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Review article

Quorum sensing-controlled gene expression in lactic acid bacteria

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Abstract

Quorum sensing in lactic acid bacteria (LAB) involves peptides that are directly sensed by membrane-located histidine kinases, after which the signal is transmitted to an intracellular response regulator. This regulator in turn activates transcription of target genes, that commonly include the structural gene for the inducer molecule. The two-component signal-transduction machinery has proven to be indispensable for transcription activation and production of several autoinducers found in LAB, which are predominantly bacteriocins or bacteriocin-like peptides. In the nisin autoregulation process in *Lactococcus lactis* the NisK protein acts as the sensor for nisin and the NisR protein as the response regulator, activating transcription of target genes. The *cis*-acting elements for NisR were identified as the *nisA* and *nisF* promoter fragments and these were further analysed for inducibility. Based on this knowledge efficient nisin-controlled expression (NICE) systems were developed for several different lactic acid bacteria. A promising application of the NICE system is the development of autolytic starter lactococci, which will lyse in an early stage during cheese ripening thereby facilitating the release of intracellular enzymes which can contribute to flavour formation.

Keywords: Quorum sensing; Signal transduction; Lactic acid bacteria; NICE

1. Introduction

The expression quorum sensing denotes a process in microorganisms that involves specific molecules that act as signals for induction of gene

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expression only when a certain threshold concentration of these molecules in the environment has been reached. It provides a way of cell-to-cell communication between these microorganisms and can be regarded as a form of multicellularity in these organisms. This phenomenon has extensively been studied in Gram-negative bacteria, in which molecules belonging to the class of *N*-acyl homoserine lactones act as the diffusible communication pheromones that are required for the regulation of cell-density-dependent phenotypes. The paradigm of quorum sensing in Gram-negative bacteria is the bioluminecence (*lux*) phenotype found in *Photobacterium fischeri*, where the synthesis of the homoserine lactone signal pheromone requires the LuxI protein. The LuxR protein can bind the homoserine lactone molecule, resulting in transcription activation of the *lux* genes. This process results in the cell-density-dependent expression of the bioluminescence genes and eventually to the bioluminescence phenotype (Salmond et al., 1995).

In Gram-positive bacteria the phenomenon of quorum sensing occurs in processes like development of genetic competence in *Bacillus subtilis* and *Streptococcus pneumoniae*, virulence development in *Staphylococcus aureus* and the production of antimicrobial peptides by several species of lactic acid bacteria (LAB; Kleerebezem et al., 1997a). In contrast to the inducing factors in Gram-negative bacteria, the Gram-positives exclusively use peptides or post-translationally modified peptides as inducer molecules.

2. Signal transduction and peptide pheromones in LAB

In LAB two main types of antimicrobial peptides can be encountered, i.e. the post-translationally modified lantibiotics belonging to class I and the linear unmodified class II bacteriocins (Klaenhammer, 1993). Prominent members of lantibiotics produced by LAB are nisin and lacticin 481, produced by *Lactococcus lactis* (Siezen et al., 1996), and lactocin S, produced by *Lactobacillus sake* (Skaugen et al., 1994). Well-characterised members of the type II bacteriocins in LAB are carnobacteriocin BM1 and B2, produced by *Carnobacterium piscicola* (Quadri et al., 1994; Saucier et al., 1995; Quadri et al., 1997), plantaricins produced by *Lactobacillus plantarum* (Diep et al., 1995, 1996) and sakacin P produced by *L. sake* (Eijsink et al., 1996; Hühne et al., 1996; Brurberg et al., 1997). Several gene clusters involved in the biosynthesis of these antimicrobial peptides have been identified and characterised (Nes et al., 1996; Siezen et al., 1996; Kleerebezem et al., 1997a). Interestingly, in several cases genes encoding two-component regulatory systems were found that were shown to be indispensable for bacteriocin production. When either the gene encoding the sensor protein, which is a histidine kinase, or the gene encoding the response regulator (for a review on these classes of proteins see Parkinson and Kofoid, 1992) were disrupted, the production of bacteriocin stopped or decreased to very low levels in some cases of sensor disruption. It was found in several cases that either the bacteriocin itself or a bacteriocin-like peptide was required as a signal to induce the transcription of target genes. These target genes include the structural gene, the biosynthesis and export genes, the immunity genes and sometimes the regulatory genes themselves. However, clear differences between the systems were also found. In the known cases of lantibiotics up till now, it is the bacteriocin itself which acts as the inducing factor (Kuipers et al., 1995a; Kleerebezem et al., 1997a), whereas in the case of linear bacteriocin production other bacteriocin-like peptides have been found which can induce several genes. In the case of carnobacteriocin production it has been found that both bacteriocin and non-bacteriocin peptides were able to induce transcription (Saucier et al., 1995; Quadri et al., 1997). For overviews on these systems, see Nes et al. (1996), Kleerebezem et al. (1997a), and Table 1.

3. Autoregulation of nisin biosynthesis

One of the best characterised cases of quorum sensing in LAB is provided by the knowledge on nisin biosynthesis (Kuipers et al., 1995b). While developing systems for production of protein-en-

Table 1
Two-component regulatory systems and their inducing peptides in LAB

Genes encoding two-component regulatory systems	
Nisin production in *L. lactis* (*nisRK*)	
Sakacin P production in *L. sake* (*sapKR*)	
Plantaricin production in *L. plantarum* (*plnBCD*)	
Carnobacteriocin in *C. piscicola* (*cnbKR*)	
Inducing peptides	
Modified: nisin	
Unmodified: PlnA, sakacin IF, CbnB2, CbnS	
Bacteriocin: nisin, CbnB2	
Bacteriocin-like: CbnS, sakacin IF, PlnA	

gineered variants of nisin to obtain optimised food-preservatives, a strain defective in the nisin structural gene was found to have lost its capacity to produce the *nisA* transcript (Kuipers et al., 1993). The production of the transcript could be restored by adding minute amounts of nisin to the culture medium during growth (Kuipers et al., 1995a). The auto-regulatory process involved in nisin biosynthesis can be considered as a special form of 'quorum sensing' in *L. lactis* (Kuipers et al., 1995a; Kleerebezem et al., 1997a). The two-component regulatory system, consisting of the response regulator NisR (van der Meer et al., 1993) and the sensor protein, the histidine kinase NisK (Engelke et al., 1994), is triggered by subinhibitory amounts of extracellular nisin (Kuipers et al., 1995a). Once the signal, i.e. the presence of extracellular nisin, is transduced by autophosphorylation of NisK and subsequent phospho-transfer to NisR, transcription of the gene(s) under control of the *nisA* and *nisF* promoters are activated (de Ruyter et al., 1996a). Transcripts comprising *nisA*, *nisABTCIP*, *nisABTCIPRK* and *nisFEG* have been found to be inducible, whereas induction-independent expression of *nisRK* was observed. An outline of the nisin gene cluster is shown in Fig. 1.

4. Nisin-controlled expression (NICE) systems

Based on these findings efficient nisin-inducible gene expression systems have been developed consisting of three essential elements: (1) a Gram-positive strain that expresses the *nisRK* genes to a desired level; (2) nisin or nisin analogs or nisin mutants as inducer molecule; and (3) plasmids containing the *nisA* or *nisF* promoter fragments, followed by convenient cloning sites to introduce the gene(s) of interest (de Ruyter et al., 1996b). The length of these promoter fragments can be as small as the region comprising 40 bp upstream of the transcription start site. The most commonly used strains and plasmids and their characteristics are listed in Table 2.

Strain NZ9700 can serve as a source of nisin to be used as inducer (production level ranging between 10 and 50 mg l^{-1}), but also as a host for constitutive gene expression using the plasmids listed in Table 2. Strains NZ9800 or NZ9000 containing any of the above-mentioned plasmids with a cloned gene of interest will not express this gene to a significant level when no nisin (or nisin analog) is present in the culture medium (Kuipers et al., 1995a; de Ruyter et al., 1996b). However, when nisin is added during log-phase (typically at OD600nm of 0.6) at concentrations ranging between 0.01 and 10 ng ml^{-1} (0.003–3 nM) concentration-dependent induction of the gene(s) of interest will take place. Because strain NZ9800 also expresses the immunity genes *nisIFEG* it can tolerate relatively high concentrations of nisin (up

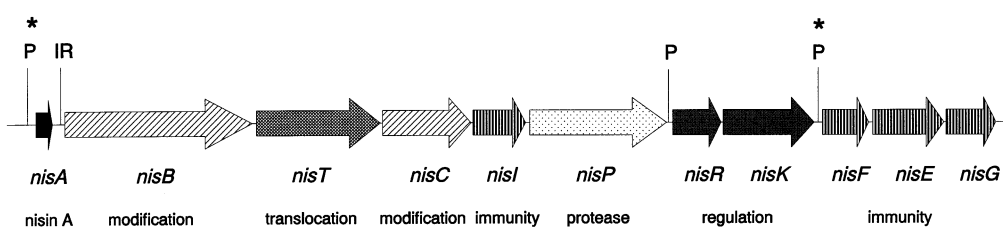

Fig. 1. An outline of the nisin gene cluster. P* denotes an inducible promotor.

Table 2
Strains and plasmids used for nisin inducible gene expression (Based on Kuipers et al., 1993, 1995a; de Ruyter et al., 1996b; Kleerebezem et al., 1997b)

	Characteristic
Strain	
L. lactis NZ9700	Nisin producer (*nisABTCIPRKFEG* present)
L. lactis NZ9800	Derivative of NZ9700; Δ*nisA*
L. lactis NZ9000	MG1363; *pepN*::*nisRK*
Plasmid examples	
pNZ273	Promoter probe vector with *gusA*
pNZ8020	P*nisA* + MCS (transcriptional fusion vector)
pNZ8037	P*nisA* + *Nco*I + MCS (translational fusion vector; *Nco*I removed from *cat*)
pNZ8048	P*nisA* + *Nco*I + MCS + terminator (translational fusion vector)
pNZ9520	*nisRK* in pIL253 (*rep* read-through; high copy number)
pNZ9530	*nisRK* in pIL252 (*rep* read-through; low copy number)
pNZ9500	Part of *nisP* and complete *nisRK* in pUC19ery (for integration purposes)

to 10 mg l^{-1} after pre-adaptation to build up a sufficient immunity level), but usually the maximum induction level is already reached at about 5–10 ng ml^{-1}. Interestingly, this is just below the MIC value found for nisin A and nisin Z against *L. lactis* MG1363, which is approximately 10 ng ml^{-1}, meaning that this concentration is the upper limit for induction of strain NZ9000. The induction efficiency in strain NZ9000 is higher than in strain NZ9800, probably because this latter strain will bind some of the nisin from the medium by NisI or any of the other immunity proteins. An alternative, but non-exclusive, explanation is that the expression level of the integrated *nisRK* genes in strain NZ9000 is higher than in NZ9800, due to upstream promoter sequences in the former case. In practice, this means that NZ9000 is the most sensitive strain for induction. Typically, production of the protein of interest is maximal at 2 h after induction and can reach levels of 60% of total soluble cellular proteins. Some protein products may cause significant growth inhibition after 30′–60′, resulting in lower total yields (e.g. 5% of total soluble intracellular protein). It is worthwhile mentioning that plasmid construction work is most conveniently performed directly in *L. lactis* as a host, since low levels of gene expression have been found to occur in several cases using *Escherichia coli* as an intermediate host, sometimes causing undesirable rearrangements in the expression plasmids.

Several examples of the efficient production of homologous and heterologous proteins have been reported (Kuipers et al., 1997), and in some cases even autolytic proteins could be produced (de Ruyter et al., 1997). Recently, it was established that the NICE system can also be functionally implemented in other lactic acid bacteria than *L. lactis*, i.e. in *Lb. helveticus* and *Leuconostoc lactis*. For this purpose transferable dual plasmid systems were developed, consisting of one plasmid expressing *nisRK* to a specific desired level and the other one containing the nisin-inducible promoter (Kleerebezem et al., 1997b). Inducibility was similar as in *L. lactis*, but the time to reach maximal protein production was significantly longer than in *L. lactis* (Kleerebezem et al., 1997b). The dose-response in three different bacteria is depicted in Fig. 2.

The advantages of the NICE system are several: (1) it is flexible, because of the linear dose-response relationship between inducer concentration and the protein production level; (2) it is low cost, because dilutions of a supernatant of a nisin-producing strain can be used for induction, or nisin-producing strains can be included in the fermentation; (3) it is food-grade, because all elements are derived from *L. lactis*; (4) it gives high production levels, even of toxic proteins; (5) it can be implemented in other Gram-positive bacteria; and (6) it can be used for detection of small amounts of nisin in products by using strains expressing suitable reporter genes and test for inducibility by adding extracts of the unknown sample. Because of these characteristics the NICE system qualifies among the best available induction systems to date for lactic acid bacteria and probably also for other Gram-positive bacteria (de Vos et al., 1997; Kleerebezem et al., 1997b; Kuipers et al., 1997).

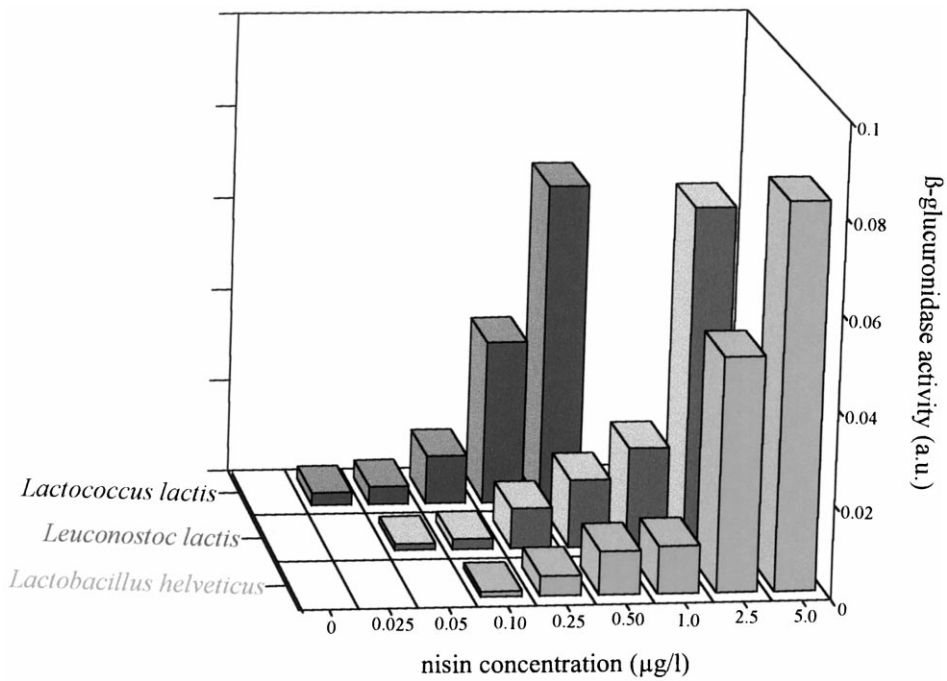

Fig. 2. Nisin-induced β-glucuronidase activities in different LAB species.

5. Controlled lysis of *L. lactis* for accelerated cheese ripening

Several phages from lactic acid bacteria have been found that express specific genes involved in the release of phage particles by the host cell and eventually in lysis. Most commonly, these genes encode phage endolysin and holin proteins. The holins are relatively small proteins which will become embedded in the cellular membrane, thereby forming pores that enable the larger sized endolysins to reach the peptidoglycan layer. Once in this layer the lysins display an effective cell-wall degrading activity which destabilises the cell and ultimately results in lysis. In lactococcal phage ΦUS3 two genes encoding holin and lysin were found, i.e. *lytH* and *lytA*, the latter of which exhibits degrading activity against lactococcal murein (Platteeuw and de Vos, 1992).

An attractive approach to accelerate cheese ripening is to induce lysis of *L. lactis* starter strains for facilitated release of intracellular enzymes involved in flavour formation. Controlled expression of the lytic genes *lytA* and *lytH* was accomplished by application of the food-grade NICE system (de Ruyter et al., 1997). A model of this system is depicted in Fig. 3. It was found that simultaneous production of the lysin and the holin is essential to obtain efficient lysis and concomitant release of intracellular enzymes. Production of the holin alone led to partial lysis of the host cells, whereas production of the lysin alone did not cause significant lysis. However, when the lysin was present externally it caused effective lysis of *L. lactis* and can display in trans activity on other lactococcal starter strains (de Ruyter et al., 1997). These features provide a flexible system suitable for a wide range of applications in dairy starter cultures. Model- and real-cheese experiments for which the inducible holin-lysin overproducing strain was used, showed that a significant increase in the release of intracellular enzymes into the curd relative to the control strain could be achieved. This will eventually result in faster flavour formation and new flavour balances in cheese, which are attractive features for both producers and consumers.

6. Conclusion

Quorum sensing phenomena occur widely in lactic acid bacteria. At present, all cases involve the production of antimicrobial peptides, either lantibiotics or linear peptides. These autoregulatory networks provide an excellent basis for the development of inducible gene expression systems. The earliest developed system in LAB based on quorum sensing is the NICE system. Because of its attractive features and ease of practical use, this system is now being exploited for the functional expression of biotechnologically important proteins (flavour enzymes, transport proteins, biopreservatives, regulatory proteins, etc.) in several different LAB hosts. It also provides a good approach for metabolic engineering endeavours, enabling precise modulation of key enzyme activities, thereby generating the desired metabolic flux-control. The NICE system also allows to make conditional knock-outs in essential genes, by use of rescue plasmids which can express the wild-type gene only when nisin is present in the medium. Moreover, because of the almost negligible expression level of the gene(s) of interest in the uninduced state, also toxic proteins can be produced in high quantities after induction at relatively high cell densities. This feature has been used to create autolytic strains of the important industrial microorganism *L. lactis*, which can be used for facilitated release of enzymes into food products like cheese, leading to accelerated ripening and product diversification methods.

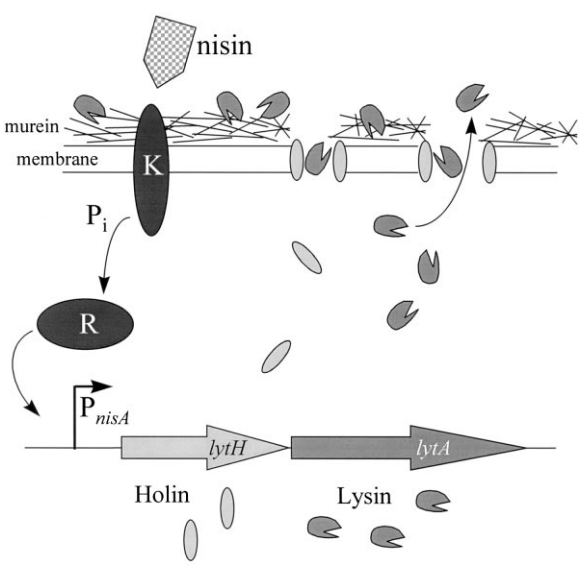

Fig. 3. Production of phage holin and lysin by the NICE system, eventually leading to cell-lysis.

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