteriocins, antimicrobial peptides and bacteriophages. *Poultry Sci.*, 82, p. 640.
- Johansen C.H., Bjerrum L., Pedersen K. (2007). Impact of salinomycin on the intestinal microflora of broiler chickens. *Acta Vet. Scand.*, 49, p. 30.
- Józefiak D., Rutkowski A., Kaczmarek S., Jensen B.B., Engberg R.M., Hojberg O. (2010 a). Effect of  $\beta$ -glucanase and xylanase supplementation of barley- and rye-based diets on caecal microbiota of broiler chickens. *Br. Poultry Sci.*, 51: 546–557.
- Józefiak D., Rutkowski A., Martin S.A. (2004). Carbohydrate fermentation in the avian ceca: a review. *Anim. Feed Sci. Technol.*, 113, pp. 1–15.
- Józefiak D., Sip A., Kaczmarek S., Rutkowski A. (2010 b). The effects of *Carnobacterium divergens* AS7 bacteriocin on gastrointestinal microflora *in vitro* and on nutrient retention in broiler chickens. *J. Anim. Feed Sci.*, 19: 460–467.
- Józefiak D., Sip A., Rawski M., Rutkowski A., Kaczmarek S., Hojberg O., Jensen B.B., Engberg R.M. (2011 a). Dietary divercin modifies gastrointestinal microbiota and improves growth performance in broiler chickens. *Br. Poultry Sci.*, 52: 492–499.
- Józefiak D., Sip A., Rawski M., Steiner T., Rutkowski A. (2011 b). The dose response effects of liquid and lyophilized *Carnobacterium divergens* AS7 bacteriocin on the nutrient retention and performance of broiler chickens. *J. Anim. Feed Sci.*, 20: 401–411.
- Józefiak D., Sip A., Rutkowski A., Rawski M., Kaczmarek S., Wolun-Chole-

- wa M., Engberg R.M., Hojberg O. (2012). Lyophilized *Carnobacterium divergens* AS7 bacteriocin preparation improves performance of broiler chickens challenged with *Clostridium perfringens*. *Poultry Sci.*, 91: 1899–1907.
- Kaldhusdal M., Evensen O., Landsverk T. (1995). *Clostridium perfringens* necrotizing enteritis of the fowl: a light microscopic, immunohistochemical and ultrastructural study of spontaneous disease. *Avian Pathol.*, 24: 421–433.
- Kaldhusdal M., Hofshagen M. (1992). Barley inclusion and avoparcin supplementation in broiler diets. 2. Clinical, pathological, and bacteriological findings in a mild form of necrotic enteritis. *Poultry Sci.*, 71: 1145–1153.
- Klaenhammer T.R. (1993). Genetics of bacteriocins produced by lactic acid bacteria. *FEMS Microbiol. Rev.*, 12: 39–85.
- Laukova A., Guba P., Nemcova R., Vasilkova Z. (2003). Reduction of *Salmonella* in gnotobiotic Japanese quails caused by the enterocin A-producing EK13 strain of *Enterococcus faecium*. *Vet. Res. Commun.*, 27: 275–280.
- Le Blay G., Lacroix C., Zihler A., Fliss I. (2007). *In vitro* inhibition activity of nisin A, nisin Z, pediocin PA-1 and antibiotics against common intestinal bacteria. *Lett. Appl. Microbiol.*, 45: 252–257.
- Leisner J.R.J., Laursen B.G., Prévost H., Drider D., Dalgaard P. (2007). *Carnobacterium*: positive and negative effects in the environment and in foods. *FEMS Microbiol. Rev.*, 31: 592–613.
- Line J.E., Svetoch E.A., Eruslanov B.V., Perelygin V.V., Mitsevich E.V., Mitsevich I.P., Levchuk V.P., Svetoch O.E., Seal B.S., Siragusa G.R., Stern N.J. (2008). Isolation and purification of enterocin E-760 with broad antimicrobial activity against gram-positive and gram-negative bacteria. *Antimicrob. Agents Chemother.*, 52: 1094–1100.
- Lu J., Hofacre C., Smith F., Lee M.D. (2008). Effects of feed additives on the development on the ileal bacterial community of the broiler chicken. *Animal*, 2: 669–676.
- Marugg J.D. (1991). Bacteriocins, their role in developing natural products. *Food Biotechnol.*, 5: 305–312.
- Montville T.J., Winkowski K., Ludescher R.D. (1995). Models and mechanisms for bacteriocin action and application. *Int. Dairy J.*, 5: 797–814.
- Musikasang H., Sohsomboon N., Tani A., Maneerat S. (2012). Bacteriocin-producing lactic acid bacteria as a probiotic potential from Thai indigenous chickens. *Czech J. Anim. Sci.*, 57: 137–149.
- Nava G.M., Bielke L.R., Callaway T.R., Castaneda M.P. (2005). Probiotic alternatives to reduce gastrointestinal infections: the poultry experience. *Anim. Health Res. Rev.*, 6: 105–118.
- Nazef L., Belguesmia Y., Tani A., Prévost H., Drider D. (2008). Identification of lactic acid bacteria from poultry feces: evidence on anti-*Campylobacter* and anti-*Listeria* activities. *Poultry Sci.*, 87, p. 329.
- Nes I.F., Diep D.B., Havarstein L.S., Brurberg M.B., Eijsink V., Holo H. (1996). Biosynthesis of bacteriocins in lactic acid bacteria. *Antonie Van Leeuwenhoek Int. J. Gen. Molec. Microbiol.*, 70: 113–128.
- O'Sullivan L., Ross R.P., Hill C. (2002). Potential of bacteriocin-producing lactic acid bacteria for improvements in food safety and quality. *Biochimie*, 84: 593–604.
- Ogunbanwo S.T., Sanni A.I., Onilude A.A. (2004). Influence of bacteriocin in the control of *Escherichia coli* infection of broiler chickens in Nigeria. *World J. Microbiol. Biotechnol.*, 20: 51–56.
- Portrait V., Cottenceau G., Pons A.M. (2000). A *Fusobacterium mortiferum* strain produces a bacteriocin-like substance(s) inhibiting *Salmonella enteritidis*. *Lett. Appl. Microbiol.*, 31: 115–117.
- Rehman H., Vahjen W., Awad W., Zentek J. (2007). Indigenous bacteria and bacterial metabolic products in the gastrointestinal tract of broiler chickens. *Archiv. Anim.* 319–335.
- Richard C., Cañon R., Naghmouchi K., Bertrand D., Prévost H., Drider D. (2006). Evidence on correlation between number of disulfide bridge and toxicity of class IIa bacteriocins. *Food Microbiol.*, 23: 175–183.
- Rihakova J., Petit V.W., Demnerova K., Prévost H., Rebuffat S., Drider D. (2009). Insights into Structure-Activity Relationships in the C-Terminal Region of Divercin V41, a Class

- Ila Bacteriocin with High-Level Antilisterial Activity. *Applied and Environmental Microbiology*, 7: 1811–1819.
- Robyn J., Rasschaert G., Messens W., Pasmans F., Heyndrickx M. (2012). Screening for lactic acid bacteria capable of inhibiting *Campylobacter jejuni* in *in vitro* simulations of the broiler chicken caecal environment. *Benef. Microbes*, 3: 299–308.
- Schillinger U., Geisen R., Holzapfel W.H. (1996). Potential of antagonistic microorganisms and bacteriocins for the biological preservation of foods. *Trends Food Sci. Technol.*, 7: 158–164.
- Shin M.S., Han S.K., Ji A.R., Kim K.S., Lee W.K. (2008). Isolation and characterization of bacteriocin-producing bacteria from the gastrointestinal tract of broiler chickens for probiotic use. *J. Appl. Microbiol.*, 105: 2203–2212.
- Sip A., Grajek W., Boyaval P. (1998). Enhancement of bacteriocin production by *Carnobacterium divergens* AS7 in the presence of a bacteriocin-sensitive strain *Carnobacterium piscicola*. *Int. J. Food Microbiol.*, 42: 63–69.
- Stern N.J., Svetoch E.A., Eruslanov B.V., Kovalev Y.N., Volodina L.I., Perelygin V.V., Mitsevich E.V., Mitsevich I.P., Levchuk V.P. (2005). Paenibacillus polymyxa purified bacteriocin to control *Campylobacter jejuni* in chickens. *J. Food Prot.*, 68: 1450–1453.
- Stern N.J., Svetoch E.A., Eruslanov B.V., Perelygin V.V., Mitsevich E.V., Mitsevich I.P., Pokhilenko V.D., Levchuk V.P., Svetoch O.E., Seal B.S. (2006). Isolation of a *Lactobacillus salivarius* strain and purification of its bacteriocin, which is inhibitory to *Campylobacter jejuni* in the chicken gastrointestinal system. *Antimicrob. Agents Chemother.*, 50: 3111–3116.
- Tahiri I., Desbiens M., Benech R., Kheadr E., Lacroix C., Thibault S., Ouellet D., Fliss I. (2004). Purification, characterization and amino acid sequencing of divergicin M35: a novel class IIa bacteriocin produced by *Carnobacterium divergens* M35. *Int. J. Food Microbiol.*, 97: 123–136.
- Totton S.C., Farrar A.M., Wilkins W., Bucher O., Waddell L.A., Wilhelm B.J., McEwen S.A., Rajic A. (2012). The effectiveness of selected feed and water additives for reducing *Salmonella* spp. of public health importance in broiler chickens: A systematic review, meta-analysis, and meta-regression approach. *Prev. Vet. Med.*, 106: 197–213.
- Van Immerseel F., Rood J., Moore R., Titball R. (2009). Rethinking our understanding of the pathogenesis of necrotic enteritis in chickens. *Trends Microbiol.*, pp. 32–36.
- Williams R.B. (2005). Intercurrent coccidiosis and necrotic enteritis of chickens: rational, integrated disease management by maintenance of gut integrity. *Avian Pathol.*, 34: 159–180.
- Wilson J., Tice G., Brash M.L., St Hilaire S. (2005). Manifestations of *Clostridium perfringens* and related bacterial enteritides in broiler chickens. *Worlds Poultry Sci. J.*, 61: 435–449.

Accepted for printing 21 III 2013

DAMIAN JÓZEFIAK, ANNA SIP

### Bakteriocyny w żywieniu drobiu – artykuł przeglądowy

#### STRESZCZENIE

W ostatnich latach ukazało się wiele prac ilustrujących ścisły związek między wynikami odchowu kurcząt reżyjnych a rozwojem endogennej mikroflory przewodu pokarmowego. Z uwagi na bioróżnorodność tego skomplikowanego mikroekosystemu, osiągnięcie potencjalnych korzyści dla ptaka-gospodarza poprzez manipulację jego flory bakteryjnej nie jest łatwym zadaniem. Bakteriocyny są substancjami białkowymi wytwarzanymi przez wiele mikroorganizmów. Ich bakteriobójcze i bakteriostatyczne właściwości są wykorzystywane od wielu lat w żywieniu ludzi. Jednak w dostępnej literaturze naukowej brakuje informacji na temat zastosowania tych związków w dietach dla kurcząt reżyjnych. Dlatego też w niniejszym artykule przeglądowym przedstawiono aktualną klasyfikację bakteriocyn, ich działanie i wykorzystanie w żywieniu drobiu.