THE SUCCESS OF USING BACTERIOCIN-PRODUCING STARTER CULTURES FOR SAUSAGE FERMENTATION IS STRONGLY STRAIN DEPENDENT

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Background

There is considerable interest for the use of bacteriocin-producing lactic acid bacteria (LAB) as novel starter cultures for the meat fermentation industry (Schillinger and Lücke, 1990; McMullen and Stiles, 1996; Hugas, 1997). Bacteriocins produced by LAB are antibacterial peptides or proteins, which can be used as natural preservatives to increase the shelf life and safety of food products (De Vuyst and Vandamme, 1994). Previously, the competitiveness of the bacteriocin-producing strains *Lactobacillus amylovorus* DCE 471, *Lactobacillus sakei* CTC 494, *Enterococcus faecium* CCM 4231 and *E. faecium* RZS C13 has been tested in Spanish-style dry sausage fermentation (Hugas *et al.*, 1995; Callewaert *et al.*, 2000). The strains were tested separately as novel starter culture and their effect on an artificially added *Listeria* population was compared with the effect of a non-bacteriocinogenic control strain. It resulted that *Lb. sakei* CTC 494 was able to lead the fermentation process and to inhibit the *Listeria* contamination to a greater extent than the control strain. Both enterococcal strains were partially competitive in the sausage batter and able to maintain themselves during the fermentation stage, but they disappeared during maturation. However, *Listeria* inhibition was stronger than with *Lb. sakei* CTC 494. On the other hand, *Lb. amylovorus* DCE 471 was immediately overgrown by the indigenous microflora of the sausage batter. Finally, both *Lb. curvatus* LTH 1174 and *E. faecium* RZS C5 seem to be interesting, novel, bacteriocin-producing starter cultures (Leroy, F. & De Vuyst, L.; Messens, W., Verluyten, J., Leroy, F., & De Vuyst, L., unpublished results). Clearly, the success of using bacteriocin-producing starters is strongly strain dependent. This study describes how this observation is related to the effect of some crucial factors prevailing in the sausage environment on the kinetics of cell growth and the lactic acid and bacteriocin production of several LAB.

Objective

The objective of this study was to investigate the suitability of several bacteriocin-producing LAB as novel starter cultures for the production of sausages

Methods

The kinetics of cell growth of and the production of lactic acid by the strains *Lb. sakei* CTC 494, *Lb. curvatus* LTH 1174, *Lb. amylovorus* DCE 471 and *E. faecium* RZS C5 were investigated during fermentation experiments *in vitro*. Fermentations were performed using a computer-controlled Biostat C fermentor (B. Braun Biotech International, Melsungen, Germany) which contained 10 litres of de Man-Rogosa-Sharpe broth (MRS; de Man *et al.*, 1960), that was used as a basic meat simulation medium. Growth (concentration of cell dry mass (CDM) in g l^{-1}), substrate consumption (residual glucose in g l^{-1}), lactic acid production (in g l^{-1}), and bacteriocin activity (in arbitrary units (AU) ml^{-1}) were determined as described elsewhere (De Vuyst *et al.*, 1996*a*,*b*; Leroy and De Vuyst, 1999). In brief, cell dry mass was measured by microfiltration, glucose and lactic acid by High Performance Liquid Chromatography, and bacteriocin activity by a critical dilution method with *Listeria innocua* LMG 13568 or *Lactobacillus delbrueckii* subsp. *bulgaricus* LMG 6901^T (in case of *Lb. amylovorus* DCE 471) as indicator organism. Maximum specific growth rate and specific bacteriocin production were estimated through modelling, as in Leroy and De Vuyst (1999*a*).

Results and discussion

Lb. amylovorus DCE 471, an isolate from corn steep liquor, produces amylovorin L (De Vuyst *et al.*, 1996*b*). The latter is a potent bacteriocin, but with a rather narrow activity spectrum, mainly towards other lactobacilli (Callewaert *et al.*, 1999). The very high activity observed during fermentations in MRS broth (up to 12.8 MAU l^{-1}, De Vuyst *et al.*, 1996*a*,*b*) suggests that *Lb. amylovorus* DCE 471 is able to obtain considerable benefit from its bacteriocin production by inhibiting the other lactobacilli present in the background microflora. However, the strain is outcompeted by the indigenous microflora of the sausage batter (Callewaert *et al.*, 2000). This might be caused by a strong reduction of the bacterial growth rate at temperatures which are typical for the production of European-style fermented sausages (Fig 1a). On the other hand, the strain still grows well at the low pH values that are achieved during sausage fermentation (Fig. 1b).

Lb. sakei CTC 494 and *Lb. curvatus* LTH 1174 are highly competitive in a sausage batter (Vogel *et al.*, 1993; Hugas *et al.*, 1995, 1996; Callewaert *et al.*, 2000). Both strains were isolated from fermented sausage, and are therefore naturally adapted to the sausage environment. It is not unlikely that a large part of this competitiveness is due to the production of their respective bacteriocins, sakacin K and curvacin A. Curvacin A is identical to sakacin K (Axelsson and Holck, 1995; Leroy and De Vuyst, 2000). They both show a strong activity towards many other LAB strains and towards the foodborne pathogen *Listeria monocytogenes* (Vogel *et al.*, 1993; Leroy and De Vuyst, 2000). It seems therefore no coincidence that the conditions of pH and temperature which prevail during sausage fermentation are optimal for the production of sakacin K (Leroy and De Vuyst, 1999a) and curvacin A (Fig 1b, 2b). The use of *Lb. sakei* CTC 494 or *Lb. curvatus* LTH 1174 as starter cultures for fermented sausages resulted in a two log unit decrease in the *Listeria* population at the end of ripening (Hugas *et al.*, 1996). Also, Callewaert *et al.* (2000) found a decrease in the *Listeria* population initially present in the sausage batter from 5.0 to 3.7 log CFU g^{-1} using *Lb. sakei* CTC 494 as starter culture during sausage fermentation. However, based on the high bacteriocin activity obtained in MRS broth under combined pH and temperature

conditions for sausage fermentation (over 3.6 MAU l^{-1}), one would expect an even more drastic decrease. In agreement with earlier observations (Stiles and Hastings, 1991; McMullen and Stiles, 1996), it seems that a bacteriocin producer in a food system is less efficient than under optimal laboratory conditions. This has (at least partially) been explained by the fact that the production of sakacin K is dramatically reduced by the low water activity of the sausage batter, originating from the addition of high amounts of salt (Leroy and De Vuyst, 1999b).

Bacteriocin-producing enterococci turned out to be surprisingly efficient in reducing *Listeria* counts (Callewaert *et al.*, 2000). *E. faecium* RZS C5 grows rapidly at sausage fermentation temperature and produces high amounts of bacteriocin (Fig.1). However, when the pH decreases during sausage fermentation, growth and bacteriocin production are dramatically reduced (Fig. 2). This leaves a possible role for *E. faecium* RZS C5 as a coculture to rapidly inhibit undesirable bacteria in the beginning of the fermentation stage, as suggested before (Callewaert *et al.*, 2000).

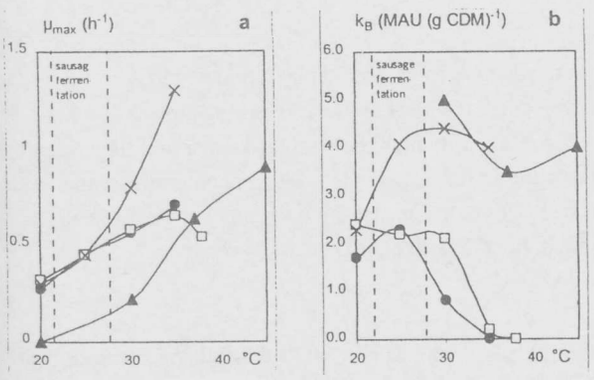

Fig 1. Influence of temperature on (a) the maximum specific growth rate and (b) the specific bacteriocin production of *Lactobacillus sakei* CTC 494 (●), *Lb. curvatus* LTH 1174 (□), *Lb. amylovorus* DCE 471 (▲), and *Enterococcus faecium* RZS C5 (×) at a constant pH 6.2 (in case of *Lb. curvatus* LTH 1174) or pH 6.5 (all other strains).

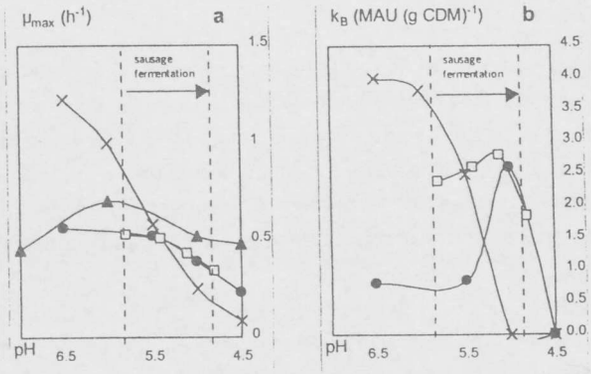

Fig 2. Influence of the pH on (a) the maximum specific growth rate and (b) the specific bacteriocin production of *Lactobacillus sakei* CTC 494 (●), *Lb. curvatus* LTH 1174 (□), *Lb. amylovorus* DCE 471 (▲), and *Enterococcus faecium* RZS C5 (×) at a temperature of 27°C (in case of *Lb. curvatus* LTH 1174), 30°C (in case of *Lb. sakei* CTC 494), 35°C (in case of *E. faecium* RZS C5), or 37°C (in case of *Lb. amylovorus* DCE 471). The data for the specific bacteriocin production of *Lb. amylovorus* DCE 471 have not been determined.

Conclusions

Bacteriocinogenic starter cultures for food fermentations get an additional competitive feature from the production of their bacteriocin(s). As a result they are able to inhibit a part of the competing background flora and hence to offer a better control of the fermentation process. Moreover, bacteriocins of LAB are in many cases particularly active towards spoilage bacteria and foodborne pathogens, such as *L. monocytogenes* and *Staphylococcus aureus*. However, bacteriocin production is no guarantee for success. It is absolutely essential that the strains, which are selected to be used as novel starter cultures, are able to express their ability to produce bacteriocins once they are actually applied in the food matrix.

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