

Chapter 2

The Phenotypes

2.1 Introduction

Once the main bacterial languages were partly decoded, the main efforts were consequently addressed to understanding the relevant phenotypes, which are coordinated in a cell density-dependent manner. N-acyl-L-homoserine lactones (AHL), autoinducing peptide (AIP, or peptide pheromone), autoinducer-2 (AI-2), through the activity of LuxS, and the new autoinducer-3 (AI-3), are all bacterial signals that may induce a large number of phenotypes. Competence, virulence, synthesis of toxins and exopolysaccharides (EPS), biofilm formation, and production of secondary metabolites are some examples of the above phenotypes, which are directly or indirectly under the control of quorum sensing circuits. Several of these phenotypic traits and the related mechanisms of control may be of marked interest in relation to foods, either in terms of sensory and nutritional quality or considering foods themselves as vehicles of pathogenic bacteria.

Although some results are still controversial, the main findings concerning some of the above phenotypes are described in the following.

2.2 Virulence

Virulence genes encode proteins whose functions are essential to effectively establish a bacterial infection in the host organism. In many Gram-negative and -positive bacteria, the expression of some virulence factors is regulated by quorum sensing.

The language of *Pseudomonas aeruginosa* comprises N-acyl-L-homoserine lactones (AHL) and 4-quinolone quorum sensing signals (see Sect. 1.3). Two systems (*las* and *rhl*) encode the transcriptions of the regulatory proteins (LasR or RhIR) and autoinducer synthases (LasI or RhII), and regulate the surface-associated or secreted virulence factors [1, 2]. As shown using animal models, when the mutation of *las*

and/or *rhl* occurred, the pathogenesis of *P. aeruginosa* decreased [3]. Comparisons between the secretome of *P. aeruginosa* wild type and one or more *las* and *rhl* mutants showed lower levels of proteins were released in the latter two [4]. This suggested that the lack of *las* or *rhl* severely disrupts the secretion of proteins and/or the expression of abundant extracellular constituents. Unknown quorum sensing regulated proteins such as aminopeptidase PA2939, endoproteinase PrpL and unique hypothetical protein PA0572 were identified. The *las* mutant did not express the major isoforms of the aminopeptidase PA2939, which contains a putative signal [5]. Under starvation conditions, PA2939 generates free amino acids from short peptides. The *rhl* mutant did not express the endoproteinase PrpL, which has the capacity to cleave lactoferrin, transferrin, elastin, and casein. The azurin precursor, chitin-binding protein (CbpD), and the hypothetical protein PA4944 were only found in *P. aeruginosa* wild type. CbpD has adhesion-like properties and is protected from proteolysis by elastase, when it is bound to chitin [6]. The hypothetical protein PA4944 has high sequence similarity to host factor I, a RNA-binding protein that regulates the synthesis of enterotoxin in *Yersinia enterocolitica* [7] and several virulence factors in *Brucella abortus* [8]. Another protein considered to belong to the family of quorum sensing regulators is PA4944. Two partner secretion exo-proteins (PA0041 and PA4625), and quorum sensing regulated extracellular proteins (LasB elastase, LasA protease and aprA alkaline metalloproteinase) were found at the highest levels in the culture supernatants of quorum sensing mutants. This suggested that quorum sensing might also negatively control the expression of some functional genes for virulence.

The regulation of virulence factors from soft-rotting plant pathogen *Erwinia carotovora* occurs via AHL signaling (see Sect. 1.3). In addition to brute force virulence factors, *E. carotovora* also produces extracellular enzymes as secondary metabolites and multiple subtle virulence factors [9]. Regulation of secondary metabolite systems AB (RsmAB) were identified in *E. carotovora* subsp. *carotovora* and *atroseptica*. A mutant defective of *rsmA* exhibited the over production of extracellular enzymes and caused disease bypassing the quorum sensing system. The Rsm system of *E. carotovora* appears to function similarly to the Csr system of *Escherichia coli*. RsmA represses extracellular enzymes by promoting transcript degradation. On the contrary, RsmB is thought to bind to RsmA and to prevent it from binding to its target transcripts, thus indirectly mediating the activation of extracellular enzymes. The quorum sensing locus of *E. carotovora* controls the Rsm system through *rsmA* and, conversely, the Rsm system affects the quorum sensing machinery by modulating the expression of *expI* (homologous to *luxI*) and the consequent synthesis of AHL. As many virulence factors are under the control of quorum sensing in *E. carotovora*, this suggests that the role of quorum sensing during infection is more complicated than simply orchestration by AHL. Examples of virulence factors are Svx, a necrosis-inducing protein, and harpin HrpN, an extracellular glycine-rich protein that elicits the hypersensitive reaction. Several other secreted proteins (e.g., ECA0852 and ECA2220) were quorum sensing dependent, which makes them good candidates for novel virulence factors.

Virulence factors are considered to be terminal virulence determinants to which the plant is exposed. Nevertheless, processes other than gene expression have to be fulfilled by AHL or other secreted virulence factors to interact with the plant. For instance, the relevant virulon has to be secreted from the bacterial cell. Hence the system of protein secretion is an accessory virulence determinant, which is also subjected to quorum sensing regulation. Lip type I of *Serratia liquefaciens* and the Xcp type II of *P. aeruginosa* were identified as secretion systems, which are quorum sensing dependent.

Burkholderia cenocepacia is a common inhabitant of soil, water, and plant surfaces where it may cause diseases such as the soft rot of onion bulbs. The bacterium is also an opportunistic pathogen, especially, in patients that are affected by cystic fibrosis. The quorum sensing system of *B. cenocepacia* uses the AHL synthase CepI, which directs the synthesis of N-octanoylhomoserine lactones [10, 11], and CepR, which activates or represses the transcription of target genes. The *cep* system regulates biofilm formation, swarming motility, synthesis of extracellular proteolytic and chitinolytic enzymes, and represses the synthesis of the siderophore ornibactin [10, 12, 13]. The proteomes of *B. cepacia* H111 wild type and that of the *cep* mutant were compared [14]. Fifty of the ca. 1,000 proteins detected were differentially expressed. Addition of AHL molecules to the culture medium restored the protein profile of the *cep* mutant. About 5 % of the *B. cepacia* proteome was down-regulated and 1% up-regulated in the *cep* mutant. A number of apparently unrelated functions seemed to be *cep* regulated, including the activity of the peroxidase RSC0754 and superoxide dismutase (SodB). The synthesis of SodB by *P. aeruginosa* increased during biofilm formation [15, 16]. This suggested that the *cep* quorum sensing system provides a regulatory link between surface colonization and development of resistance against oxidative stress.

Vibrio vulnificus is a Gram-negative human pathogen, in some cases conveyed by foods. A number of factors are implicated in its virulence and pathogenesis: capsular polysaccharide, lipopolysaccharide (LPS), elastase, cytolysin, metalloprotease, siderophores, and phospholipase [17, 18]. The quorum sensing of *V. vulnificus* is controlled through a hierarchical circuit via *luxS* and *smcR* (homologous to *luxR*). The proteome profile of the *luxS-smcR* double mutant was compared to that of the wild type [19]. Some proteins were repressed by double mutation. They included Zn-dependent protease (VVP), which is responsible for skin lesions [20], periplasmic ABC-type Fe³⁺ transport system and deoxyribose-phosphate aldolase (DERA), which determine the adaptation to starvation and/or the deoxynucleoside catabolism [21], and phosphomannomutase (PMM), which is responsible for the biosynthesis of EPS and LPS.

The global protein expression was compared between the pathogenic wild type *Escherichia coli* O157:H7 and its isogenic *luxS* mutant, and between the *luxS* mutant and *luxS* mutant supplemented with AI-2 [22]; 11 and 18 proteins were differentially expressed, respectively. Both comparisons showed differential expression of the tryptophan repressor binding protein (WrbA), phosphoglycerate mutase (GpmA), and putative protein YbbN. The up-regulation of the FliC protein, which is

responsible for flagellar synthesis and motility, was only found in the wild type. The addition of AI-2 did not influence the synthesis of FlhC by the *luxS* mutant. This suggested that signaling molecules other than AI-2 are involved in flagellar synthesis and motility. Overall, flagellar synthesis and motility are strictly related to virulence phenotypes. A comparison was also made of *E. coli* O157:H7 and its *luxS* mutant under the probiotic effect (inhibition of AI-2 like activity) of the cell extract of *Lactobacillus acidophilus* A4. Five proteins (NifU, PapC, FlgI, MdaB, and DsbA), which are responsible for pathogenesis, were up-regulated in the presence of AI-2 activity (wild type) or down-regulated in the *luxS* mutant and wild type subjected to the probiotic effect. These findings showed the relationship between AI-2 and the virulence of *E. coli* O157:H7 as well as the potential role of *L. acidophilus* as a quenching agent.

PlcR is the major virulence regulator of the *Bacillus cereus* group, which includes species that very often contaminate vegetable foods [23]. In addition to *B. cereus* sensu stricto, an opportunistic pathogen that causes gastroenteritis, pneumonia and endophthalmitis, this group includes *Bacillus thuringiensis*, an entomopathogenic bacterium used to produce biopesticides, and *Bacillus anthracis*, the causative agent of anthrax [24, 25]. The activity of PlcR depends on PapR, a secreted signaling peptide re-imported into the bacterial cell through the Opp transport system [26]. When high bacterial density is reached, the intracellular concentration of PapR increases, which promotes its interaction with PlcR. Then, the PapR: PlcR complex binds to its DNA recognition site, the palindromic PlcR box, and triggers a positive feedback loop that up-regulates the expression of *plcR*, *papR*, and various virulence factors [26]. The molecular basis for transcriptional control by PapR: PlcR is still unknown.

Clostridium perfringens uses AI-2/LuxS to regulate the toxin production [27]. The timing of toxin production is critical for the virulence of this species, which occurs at the mid-late exponential phase of growth. This maximum synthesis of the toxin coincides with the maximum synthesis of AI-2. Compared to wild type, *C. perfringens luxS* mutants have reduced toxin transcription at the mid-late exponential phase of growth, whereas levels of the toxin mRNA were similar in the stationary phase of growth.

2.3 Biofilm Formation

Bacteria develop a biofilm on a number of different surfaces, such as natural aquatic and soil environments, living tissues, vegetables and fruits, medical devices or industrial or potable water piping systems [28, 29]. Biofilm formation is a prerequisite for the existence and survival of microbial aggregates [29, 30]. EPS are the main components of biofilms, even though the type of EPS varies according to the status of bacterial growth and the substrate for microbial metabolism. As almost all bacterial species that form biofilms may synthesize and degrade EPS, these latter are

considered tools for communication. Bacteria living attached to surfaces and enveloped within biofilms substantially differ from planktonic cells [31]. EPS provide shelter to bacteria, block harmful agents, and trap nutrients from the environment thereby increasing the local concentration.

Formation of a biofilm is a complex process, which is regulated at different stages via diverse mechanisms [32, 33]. The most-studied regulatory mechanism is quorum sensing [28, 32–35]. At a given population density, the genes responsible for biofilm differentiation and maturation are activated [28, 33]. During growth within a biofilm, cells are in close contact with their neighbors and this promotes communication [36]. A few examples were described from mixed cultures during food and beverage fermentation [37]. The synthesis of the capsular kefiran (a type of EPS) promoted physical contact between *Lactobacillus kefiranofaciens* and *Saccharomyces cerevisiae*, the usual natural starters for making kefir. It was postulated that bacteria and yeasts benefit from the synthesis of kefiran, which promote interactions within the kefir grains, where the exchange of growth factors is facilitated. Under biofilm conditions, the synthesis and activity of bacteriocins is more efficient. Killing sensitive strains within a delimited zone, around the bacteriocin-producing strain, favors a more efficient increase of available nutrients than that found under broth culture conditions [38].

The role of quorum sensing in biofilm formation cannot be described in general terms but it varies depending on the bacterial species [39]. Quorum sensing is essential for adhesion, biofilm formation, and virulence of *P. aeruginosa* [33]. Mutants of *P. aeruginosa* that did not synthesize quorum sensing signals formed thinner biofilms than the wild type. Mutation of the *lasI* gene also resulted in an abnormal and undifferentiated biofilm formation [40]. The link between quorum sensing and the biofilm seemed to be mediated via the synthesis of EPS, with the unknown protein PA1324 having the role of binding and transporting EPS during biofilm formation [41]. Another important biofilm component is the polysaccharide intercellular adhesin (PIA), which mediates cell-to-cell adhesion [42]. Glucose is required to synthesize PIA [43], and uridine diphosphate-N-acetylglucosamine (UDP-GlcNAc) is the precursor of the polysaccharide matrix [44]. Indeed, the addition of glucose and UDP-GlcNAc in the culture medium stimulated the synthesis of PIA and the formation of a biofilm by *P. aeruginosa* [44]. N-acetylglucosamine is also the repeating unit within the heparin molecule, which stimulates the formation of a biofilm [45]. Heparin favors the adherence of *P. aeruginosa* to epithelial respiratory cells [46]. *Pseudomonas aeruginosa* also synthesizes alginate as the main biofilm component, which is made up of glucose, galactose, and pyruvate [47].

LuxS is required for biofilm formation on human gallstones by *Salmonella* Enteritidis [48]. Formation of a biofilm on gallstone surfaces should offer long-term protection against antimicrobial agents and high concentrations of bile. *Salmonella* Enteritidis senses the presence of bile as a signal. This induces the synthesis of bacterial surface organelles (e.g., fimbriae, flagella), which promote the formation of a biofilm. Flagella play a role in the secretion or synthesis of EPS as well as in the

initial adherence and formation of micro-colonies. The comparison of the global protein expression between the wild type and *luxS* mutant of *Salmonella* Enteritidis showed the negative effect of LuxS on the synthesis of flagellin [22]. The proteome of *Salmonella* Enteritidis was studied under conditions that mimicked the in vivo infection [49]. Two-dimensional differential in gel electrophoresis (2-D DIGE) analysis showed that adaptation was mediated through up- and down-regulation of several proteins. In particular, the uptake of AI-2, and the expression of LsrF, LsrA, LsrB, and LsrR were up-regulated. LsrA and LsrB are part of the AI-2 uptake transporter. Once AI-2 is phosphorylated, it binds to the transcriptional repressor LsrR. As such, it alleviates the repression of the *lsr* operon and allows the increased transcription of the *lsr*-genes, which resulted in an increased internalization of AI-2 [50]. It is supposed that stream of AI-2 is executed by LsrF, LsrE, and LsrG [50]. The up-regulation of LsrA and LsrB was related to the pathogenesis of *Salmonella* Enteritidis via the activation of the transcriptional regulator PhoP. This is a part of the two-component regulatory system (2CRS), which senses the concentration of extracellular Mg^{2+} [51].

Biofilm formation and architecture, and cell fimbriae were significantly altered in *lsrR* and *lsrK* mutants (see Sect. 1.5) of *E. coli* [52]. While *H. pylori* secretes EPS during biofilm formation [53] and other enteric pathogens such as *Salmonella* also use carbohydrates extensively [54], the matrix surrounding the biofilm of *Campylobacter jejuni* remains to be defined. The genome of *C. jejuni* encodes a limited repertoire of regulatory elements, which include a relatively small number of 2CRS [seven histidine protein kinase (HPK) and 12 response regulators (RR)] [55]. The CprRS sensor kinase mutant of *C. jejuni* displayed an apparent growth defect, and formed an enhanced and accelerated biofilm [56]. Modifications were consistent with the modulation of essential metabolic genes, and up-regulation of stress-tolerance proteins and cell surface structures. Oxidative stress-tolerance proteins such as catalase (Kat), thioredoxin reductase (TrxR), and alkyl hydroperoxide reductase (Ahp) were up-regulated. The major outer membrane protein and flagellar filament protein FlaA were also up-regulated. Down-regulation was found for the orphan RR and LuxS. The diversity of the deregulated proteins suggested that CprRS controls various aspects of *C. jejuni*, and the hypothesis was that nutrient availability might influence the formation of a biofilm.

An *agr*-like 2CRS, which encodes a cyclic thiolactone autoinducing peptide (AIP, LamD558), was found in *Lactobacillus plantarum* WCFS1 (Fig. 2.1) [57] (see Sect. 1.4). LamD558 has a ring structure similar to that of AIP from the staphylococcal *agr* system and it is involved in the regulation of adherence. Complete *agrBDCA*-like systems were found only for pathogenic bacteria such as staphylococci [58], *Enterococcus faecalis* [59] and *Listeria monocytogenes* [60]. Similarly, the *lamBDCA* system of *L. plantarum* may play a role in commensal host-microbe interaction [61].

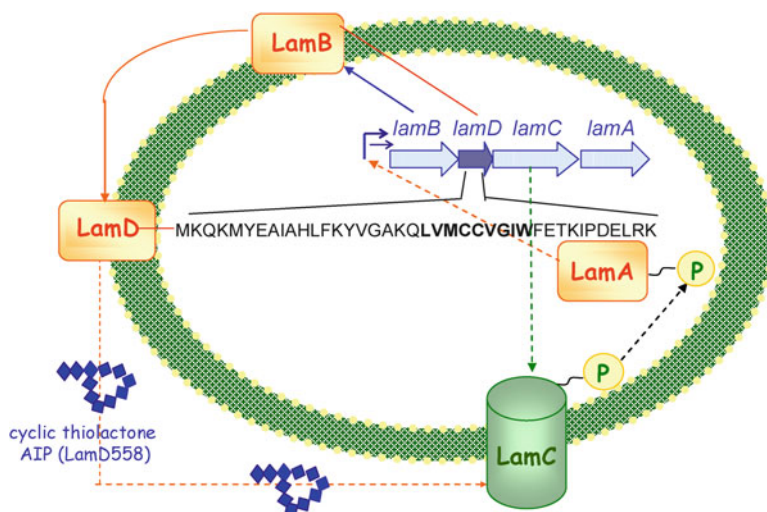


Fig. 2.1 Schematic representation of an agr-like two-component regulatory system (2CRS) found in *Lactobacillus plantarum* WCFS1. The *lam* quorum sensing system encodes the two-component histidine protein kinase LamC and response regulator LamA, an autoinducing pentapeptide (AIP) cyclic thiolactone derived from precursor peptide LamD and additionally LamB, a protein involved in processing and post-translational modification of LamD. The signal cyclic thiolactone pentapeptide with a ring structure was designated as LamD558. Amino acids of predicted AIP sequence is shown in bold type (Adapted from [22])

2.4 Bacteriocin Synthesis

Bacterial communities produce antimicrobial compounds to compete with other similar microorganisms. On the basis of biosynthetic mechanisms, bacteria produce two types of antimicrobial peptides: ribosomally synthesized peptides, or bacteriocins, which exhibit a relatively narrow range of antimicrobial activity, mainly inhibiting closely related bacteria that share the same ecological niche [62]; and nonribosomally synthesized peptides that show broader spectra of activities, inhibiting bacteria or fungi. On the basis of biochemical and genetic properties, bacteriocins are grouped into four classes (I–IV) [63]. Both class I and II bacteriocins are small (3–10 kDa), cationic, amphiphilic, and membrane-active peptides. Class I bacteriocins, or lantibiotics, contain the unusual amino acids lanthionine and methylanthionine. On the contrary, class II bacteriocins do not contain these modified amino acids. They are subdivided into three classes: IIa, *Listeria*-active peptides with the consensus sequence -Y-G-N-G-V-X-C- near the N-terminus; IIb, two-peptide bacteriocins, in which both components are required for antimicrobial activity; and IIc, thiol-activated peptides that require reduced cysteine residues for activity. Class III bacteriocins are high molecular mass (>30 kDa), heat-labile proteins. Class IV bacteriocins are complex peptides containing lipid or carbohydrate moieties, which are essential for activity.

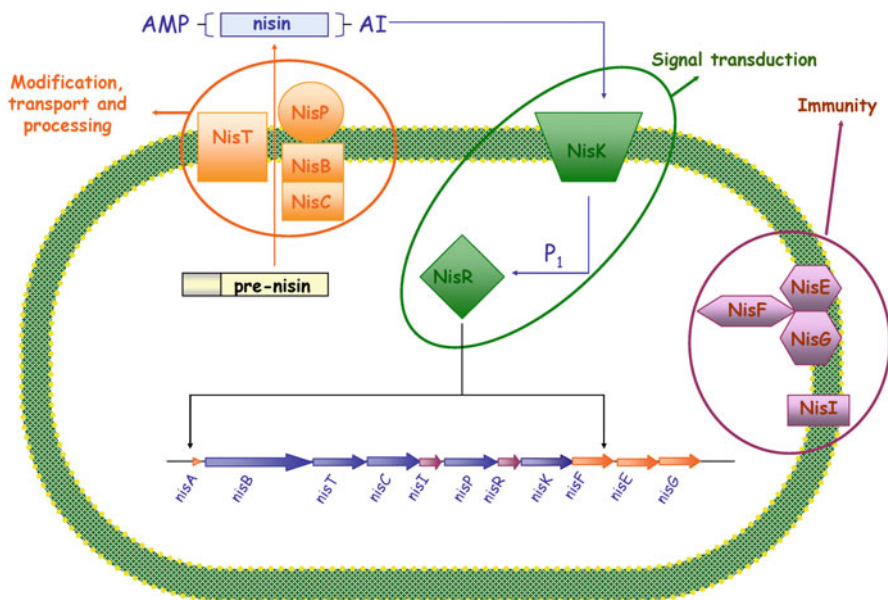


Fig. 2.2 Quorum sensing regulation of class I antimicrobial peptides (AMP) in lactic acid bacteria. *NisABTCIPRKFEF*, gene cluster encoding nisin; NisB and NisC, proteins involved in the intracellular post-translational modification reactions; NisT, putative transport protein of the ABC translocator family; NisP, extracellular protease for removing the leader peptide; AI, autoinducer; NisK, transmembrane-associated signal transducer; NisR, response regulator; NisF, NisE and NisG, ABC exporter system that generates immunity through active cell extrusion from the cell; and NisI, lipoprotein that contributes to producer immunity. For the quorum sensing mechanism see the text (Adapted from [87])

Many Gram-positive bacteria, especially lactic acid bacteria, secrete small antimicrobial peptides (AMP) or bacteriocins, which are regulated via quorum sensing mechanisms [64]. These compounds are of marked interest as natural food preservatives [65] and/or because they exert inhibitory activity against pathogens at the gastrointestinal level of humans and animals [66]. Nisin, which is synthesized by *Lactococcus lactis*, is the best known and most used lantibiotic [67]. Nisin is produced as the 57-residue precursor that contains the 23-residue N-terminal extension, called the leader peptide, which is absent in the mature molecule. The biosynthesis of nisin is encoded by the gene cluster *nisABTCIPRKFEF* [68]. Besides the structural, processing and producer-immunity genes, the cluster also contains elements of the 2CRS system, RR (*nisR*) and HPK (*nisK*), which are responsible for the regulation of nisin biosynthesis (Fig. 2.2). The synthesis of nisin starts at the early to mid logarithmic phase of growth and increases to the maximal level at the early stationary phase of growth, when the highest cell density is reached. Introduction of a 4 bp deletion on the structural *nisA* gene (Δ *nisA*) of *Lc. lactis* resulted not only in the loss of the capacity to synthesize nisin but also in the abolition of Δ *nisA* transcription. The transcription of Δ *nisA* was restored by the addition of sub-inhibitory levels of

nisin [69]. Therefore, besides its function as AMP, nisin also acts as a secreted signal molecule that induces the transcription of the genes involved in its biosynthesis. The signal transduction is mediated via NisK and NisR. The lantibiotic subtilisin of *Bacillus subtilis* is subjected to a similar quorum sensing circuit, which contains genes encoding HPK (*spaK*) and RR (*spaR*) [70]. A dual mechanism regulates the expression of subtilin. First, the σ factor H allows the low level of expression of the 2CRS *SpaR/SpaK* [71]. Further, subtilin auto-induces the histidine kinase *SpaK*, which, in turn, phosphorylates the response regulator *SpaR* and up-regulates the transcription of subtilin and immunity genes [71]. A novel subtilin-like lantibiotic, termed entianin, was identified in *B. subtilis* [72]. Combining DNA and mass spectrometry (MS/MS) sequencing data, it was shown that entianin exhibits the primary sequence of subtilin, except for the amino acid exchanges between Leu6 and Val6, Ala15 and Leu15, and Leu24 and Ile24. It represents the third subtilin-like lantibiotic along with ericin [73]. Entianin is synthesized in succinylated or unsuccinylated forms. In the latter case, the antimicrobial activity is much higher. Succinylation seems to dramatically decrease the antimicrobial activity. This is probably due to the diminished interaction between lipid II and lantibiotic or to the hampered integration of the complex into the cytoplasmic membrane. On the contrary, auto-induction is not adversely affected by succinylation. The *etn* gene cluster, which is responsible for entianin biosynthesis, regulation and autoimmunity, showed a high degree of homology (ca. 93 %) with the *spa* gene cluster that is responsible for subtilin biosynthesis. On the basis of genome sequences, the 2CRS of *Streptococcus thermophilus*, which consists of response regulator (RR) 04 (2CRS04), displays high homology with *SpaK/SpaR* of *B. subtilis* and *NisK/NisR* of *Lc. lactis* [74]. The biological relevance of this general regulatory mechanism, which is quite common to the above bacterial species, was based on the following considerations: (1) it ensures that the environmental concentration of AMP rapidly reaches levels, which are efficient to kill competitors; (2) the rapid increase of the concentration of AMP prevents the development of immunity mechanisms into target cells; and (3) it protects the producing cells from the ineffective activity, which may occur when AMP diffuses away from the environment [68].

Class II bacteriocins are synthesized as precursor peptides that contain an N-terminal extension, which is removed during or shortly after secretion of the peptide. Pro-peptides share the common feature of having two glycine residues (Gly-Gly motif) that precede the cleavage site. The genetic characterization of several strains of *L. plantarum*, which were variously isolated from vegetables, fermented foods and human saliva, showed that the same determinants were responsible for bacteriocin biosynthesis and gene regulation. These strains synthesized bacteriocins belonging to the group of plantaricins and their *pln* loci is bi-faceted, one part being highly conserved and the other mosaic like. The *pln* loci encode class IIb (plantaricins EF, JK, NC8, and J51) or class IIc (pheromone peptide plantaricin A, *plnA*) bacteriocins, one conserved ABC-transporter dedicated to export peptides, with the so called double-glycine leader, and two divergent quorum sensing networks. Many bacteriocins from lactic acid bacteria are only synthesized in broth cultures. This occurs when specific inoculum size and growth conditions are achieved, and a dedicated

three-component regulatory system (3CRS), involved in quorum sensing mechanisms, is switched on. On the contrary, a few other bacteriocins are phenotypically constitutive, and they are synthesized on solid but not in liquid media. Such divergence in biosynthesis is usually attributed to differences in the rate of diffusion. Compared to a liquid medium, cells growing on the agar surface are in closer contact with the secreted bacteriocins. The question about constitutive and regulated bacteriocins was highlighted constructing knockout mutants for regulatory operons [75]. It was revealed that the synthesis of bacteriocins is under the control of quorum sensing mechanisms both on solid and liquid media. During growth in a liquid medium, the synthesis of bacteriocins occurs only in the presence of an elevated inoculum size or if an external source of bacteriocin is added to the medium. This confirmed the auto-induction mechanism. Such a regulatory mechanism was also shown for the synthesis of carnobacteriocin A, B2 and BM1 by *Carnobacterium piscicola* [76], several different putative plantaricins (PlnJK, PlnEF and PlnN) by *L. plantarum* [77], and sakacin P by *Lactobacillus sakei* [78]. The phenotype (Bac⁺) was lost upon inoculation of an overnight culture into fresh culture medium at the level below the threshold of inoculum size (10^6 – 10^4 cfu/ml). The Bac⁻ phenotype persisted during subsequent cultivation but it was recovered by addition of cell-free Bac⁺ culture supernatant. Other environmental factors are, probably, responsible for the synthesis of phenotypically constitutive bacteriocins into solid media [79]. Whatever be the case, most of these bacteriocins are synthesized in those culture conditions, which better mimic the natural ecological niche of lactic acid bacteria (e.g., growth on a solid surface and presence of inducing microorganisms) [80]. This phenotype should be of importance in food fermentation, especially for vegetables (e.g., olive fermentation), where solid matrices represent enormous surfaces for bacteria to adhere via biofilm formation. Under these ecological conditions, bacteria may find suitable environmental parameters to synthesize bacteriocins. Selection of starter cultures of *L. plantarum* for vegetable fermentations should also consider these features.

2.4.1 The Regulatory Operons and Their Regulated Promoters

As stated above the synthesis of bacteriocins is regulated through a quorum sensing pathway via 3CRS. Usually, this regulation involves three proteins: the secreted peptide autoinducing pheromone (AIP), the membrane-located histidine protein kinase (HPK), and the response regulator (RR). The secreted pheromone serves as a tool for measuring the cell density of the producer strain. At a certain cell density, AIP reaches the critical threshold concentration and triggers a cascade of phosphorylation, which culminated with the phosphorylated RR. This latter binds to the promoters of the bacteriocin regulon and activates the genes for biosynthesis. The *pln* regulon of *L. plantarum* C11 was studied in detail. The regulatory operon *plnABCD* codes for an auto-regulatory circuit, which activates its own transcription as well as the transcription of another four operons at the *pln* locus [77]. *plnABCD*

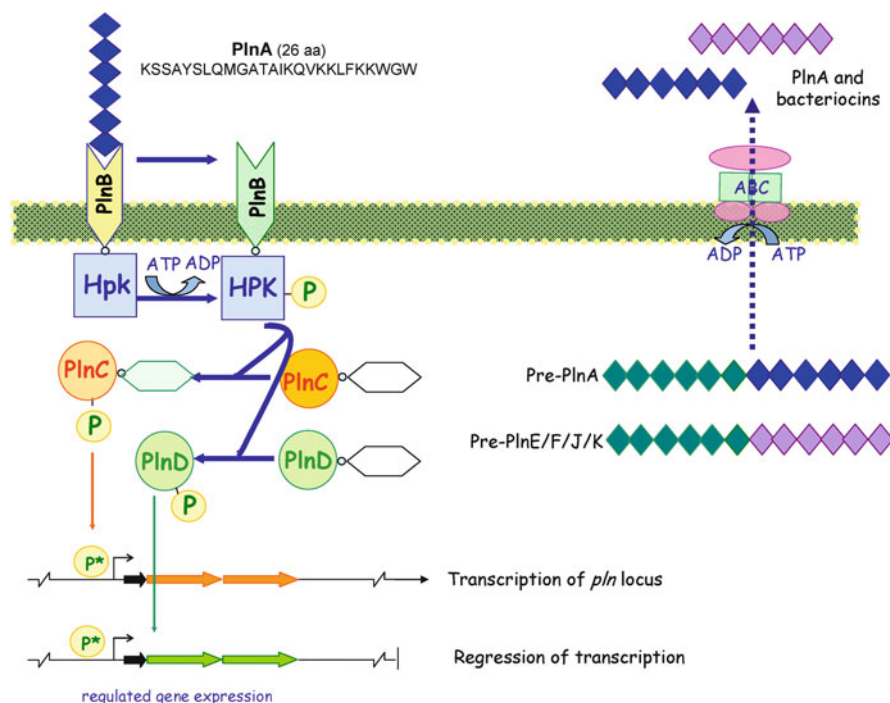


Fig. 2.3 Auto-regulatory network of the *pln* regulon in *Lactobacillus plantarum* C11. Binding of the inducing factor (*PlnA*) to the membrane domain of the histidine protein kinase *PlnB* leads to auto-phosphorylation of the cytoplasmic domain of *PlnB* and the subsequent transfer of the phosphoryl group to the gene regulators *PlnC* and *PlnD*. Phosphorylated regulators bind to regulated promoters to activate (by *PlnC*) or repress (by *PlnD*) expression of the genes involved in bacteriocin synthesis, including the auto-regulatory operon (*plnABCD*). All bacteriocins and the inducing peptide *PlnA* apply double-glycine leaders for export through a dedicated ABC transporter (Adapted from [81])

codes for plantaricin A (AIP), *PlnB* (homologous to HPK), and *PlnC* and *PlnD* (homologues to RR) [81] (Fig. 2.3). Almost the same regulatory network was found for *L. plantarum* NC8 and DC400, which were isolated from vegetables and Italian sourdoughs, respectively [81, 82]. Unlike the *pln* regulatory operon of *L. plantarum* C11, that of strain NC8 contains only three genes: *NC8-IF*, *NC8-HK*, and *NC8-plnD* that code for AIP, HPK, and RR, respectively. In general, the interactions between the peptide pheromones (plantaricin A or NC8-IF) and their cognate HPK (*PlnB* or NC8-HK) is specific and no cross-talk occurs between the same pheromones and noncognate HPK molecules. In vitro studies showed that both response regulators, *PlnC* and *PlnD*, bind as homo-dimers in a cooperative manner. When examined in a heterologous host (e.g., *L. sakei*), *PlnC* and *PlnD* act as positive regulators, the first being much stronger [81]. Nevertheless, when these regulators were individually overexpressed in the endogenous host (*L. plantarum* C11), they acted differently. *PlnC* activated, while *PlnD* repressed the biosynthesis of the bacteriocin [81].

The factors that cause such variable biological functions are still unknown. A hypothetical scenario was proposed upon activation of the *pln* locus of *L. plantarum* C11. During initial gene activation (the level of regulators is low), PlnC strongly binds to the regulatory operon, which activates the expression of the remaining operons at the *pln* locus and leads to a burst of bacteriocin production. During later stages (regulators are accumulated), PlnD ousts PlnC from promoter binding, especially from the transport promoter. As the encoded transport system is dedicated to export of bacteriocin, its inactivation causes an adverse effect on the auto-regulatory network. This leads to cessation of bacteriocin biosynthesis, which usually occurs during the late exponential phase of growth.

Recently, another mechanism was proposed for down-regulation of *plnABCD* from *L. plantarum* C11 [83]. Truncated versions of the activator PlnC resulting from translation from alternative start codons within *plnC* in cells, were found to exhibit repression of the bacteriocin regulon, which completely changed its functionality. It exhibited repression of the bacteriocin regulon. The same finding was observed for the bacteriocin systems of *L. sakei* LTH673 and *L. plantarum* NC8. This mode of repression may represent a common tool used by bacteria to down-regulate certain quorum sensing-based pathways.

2.4.2 The Peptide Pheromone Plantaricin A

Plantaricin A (PlnA) has a dual function in the plantaricin system. It works as an induction factor in gene regulation and as an antimicrobial peptide [84]. Plantaricin A was originally described as bacteriocin [85], and, in this context, it should be considered as belonging to class IIc: non-pediocin-like, one-peptide bacteriocin without post-translational modifications. The antimicrobial spectrum of PlnA is relatively narrow. It mainly comprises *Lactobacillus* species, for instance *Lactobacillus casei*, *L. sakei* and *Lactobacillus viridescens*, in addition to *L. plantarum* strains. Compared to other plantaricins (EF and JK), PlnA shows significantly lower activity, being 10–100-fold less potent [81]. Contrary to most of the bacteriocins, it lacks a dedicated immunity protein. These features suggest that PlnA is primarily an induction factor and that the antimicrobial activity is secondary, probably caused by the amphiphilic characteristics of its secondary structure.

From the structural point of view, PlnA is unstructured in aqueous solution, but it adopts an amphiphilic α -helix, from residue 12 to 21 (C-terminal part), when it comes in contact with negative charges into the membrane. The α -helix conformation is essential for pheromone and antimicrobial activities. Regarding the pheromone function, the α -helix facilitates the positioning of the N-terminal part of PlnA, which engages chiral interactions with the receptor PlnB. For antimicrobial activity, no chiral interactions take place and only the α -helix structure is sufficient to permeabilize sensitive cells [84]. Because of the necessity for the contact of PlnA with the membrane for it to act as a pheromone, it was suggested that the antimicrobial activity is a side effect, which is indirectly caused by the mode of action of the pheromone peptide.

As shown for other strains that populate other food ecosystems [86], multidimensional high-performance liquid chromatography (MDLC) coupled with electrospray-ionization (ESI)-ion trap mass spectrometry (nano-ESI-MS/MS) analyses revealed the synthesis of the pheromone PlnA in sourdough *L. plantarum* DC400 [82]. The main features of its activity and the ecological relevance are described in the Sect. 3.2.2.

References

1. Hentzer M, Wu H, Andersen JB, Riedel K, Rasmussen TB, Bagge N, Schembri MA, Song Z, Kristoffersen P, Manefield M, Costerton JW, Molin S, Eberl L, Steinberg P, Kjelleberg S, Høiby N, Givskov M (2003) Attenuation of *Pseudomonas aeruginosa* virulence by quorum sensing inhibitors. *EMBO J* 22:3803–3815
2. Wagner VE, Gillis RJ, Iglewski B (2004) Transcriptome analysis of quorum sensing regulation and virulence factor expression in *Pseudomonas aeruginosa*. *Vaccine* 22:S15–S20
3. Wu L, Estrada O, Zaborina O, Bains M, Shen L, Kohler JE, Patel N, Musch MW, Chang EB, Fu Y-X, Jacobs MA, Nishimura MI, Hancock REW, Turner JR, Alverdy JC (2005) Recognition of host immune activation by *Pseudomonas aeruginosa*. *Science* 309:774–777
4. Nouwens AS, Beatson SA, Whitchurch CB, Walsh BJ, Schweizer HP, Mattick JS, Cordwell SJ (2003) Proteome analysis of extracellular proteins regulated by the las and rhl quorum sensing system in *Pseudomonas aeruginosa* PAO1. *Microbiology* 149:1311–1322
5. Braun P, de Groot A, Bitter W, Tommassen J (1998) Secretion of elastinolytic enzymes and their propeptides by *Pseudomonas aeruginosa*. *J Bacteriol* 180:3467–3469
6. Folders J, Tommassen J, Van Loon LC, Bitter W (2000) Identification of a chitin-binding protein secreted by *Pseudomonas aeruginosa*. *J Bacteriol* 182:1257–1263
7. Nakao H, Watanabe H, Nakayama S, Takeda T (1995) *yst* gene expression in *Yersinia enterocolitica* is positively regulated by a chromosomal region that is highly homologous to *Escherichia coli* host factor 1 gene (*hfq*). *Mol Microbiol* 18:859–865
8. Robertson GT, Loop RM Jr (1999) The *Brucella abortus* host factor I (HF-I) protein contributes to stress resistance during stationary phase and is a major determinant of virulence in mice. *Mol Microbiol* 34:690–700
9. Coulthurst SJ, Monson RE, Salmond GPC (2008) Quorum sensing in the soft-rot *Erwinias*. In: Winans SC, Bassler BL (eds) *Chemical communication among bacteria*. ASM Press, Washington, DC, p 185
10. Lewenza S, Conway B, Greenberg EP, Sokol PA (1999) Quorum sensing in *Burkholderia cepacia*: identification of the LuxRI homologs CepRI. *J Bacteriol* 181:748–756
11. Gotschlich A, Huber B, Geisenberger O, Tögl A, Steidle A, Riedel K, Hill P, Tümmler B, Vandamme P, Middleton B, Camara M, Williams P, Hardman A, Eberl L (2000) Synthesis of multiple N-acyl-homoserine lactones is wide-spread among the members of the *Burkholderia cepacia* complex. *Syst Appl Microbiol* 24:1–14
12. Huber B, Riedel K, Hentzer M, Heydorn A, Gotschlich A, Givskov M, Molin S, Eberl L (2001) The cep quorum sensing system of *Burkholderia cepacia* H111 controls biofilm formation and swarming motility. *Microbiology* 147:2517–2528
13. Lewenza S, Sokol PA (2001) Regulation of ornibactin biosynthesis and N-acyl-L-homoserine lactone production by CepR in *Burkholderia cepacia*. *J Bacteriol* 183:2212–2218
14. Riedel K, Aravalo-Ferro C, Reil G, Gorg A, Lottspeich F, Eberl L (2003) Analysis of the quorum sensing *Burkholderia cepacia* H111 by proteomics. *Electrophoresis* 24:740–750
15. Hanna SL, Sherman NE, Kinter MT, Goldberg JB (2000) Comparison of proteins expressed by *Pseudomonas aeruginosa* strains representing initial and chronic isolates from a cystic fibrosis

- patient: an analysis by 2-D gel electrophoresis and capillary column liquid chromatography-tandem mass spectrometry. *Microbiology* 146:2495–2508
16. Sauer K, Camper AK, Ehrlich GD, Costerton JW, Davies DG (2002) *Pseudomonas aeruginosa* displays multiple phenotypes during development as a biofilm. *J Bacteriol* 184:1140–1154
 17. Shao CP, Hor LI (2000) Metalloprotease is not essential for *Vibrio vulnificus* virulence in mice. *Inf Imm* 68:3569–3573
 18. Jeong HS, Rhee JE, Lee JH, Choi HK, Kim DI, Lee MH, Park S-J, Choi SH (2003) Identification of *Vibrio vulnificus* Irp and its influence on survival under various stresses. *J Microbiol Biotechnol* 13:159–163
 19. Shin NR, Lee DY, Yoo HS (2007) Identification of quorum sensing-related regulons in *Vibrio vulnificus* by two-dimensional gel electrophoresis and differentially displayed reverse transcriptase PCR. *FEMS Immunol Med Microbiol* 50:94–103
 20. Miyoshi N, Shinoda S (1997) Bacterial metalloprotease as the toxic factor in infection. *J Toxicol Toxin Rev* 16:177–194
 21. Sgarrella F, Poddie FP, Meloni MA, Sciola L, Pippia P, Tozzi MG (1997) Channelling of deoxyribose moiety of exogenous DNA into carbohydrate metabolism: role of deoxyriboaldolase. *Comp Biochem Physiol B Biochem Mol Biol* 117:253–257
 22. Di Cagno R, De Angelis M, Calasso M, Gobetti M (2011) Proteomics of the bacterial cross-talk by quorum sensing. *J Proteomics* 74:19–34
 23. Lereclus D, Agaisse H, Gominet M, Salameitou S, Sanchis V (1996) Identification of a *Bacillus thuringiensis* gene that positively regulates transcription of the phosphatidylinositol-specific phospholipase C gene at the onset of the stationary phase. *J Bacteriol* 178:2749–2756
 24. Rasko DA, Altherr MR, Han CS, Ravel J (2005) Genomics of the *Bacillus cereus* group of organisms. *FEMS Microbiol Rev* 29:303–329
 25. Dixon TC, Meselson M, Guillemin J, Hanna PC (1999) Anthrax. *N Engl J Med* 341:815–826
 26. Ramtani L, Lereclus D (2002) A cell-cell signaling peptide activates the PlcR virulence regulon in bacteria of the *Bacillus cereus* group. *EMBO J* 21:4550–4559
 27. Ohtani K, Hayashi H, Shimizu T (2002) The luxS gene is involved in cell-cell signalling for toxin production in *Clostridium perfringens*. *Mol Microbiol* 44:171–179
 28. Donlan RM (2002) Biofilms: microbial life on surfaces. *Emerging Infect Dis* 8:881–890
 29. Flemming HC, Wingender J (2001) Relevance of microbial extracellular polymeric substances (EPSs)-part I: structural and ecological aspects. *Water Sci Technol* 43:1–8
 30. Sutherland IW (2001) Biofilm exopolysaccharides: a strong and sticky framework. *Microbiology* 147:3–9
 31. Korber DR, Lawrence JR, Lappin-Scott HM, Costerton JW (1995) Growth of microorganisms on surfaces. In: Lappin-Scott HM, Costerton JW (eds) *Microbial biofilms, plant and microbial biotechnology research*, vol 5. Cambridge University Press, Cambridge, UK, p 15
 32. Ruiz LM, Valenzuela S, Castro M, Gonzalez A, Frezza M, Soule L, Rohwerder T, Queneau Y, Doutheau A, Sand W, Jerez CA, Guiliani N (2008) AHL communication is a widespread phenomenon in biomineralizing bacteria and seems to be involved in mineral-adhesion efficiency. *Hydrometallurgy* 94:133–137
 33. Waters CM, Bassler BL (2005) Quorum sensing: cell-to-cell communication in bacteria. *Annu Rev Cell Dev Biol* 21:319–346
 34. von Bodman SB, Majerczak DR, Coplin DL (1998) A negative regulator mediates quorum sensing control of exopolysaccharide production in *Pantoea stewartii* subsp. *stewartii*. *Proc Natl Acad Sci USA* 95:7687–7692
 35. Rivas M, Seeger M, Holmes DS, Jedlicki E (2005) A Lux-like quorum sensing system in the extreme acidophile *Acidithiobacillus ferrooxidans*. *Biol Res* 38:283–297
 36. Miller MB, Bassler BL (2001) Quorum sensing in bacteria. *Annu Rev Microbiol* 55:165–199
 37. Cheirsilp B, Shoji H, Shimizu H, Shioya S (2003) Interactions between *Lactobacillus kefirifaciens* and *Saccharomyces cerevisiae* in mixed culture for kefir production. *J Biosci Bioeng* 96:279–284

38. Chao L, Levin BR (1981) Structured habitats and the evolution of anticompetitor toxins in bacteria. *Proc Natl Acad Sci USA* 78:6324–6328
39. Hooshangi S, Bentley WE (2008) From unicellular properties to multicellular behavior: bacteria quorum sensing circuitry and applications. *Curr Opin Biotechnol* 19:550–555
40. Micheli L, Uccelletti D, Palleschi C, Crescenzi V (1999) Isolation and characterisation of a ropy *Lactobacillus* strain producing the exopolysaccharide kefiran. *Appl Environ Microbiol* 53:69–74
41. Mercier KA, Cort JR, Kennedy MA, Lockert EE, Ni S, Shortridge MD, Powers R (2009) Structure and function of *Pseudomonas aeruginosa* protein PA1324 (21–170). *Prot Sci* 18:606–618
42. Cramton SE, Gerke C, Schnell NF, Nichols WW, Gotz F (1999) The intercellular adhesion (ica) locus is present in *Staphylococcus aureus* and is required for biofilm formation. *Infect Immun* 67:5427–5433
43. Dobinsky S, Kiel K, Rohde H, Bartscht K, Knobloch JKM, Horstkotte MA, Mack D (2003) Glucose-related dissociation between icaADBC transcription and biofilm expression by *Staphylococcus epidermidis*: evidence for an additional factor required for polysaccharide intercellular adhesin synthesis. *J Bacteriol* 185:2879–2886
44. Gerke C, Kraft A, Sussmuth R, Schweitzer O, Gotz F (1998) Characterization of the N-acetylglucosaminyltransferase activity involved in the biosynthesis of the *Staphylococcus epidermidis* polysaccharide intercellular adhesin. *J Biol Chem* 273:18586–18593
45. Shanks RMQ, Donegan NP, Graber ML, Buckingham SE, Zegans ME, Cheung AL, O'Toole GA (2005) Heparin stimulates *Staphylococcus aureus* biofilm formation. *Infect Immun* 73:4596–4606
46. Plotkowski MC, Costa AO, Morandi V, Barbosa HS, Nader HB, De Benizmann S, Puchelle E (2001) Role of heparan sulphate proteoglycans as potential receptors for non-piliated *Pseudomonas aeruginosa* adherence to non-polarised airway epithelial cells. *J Med Microbiol* 50:183–190
47. Read RR, Costerton JW (1987) Purification and characterization of adhesive exopolysaccharides from *Pseudomonas putida* and *Pseudomonas fluorescens*. *Can J Microbiol* 33:1080–1090
48. Prouty AM, Schwesinger WH, Gunn JS (2002) Biofilm formation and interaction with the surfaces of gallstones by *Salmonella* spp. *Infect Immun* 70:2640–2649
49. Sonck KAJ, Kint G, Schoofs G, Wauden CV, Vanderleyden J, De Keersmaecker SCJ (2009) The proteome of *Salmonella typhimurium* grown under in vivo-mimicking conditions. *Proteomics* 9:565–579
50. Taga ME, Semmelhack JL, Bassler BL (2001) The LuxS-dependent autoinducer AI-2 controls the expression of an ABC transporter that functions in AI-2 uptake in *Salmonella typhimurium*. *Mol Microbiol* 42:777–793
51. Groisman EA (2001) The pleiotropic two-component regulatory system PhoP-PhoQ. *J Bacteriol* 183:1835–1842
52. Agudo D, Mendoza MT, Castañares C, Nombela C, Rotger R (2004) A proteomic approach to study *Salmonella typhi* periplasmic proteins altered by a lack of the DsbA thiol: disulfide isomerase. *Proteomics* 4:355–363
53. Stark RM, Gerwig GJ, Pitman RS, Potts LF, Williams NA, Greenman J, Weinzwieg IP, Hirst TR, Millar MR (1999) Biofilm formation by *Helicobacter pylori*. *Lett Appl Microbiol* 28:121–126
54. Romling U (2005) Characterization of the rdar morphotype, a multicellular behaviour in *Enterobacteriaceae*. *Cell Mol Life Sci* 62:1234–1246
55. Parkhill J, Wren BW, Mungall K, Ketley JM, Churcher C, Basham D, Chillingworth T, Davies RM, Feltwell T, Holroyd S, Jagels K, Karlyshev AV, Moule S, Pallen MJ, Penn CW, Quail MA, Rajandream MA, Rutherford KM, van Vliet AH, Whitehead S, Barrell BG (2000) The genome sequence of the food-borne pathogen *Campylobacter jejuni* reveals hypervariable sequences. *Nature* 403:665–668

56. Svensson SL, Davis LM, Mac Kichan JK, Allan BJ, Pajaniappan M, Thompson SA, Gaynor EC (2009) The CprS sensor kinase of the zoonotic pathogen *Campylobacter jejuni* influences biofilm formation and is required for optimal chick colonization. *Mol Microbiol* 71:253–272
57. Sturme MHJ, Nakayama J, Molenaar D, Murakami Y, Kunugi R, Fujii T, Vaughan EE, Kleerebezem M, de Vos WM (2005) An agr-like two-component regulatory system in *Lactobacillus plantarum* is involved in production of a novel cyclic peptide and regulation of adherence. *J Bacteriol* 187:5224–5235
58. Dufour P, Jarraud S, Vandenesch F, Greenland T, Novick RP, Bes M, Etienne J (2002) High genetic variability of the agr locus in *Staphylococcus species*. *J Bacteriol* 184:1180–1186
59. Nakayama J, Kariyama R, Kumon H (2002) Description of a 23.9-kilobase chromosomal deletion containing a region encoding fsr genes which mainly determines the gelatinase-negative phenotype of clinical isolates of *Enterococcus faecalis* in urine. *Appl Environ Microbiol* 68:3152–3155
60. Autret N, Raynaud C, Dubail I, Berche P, Charbit A (2003) Identification of the agr locus of *Listeria monocytogenes*: role in bacterial virulence. *Infect Immun* 71:4463–4471
61. De Vos WM, Bron PA, Kleerebezem M (2004) Post-genomics of lactic acid bacteria and other food-grade bacteria to discover gut functionality. *Curr Opin Biotechnol* 15:86–93
62. Nissen-Meyer J, Nes IF (1997) Ribosomally synthesized antimicrobial peptides: their function, structure, biogenesis, and mechanism of action. *Arch Microbiol* 167:67–77
63. Nes IF, Diep DB, Havarstein LS, Brurberg MB, Eijsink V, Holo H (1996) Biosynthesis of bacteriocins in lactic acid bacteria. *Ant Van Leeuw* 70:113–128
64. Klaenhammer TR (1993) Genetics of bacteriocins produced by lactic acid bacteria. *FEMS Microbiol Rev* 12:39–86
65. Cotter PD, Hill C, Ross RP (2005) Bacteriocins: developing innate immunity for food. *Nat Rev Microbiol* 3:777–788
66. Klarin B, Johansson ML, Molin G, Larsson A, Jeppsson B (2005) Adhesion of the probiotic bacterium *Lactobacillus plantarum* 299v onto the gut mucosa in critically ill patients: a randomised open trial. *Crit Care* 9:R285–R293
67. Hansen JN (1994) Nisin as a model food preservative. *Crit Rev Food Sci Nutr* 34:69–93
68. Kleerebezem M, Quadri LE (2001) Peptide pheromone-dependent regulation of antimicrobial peptide production in Gram-positive bacteria: a case of multicellular behaviour. *Peptides* 22:1579–1596
69. Kuipers OP, Beerthuis MM, de Ruyter PGGA, Luesink EJ, de Vos WM (1995) Autoregulation of nisin biosynthesis in *Lactococcus lactis* by a signal transduction. *J Biol Chem* 270:27299–27304
70. Gutowski-Eckel Z, Klei C, Siegers K, Bhom K, Hammalmen M, Entian KD (1994) Growth phase-dependent regulation and membrane localization of SpaB, a protein involved in biosynthesis of the lantibiotic subtilin. *Appl Environ Microbiol* 60:1–11
71. Heinzmann S, Entian KD, Stein T (2006) Engineering *Bacillus subtilis* ATCC 6633 for improved production of the lantibiotic subtilin. *Appl Microbiol Biotechnol* 69:532–536
72. Fuchs SW, Jaskolla TW, Bochmann S, Kötter P, Wichelhaus T, Karas M, Stein T, Entian K-D (2011) Entianin, a novel subtilin-like lantibiotic from *Bacillus subtilis* subsp. *spizizenii* DSM 15029 T with high antimicrobial activity. *Appl Environ Microbiol* 77:1698–1707
73. Stein T, Borchert S, Conrad B, Feesche J, Hofemeister B, Hofemeister J, Entian KD (2002) Two different lantibiotic-like peptides originate from the ericin gene cluster of *Bacillus subtilis* A1/3. *J Bacteriol* 184:1703–1711
74. Hols P, Hancy F, Fontaine L, Grossiord B, Prozzi D, Leblond-Bourget N, Decaris B, Bolutin A, Delborme C, Ehrlich SD, Guèdon E, Monnet V, Renault P, Kleerebezem M (2005) New insights in the molecular biology and physiology of *Streptococcus thermophilus* revealed by comparative genomics. *FEMS Microbiol Rev* 29:435–463
75. Maldonado-Barragan A, Ruiz-Barba JL, Jimenez-Diaz R (2009) Knockout of three-component regulatory systems reveals that the apparently constitutive plantaricin production phenotype

- shown by *Lactobacillus plantarum* on solid medium is regulated via quorum sensing. *Int J Food Microbiol* 130:35–42
76. Quadri LEN, Kleerebezem M, Kuipers OP, De Vos WM, Roy KL, Vederas JC, Stiles ME (1997) Characterization of a locus from *Carnobacterium piscicola* LV17B involved in bacteriocin production and immunity: evidence for global inducer-mediated transcriptional regulation. *J Bacteriol* 179:6163–6171
 77. Diep DB, Håvarstein LS, Nes IF (1996) Characterization of the locus responsible for the bacteriocin production in *Lactobacillus plantarum* C11. *J Bacteriol* 178:4472–4483
 78. Eijsink VGH, Brurberg MB, Middelhoven PH, Nes IF (1996) Induction of bacteriocin production in *Lactobacillus sake* by a secreted peptide. *J Bacteriol* 178:2232–2237
 79. Nes IF, Eijsink VGH (1999) Regulation of group II peptide bacteriocin synthesis by quorum sensing mechanisms. In: Dunny GM, Winans SC (eds) *Cell-cell signaling in bacteria*. ASM Press, Washington, DC, p 175
 80. Maldonado A, Ruiz-Barba JL, Jiménez-Díaz R (2004) Production of plantaricin NC8 by *Lactobacillus plantarum* NC8 is induced in the presence of different types of Gram-positive bacteria. *Arch Microbiol* 181:8–16
 81. Diep DB, Straume D, Kjos M, Torres C, Nes IF (2009) An overview of the mosaic bacteriocin *pln* loci from *Lactobacillus plantarum*. *Peptides* 30:1562–1574
 82. Di Cagno R, De Angelis M, Calasso M, Vincentini O, Vernocchi P, Ndagijimana M, De Vincenzi M, Dessi MR, Guerzoni ME, Gobetti M (2010) Quorum sensing in sourdough *Lactobacillus plantarum* DC400: induction of plantaricin A (PlnA) under co-cultivation with other lactic acid bacteria and effect of PlnA on bacterial and Caco-2 cells. *Proteomics* 10:2175–2190
 83. Straume D, Kjos M, Nes IF, Diep DB (2007) Quorum sensing based bacteriocin production is down-regulated by N-terminally truncated species of gene activators. *Mol Genet Genomics* 278:283–293
 84. Hauge HH, Mantzilas D, Moll GN, Konings WN, Driessen AJ, Eijsink VG, Nissen-Meyer J (1998) Plantaricin A is an amphiphilic alpha-helical bacteriocin-like pheromone which exerts antimicrobial and pheromone activities through different mechanisms. *Biochemistry* 37:16026–16032
 85. Nissen-Meyer J, Larsen AG, Sletten K, Daeschel M, Nes IF (1993) Purification and characterization of plantaricin A, a *Lactobacillus plantarum* bacteriocin whose activity depends on the action of two peptides. *J Gen Microbiol* 139:1973–1978
 86. Fimland N, Rogne P, Fimland G, Nissen-Meyer J, Kristiansen PE (2008) Three-dimensional structure of the two peptides that constitute the two-peptide bacteriocin plantaricin EF. *Biochim Biophys Acta* 1784:1711–1719
 87. Quadri LEN (2002) Regulation of antimicrobial peptide production by autoinducer-mediated quorum sensing in lactic acid bacteria. *Ant Van Leeuwen* 82:133–145

Bacterial Communication in Foods

Gobbetti, M.; Di Cagno, R.

2013, X, 77 p. 20 illus., 18 illus. in color., Softcover

ISBN: 978-1-4614-5655-1