GENETICS OF BACTERIOCINS BIOSYNTHESIS BY LACTIC ACID BACTERIA

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Introduction

Many studies have been focused on bacteriocins produced by lactic acid bacteria (LAB), especially those produced by many dairy starter cultures strains because of their potential industrial application as natural food preservatives. Bacteriocins are toxins with protein nature that are ribosomally synthesized by gram-positive bacteria and excreted out of the cell. They can be divided into 4 major classes: Class I, lantibiotics (1) (containing posttranslationally modified amino acids such as lanthionine and β-methyllanthionine); Class II – small unmodified thermo stable usually positively charged at a neutral pH peptides (2); Class III – large unmodified thermo labile peptides (3), and Class IV – complex bacteriocins which possess a carbohydrate or lipid component (4). Most of the investigations were focused on the protein nature of bacteriocins, nevertheless in the last years many data were accumulated regarding the genetic determinants of bacteriocin synthesis as well as its regulation. This paper is a attempt for a concise overview concerning the localization, the organization and the regulation of the expression of the genes related to bacteriocins synthesis, procession, export and immunity.

Localization of bacteriocins genetic determinants

Three types of localizations of the bacteriocins operons have been reported till now by the authors: on the bacterial chromosome, on plasmids and on transposons (both plasmids and chromosome carried). Chromosomal localization of the bacteriocins operons have been described for the Cass I, lantibiotics, as well for the Class II small heat stable peptides. Examples for chromosomal lantibiotc genes are those encoding mutacin II and mutacin III produced by Streptococcus mutans (5, 6), as well the Streptococcus salivarius salivaricin A (7). Chromosomal location of the class II bacteriocins operons is more widespread. Typical cases are those for enterocins A and B produced by Enterococcus faecium (8, 9), divercin V41 excreted by Carnobacterium divergens (10), Lactobacillus johnsonii lactacin F (11), Lactobacillus plantarum plantaricin S (12) and plantaricin A (13).

Most of the bacteriocins operons are located on plasmids. It has been suggested that this plasmid location helps the intra- and inter-species phylogenetic dissemination of bacteriocins among LAB.

As examples for lantibiotics with plasmid genetic determinants emerge some well characterized ones: lacticin 481 (14) and the two-component lacticin 3147 (15, 16), both produced by Lactobacillus lactis, and encoded by genes located on a 70 kb and 63 kb plasmids respectively. Another plasmid determined lantibiotic is the two-component staphylococcin C55 (17) produced by Staphylococcus aureus C55 harboring a 32 kb plasmid.

Numerous non-lantibiotic bacteriocins have structural genes located on plasmids. Typical examples are those for pediocin PA-1 (18) and pediocin AcH (19) produced by strains of Pediococcus acidilactici, pos-
sessing plasmids with sizes 9.4 kb and 8.9 kb correspondingly; sakicin A determined by a 60 kb *Lactobacillus sakei* plasmid (20); divergicin A from *Carnobacterium divergens* (21); the two-component bacteriocin enterocin P synthesized by *Enterococcus faecium* (22) and many others.

Probably one of the best characterized bacteriocins is the lantibiotic nisin allowed in many countries as a food preservative. Nisin is produced by *Lactococcus lactis* and its genetic determinants are located on the conjugative transposon *Tn*5276 within the bacterial chromosome (23). Location on a mobile genetic element, the transposon *Tn*3721, has been also demonstrated for the lantibiotic lacticin 481 produced also by *Lactococcus lactis* but in this case the transposon is located on a 70 kb plasmid (24). In the last two cases the bacteriocin genes are flanked by intact insertion sequences (IS) or inverted repeats (IR). Many other bacteriocin genes are located in proximity to defect IS or IR suggesting that they must be evolved eventually as well from ancestors on mobile genetic elements.

An unusual genomic organization is described for the non-lantibiotic bacteriocin carnobacteriocin BM1 produced by *Carnobacterium piscicola*. While its structural gene is located on the bacterial chromosome, its expression is dependent on the presence of a 61 kb plasmid which carries some of the genes required for the export and the immunity (25).

**Production of more than one bacteriocin**

Usually LAB produce only one bacteriocin. But there are many reports for strains producing more than one. Examples for the secretion of two bacteriocins are the *Carnobacterium piscicola* carnobacteriocins BM1 and B2 (25), the *Lactococcus lactis* bacteriocins LsbA and LsbB (which can act synergetically) (26), the *Enterococcus faecium* enterocins A and B (27) etc. It was established that three different bacteriocins are produced by *Lactobacillus plantarum* (two two-peptide bacteriocins – plantaricins E/F and J/K, as well as the single peptide bacteriocin, plantaricin N) (28), and *Enterococcus faecium* (one two-peptide bacteriocin, enterocin L50, and two single-peptide bacteriocins: enterocin P and enterocin Q) (22).

It is possible that, when more than one bacteriocin is produced, the peptides can belong to different classes. For example *Staphylococcus aureus* C55 produces the two-peptide synergetic lantibiotic staphylococccins C55α and C55β, and the non-lantibiotic staphylococcin C55γ (17), while *Streptococcus mutans* UA140 produces the lantibiotic mutacin I and the two-peptide class II bacteriocin mutacin IV (29).

**Operon-like organization of bacteriocin genes**

Till now scientific data show that almost all bacteriocin genetic determinants are clustered in operons or regulons (term proposed by some authors on the basis of the regulation of the gene expression). This is not unexpected because in the simplest case the bacteriocin expression needs at least two genes: one structural gene and another one that encodes an immunity protein specific to the produced bacteriocin. In most cases bacteriocin production needs also a specific export machinery, and is subjected to some regulation factors, which make bacteriocin operons much more complex. Based on the data obtained, there is a tendency that in general the lantibiotics operons are more complex than those encoding non-lantibiotics because they need additional genes encoding enzymes for posttranslational modifications. With very few exceptions all bacteriocins are synthesized as prepeptides containing leader sequences.

Some simple bacteriocin operons composed of only two genes are found in the so called class IIc or sec-dependent bacteriocins. Typical examples are divergicin A...
operon located on 3.4 kb plasmid in Carnobacterium divergens (21), and the Enterococcus faecalis bacteriocin 31 operon which is located on 57.5 kb conjugative plasmid (30). These two operons are composed of only two genes: the structural bacteriocin gene immediately followed downstream by the gene for the immunity protein. This simplicity is possible because the sec-dependent bacteriocins use the general secretory pathway and do not require dedicated export proteins. There are no data that the expression of the above two bacteriocins is under regulation by some additional factors, that is also confirmed by cloning and heterologous expression experiments.

More complex is the structure of the class IIa, pediocin-like bacteriocins. They contain double-glycine leader peptide and are exported by dedicated ABC-transporter (ATP-binding cassette). This subclass of bacteriocins have their name from some of the best characterized pediocins PA-1 and AcH. Pediocin AcH genes are plasmid located and they are arranged in a gene cluster of 3500 bp sharing a common promoter and rho-independent stem-loop terminator. The four genes, each with independent ribosome binding sites (rbs), initiation and termination codons and spacer sequences in between, were designated as papA, papB, papC and papD (31). PapA is the structural gene for the pre-pediocin, while papB encodes the immunity protein. PapC and papD form the export machinery, and are required for the membrane translocation and the removal of the leader peptide. PapD shares a double-glycine protease domain with other ABC export proteins active in transport of bacteriocins.

Sakicin A is another pediocin-like bacteriocin. The sakicin A genes are organized in two divergent operons (Fig. 1) divided by an insertion sequence IS1163 (20). There are six genes participating in the sakicin A synthesis: sapA – the gene for the pre-sakicin A, saiA – the gene, encoding the sakicin A immunity protein, sapK – the histidine protein kinase, sapR – the response regulator, sapT – the ATP binding cassette exporter, and sapE – the transport protein. SapT and sapE are dedicated to the export of the bacteriocin while sapK and sapR play a role in the regulation of the expression via signal transduction mechanisms.

The operons encoding the two-peptide
non-lantibiotic bacteriocins of class IIb, are in general transcribed in polycistronic mRNA. Two examples of such operons are the relatively simple lactacin F operon in *Lactobacillus johnsonii* (composed of only three ORF: *lafA* and *lafX* encoding the prepeptides, and the third, *lafI* or ORFZ, encoding the immunity protein (11, 32)), and the brochocin-C operon in *Brochothrix campestris* (33). The second one contains the structural genes *brcA* and *brcB* immediately followed by the gene encoding an immunity protein *brcI*. In addition two other genes – *brcD* and *brcT* are located downstream. *BrcD* determines an ABC-translocator while *brcT* a response regulator. Both operons are compared on Fig. 2. Similar to brochocin-C operon structure is found for the divercin V41 operon (10).

Because lantibiotics functionality depends on posttranslational modifications other than cleavage of the leader sequences, their operons are some of the most complex among bacteriocins. One of the best characterized bacteriocin operon is that encoding the biosynthesis of nisin by *Lactococcus lactis* (34). The nisin gene cluster contains eleven genes and is usually designed as *nisABTCIPRKFEG* (Fig. 3).

Of these genes, *nisA* encodes the nisin A precursor peptide; *nisB* and *nisC* encode putative enzymes involved in the post-translational modification reactions; *nisT* encodes a putative transport protein of the ABC translocator family that is probably involved in the extrusion of the modified nisin precursor; *nisP* encodes an extracellular protease involved in precursor processing; *nisI* encodes a lipoprotein involved in the producer self-protection against nisin; and *nisFEG* encode putative transporter proteins that have also been implied in immunity. The proteins encoded by *nisR* and *nisK* have shown to be involved in the regulation of nisin biosynthesis. *NisR*, a response regulator, and *nisK*, is a sensor histidine protein kinase which belong to the class of the signal transduction regulatory systems proteins.

The gene cluster contains two promoters. The first one is located upstream the *nisA* gene, and the second one between the genes *nisP* and *nisR*. It is interesting that an inverted repeat located between the genes *nisA* and *nisB* could act as a rho-independent terminator suggesting sophisticated regulation of the expression of the genes within the nisin cluster.

The following path for nisin biosynthesis has been proposed. 1) *nisA* is translated to...
Nisin precursor structural gene

Genes for the enzymes for posttranslational modifications

ABC translocator protein

Transporter proteins implied also in immunity

Extracellular protease involved in the precursor processing

Proteins involved in the regulation of nisin synthesis

Fig. 3. Organization of the gene cluster responsible for nisin synthesis. The genes are marked with different patterns depending on the function of their protein products (34).

pre-nisin A. 2) Pre-nisin A is transformed to precursor nisin A by the products of the genes nisB and nisC. At this stage several disulfide bridges are made, and as some amino-acids are transformed to unusual ones as well. 3) Finally the precursor nisin A is exported out of the cell by the products of the genes nisT and nisP at the same time while the leader peptide is cleaved and the final product, nisin A is obtained.

Two other well characterized lantibiotic clusters are those determining lactococcal lacticin 481 (24) and lacticin 3147 (16). The first one has analogical structure with the nisin genes cluster but it contains only 6 genes, all of them transcribed in the same direction. As the nisin cluster, the lacticin 481 is also framed by IS as a part of the transposon Tn5721. The lacticin 3147 gene cluster has a different structure. Although it contains the same functional groups of genes there are two major differences: 1) there are two structural genes for the two pre-lacticins because lactacin 3147 is a two peptide lantibiotic, and 2) the genes are transcribed in two divergent directions.

Regulation of bacteriocins expression

Expression of bacteriocins genes is usually subjected to regulation by external induction factors (IF), in most cases small peptides secreted by the producer strain itself or by its concurrents for the ecological recesses. However in some cases bacteriocins production depends on the environmental conditions (temperature, pH etc) or even it can be constitutive.

The Enterococcus faecium enterocin B (9) is an example for constitutive bacteriocin production, while it has been shown that the expression of enterocins L50A, L50B and Q produced by another strain of Enterococcus faecium is affected by environmental changes such as temperature, ionic strength, and media (22). However environmental conditions often affect only the bacteriocin biosynthesis, while the primary regulation is effectuated by appropriate an IF. Such dual mechanism has been proved for sakacin A in Lactobacillus sakei (35).

In many LAB bacteriocin production is “quorum-sensing” mechanisms controlled. Quorum-sensing systems control plethora of different important biological processes in bacteria, such as natural genetic transformation, virulence, sporulation and many others. In gram-positive bacteria quorum-sensing is predominantly mediated by peptide pheromones or IF. IF are the first component of a three-component signal transduction pathway (Fig. 4).

The induction factor is believed to bind specifically to the correspondent histidine protein kinase, and to activate it to phosphorylate the response regulator, which
Fig. 4. Three component signal transduction pathway used by many bacteriocin operons in quorum-sensing regulation. Abbreviations: IF - induction factor; HPK – histidine kinase; RR and RR* - response regulators.

than stimulates transcription of the target genes, most probably by binding to specific imperfect direct repeats found in many bacteriocin genes clusters. IF for a given bacteriocin operon can be produced either by the bacteriocin producer strain, or by other strains belonging to the same or other species or genera.

When the inducer peptide is produced by the bacteriocin producer itself there is an autoregulation of bacteriocin biosynthesis. The IF can be a dedicated peptide encoded by a respective gene (exported by specific ABC-translocator or by the general secretory pathway), or the bacteriocin molecule itself. Production of the bacteriocin sakacin P by Lactobacillus sakei LTH673 is dependent on a secreted 19-residue peptide pheromone (IP-673) (36). The gene encoding IP-673, SppIP, is co-transcribed with the genes encoding the histidine protein kinase (sppK) and the response regulator (sppR). IP-673 induces the transcription of its own gene and of what are often considered to be of all genes necessary for the bacteriocin production and immunity. Studies with a reporter gene showed that the promoter in front of the sakacin P structural gene (sppA) is strictly regulated. The promoter in front of sppIP turned out to be less strictly regulated, and low basal promoter activity could be detected in uninduced cells. These results show that bacteriocin production in lactobacilli is regulated using a short, strain-specific peptide pheromone. Similar results have been obtained for another Lactobacillus sakei bacteriocin, sakacin A, where the IF is a pheromone peptide termed Sap-Ph (35). But it should also be noticed that in both sakacin cases the expression is strongly influenced by the temperature conditions.

Typical case in which the bacteriocin molecule itself plays a role of an IF is nisin (34). In this case the mature nisin molecule interacts with a sensor histidine protein kinase (HPK), produced by the gene nisK, and disposed on the cell membrane. The HPK phosphorylates the response regulator (RR), product of the gene nisR, followed by binding to the imperfect direct repeats found in the promoter regions. This event leads to a transcription activation of the nisin gene cluster, and finally to the secretion of more nisin molecules, thus closing the cycle of autoactivation.

Conclusions
Bacteriocins represent enormous interest for food industry because they are produced by many dairy starter culture strains,
and because of their capability to repress the development of pathogenic microflora in dairy products. Their potential application as natural food preservatives depends on the capacity of expression of bacteriocin genetic determinants by genetically modified heterologous host strains at industrial level. This task will be unimaginable without a deep understanding of the bacteriocins genetics. In the last decades many scientific results were obtained which inevitably led to the industrial production and introduction of several bacteriocins as safe preservatives against food spoilage. The first step was the approval of nisin as safe preservative by the US "Food and Drug Administration" about 25 years ago.

REFERENCES