MINI-REVIEW

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Production, recovery and purification of bacteriocins from lactic acid bacteria

Received: 29 December 1998 / Received revision: 23 April 1999 / Accepted: 23 April 1999

Abstract Bacteriocins produced by lactic acid bacteria are a heterogeneous group of peptide inhibitors which include lantibiotics (class I, e.g. nisin), small heat-stable peptides (class II, e.g. pediocin AcH/PA1) and large heat-labile proteins (class III, e.g. helveticin J). Many bacteriocins belonging to the first two groups can be successfully used to inhibit undesirable microorganisms in foods, but only nisin is produced industrially and is licensed for use as a food preservative in a partially purified form. This review focuses on the production and purification of class I and class II bacteriocins from lactic acid bacteria. Bacteriocin production is growth associated but the yield of bacteriocin per unit biomass is affected by several factors, including the producing strain, media (carbohydrate and nitrogen sources, cations, etc.) and fermentation conditions (pH, temperature, agitation, aeration and dilution rate in continuous fermentations). Continuous fermentation processes with cell recycle or immobilized cells can result in a dramatic improvement in productivity over batch fermentations. Several simple recovery processes, based on adsorbing bacteriocin on resins or silica compounds, have been developed and can be used to build integrated production processes.

Introduction

Mankind has (consciously or unconsciously) exploited lactic acid bacteria (LAB) for thousands of years in the production of fermented foods because of their ability to produce desirable changes in the taste, flavour and texture and to inhibit pathogenic and spoilage microorganisms. The inhibitory activity of LAB is due to pH decrease, competition for substrates and to a variety of antimicrobial compounds, including bacteriocins. Bacteriocins have been defined as “extracellularly released primary or modified products of bacterial ribosomal synthesis, which can have a relatively narrow spectrum of bactericidal activity, characterized by inclusion of at least some strains of the same species as the producer bacterium and against which the producer strain has some mechanism(s) of specific protection” (Jack et al. 1995). The discovery of nisin, the first bacteriocin used on a commercial scale as a food preservative, dates back to the first half of this century (Rogers 1928; De Vuyst and Vandamme 1994) but research on bacteriocins of LAB has expanded in the last 15 years, prompted by their potential application as natural food preservatives and/or as food grade markers for the development of cloning vectors (Klaenhammer 1988). Bacteriocins have recently been grouped into three classes (class I: lantibiotics; class II: small heat-stable non-lantibiotics; class III: large heat-labile proteins) on the basis of the sequence of the mature peptides and prepeptides (Nes et al. 1996; Table 1).

Nisin (class I) is licensed for use in foods in more than 40 countries and has been produced industrially since 1953 (De Vuyst and Vandamme 1994). The largest producer is Aplin & Barrett Ltd (Dorset, England, a subsidiary of Cultor Food Science, Ardsley, N.Y., USA), which markets a standardized (1 × 10^6 IU g⁻¹) preparation of nisin (Nisaplin). The market price for Nisaplin is 810 DM kg⁻¹. In the current industrial process, pasteurized milk plus added yeast extract is treated with a protease and used as a substrate in batch fermentation at controlled pH and temperature. An extraction process, including a spray-drying step, follows and the resulting powder is standardized with NaCl to 1 × 10^6 IU g⁻¹. Further purification is costly and not commercially viable for food use (Trigg, Aplin and Barrett, personal communication). A standardized (minimum 0.9 × 10^6 IU g⁻¹) nisin preparation (Chrisin) is also marketed by Chr Hansen (Hørsholm, Denmark).
Although several class II bacteriocins have been shown to be effective in the biopreservation of foods (Stiles 1996), none is licensed or marketed as a food additive in a partially purified form. Some undefined antimicrobial products (ALTA 2341, Quest Biotechnology Inc., Sarasota, Fla., USA; Microgard, Rhône-Poulenc, Courbevoie, France) are licensed for food use but no details are available on their composition and production, although it has been reported that ALTA 2341 may contain a class II bacteriocin (Szabo and Cahill 1998).

In this paper the factors affecting the production of class I and II bacteriocins from LAB and the techniques for their recovery and purification are reviewed. Since nisin production was recently reviewed by De Vuyst and Vandamme (1994), only some aspects of nisin production and purification will be addressed.

**Biosynthesis of bacteriocins from LAB**

Bacteriocins are synthesized as pre-propeptides, which are processed and externalized by dedicated transport machinery or by the sec-dependent mechanism (Nes et al. 1996). Table 1 shows the amino acid sequence of the prepeptides of some class I and class II bacteriocins. Cleavage of leader peptides is carried out by specific peptidases or by a proteolytic domain of the dedicated ABC transporter (Nes et al. 1996), which recognize highly conserved sequences in the leader peptide. In addition, the synthesis of lantibiotics, like nisin, requires post-translational modification of selected amino acid residues prior to secretion (De Vuyst and Vandamme 1994). In addition to structural and secretion/modification machinery genes, bacteriocin operons always include genes for specific immunity proteins (Jack et al. 1995; Nes et al. 1996) which protect the producer cells from their own bacteriocins. However, little is known on their mode of action. The genes *nisI* and *nisFEG* have been implicated in nisin immunity. *nisI* is a 32 kDa protein which is postulated to be lipid modified and extracellularly anchored to the membrane (Kuipers et al. 1993). *nisF* and *nisE* encode for an ABC transporter which shows homology with proteins implicated in resistance to subtilin and microcin B17, while the predicted product of *nisG* is a hydrophobic protein which may interact directly with the pore-forming domain of nisin in a way similar to the immunity proteins of colicins (Siegers and Entian 1995). Immunity proteins of class II bacteriocins are usually small (51–150 amino acids) and show a low degree of homology, even when the bacteriocins are closely related or identical. This may suggest that they do not interact directly with the bacteriocins (Nes et al. 1996). However, it has been pointed out that many immunity proteins may actually resemble each other, at least in structure (Eijsink et al. 1998). Some immunity proteins may integrate in the membrane of the producer strain (Axelsson et al. 1993; Fremaux et al. 1993) but many others do not present transmembrane helices (Eijsink et al. 1998). Many bacteriocin operons are regulated by a quorum sensing system (for a review see Nes et al. 1996; Kleerebeem et al. 1997). The extracellular accumulation of an induction factor (IF) is sensed by a two-component signal transduction system consisting of a membrane-located histidine kinase (HK) which phosphorylates a response regulator (RR), which in turn interacts with promoters of structural, biosynthetic and regulatory operons and induces gene expression. The lantibiotic nisin autoregulates its own production (Kuipers et al. 1995; de Ruyter et al. 1996a; Quiao et al. 1996). In *Lactococcus lactis* N8 (a nisin Z producer) both *nisZBTCIPRK* and *nisFEG* operons are induced by nisin (Quiao et al. 1996). In nisin A producers, two inducible promoters are located upstream of *nisA* and *nisF*; a third promoter is located upstream of *nisR* (Kuipers et al. 1995; de Ruyter et al. 1996a). In many class II bacteriocins, the IF, HK and RR are organized in an autoinducible regulatory operon (Nes et al. 1996) and the IF is a bacteriocin-like peptide which may have no inhibitory activity. However, it has recently been found that the IF of the plantaricin operons in *Lactobacillus plantarum* C11, PlnA, has indeed bacteriocin-like activity (Anderssen et al. 1998) and that bacteriocin operons in *Carnobacterium piscicola* LV17B can be induced by *carnobacteriocin* CB2 and by a bacteriocin-like IF (CnbS) whose gene is organized in an operon with *cnbK* (HK) and *cnbR* (RR) (Quadri et al. 1997).

Induction is not the only factor affecting the expression of bacteriocin operons: carbon source regulation and the level of cell-adhered nisin have been shown to affect nisin synthesis (De Vuyst and Vandamme 1992; Meghrous et al. 1992) and catabolite repression has been claimed to operate in the regulation of plantaricin C production (Bárcena et al. 1998). There are also a few reports of induction of bacteriocin production caused by cells and extracts of sensitive strains (Barefoot et al. 1994; Sip et al. 1998).

**Kinetics of bacteriocin production**

Bacteriocin production in LAB is growth-associated: it usually occurs throughout the growth phase; and ceases at the end of the exponential phase (or sometimes before the end of growth: Parente et al. 1997; Lejeune et al. 1998). A decrease of bacteriocin titre usually follows. This may be attributed to adsorption on producer cells or to degradation by specific or non-specific proteases. To our knowledge, the latter has never been proven, while adsorption to cells occurs for most bacteriocins (see for example Meghrous et al. 1992; Yang et al. 1992; Parente et al. 1994; Parente and Ricciardi 1994a; De Vuyst et al. 1996; Lejeune et al. 1998; Chinachoti et al. 1997d). Since adsorption of bacteriocins to cells is maximal at pH 5.5–6.5 (Yang et al. 1992) and decreases at low pH, it is not surprising that no reduction of bacteriocin titre is sometimes observed in fermentations.