Production of antilisterial bacteriocin by *Pediococcus acidilactici* HA-6111-2 under different stress conditions

Tekla Engelhardt[1] - Helena Albano[2] - Gabriella Kiskó[1] - Csilla Mohácsi-Farkas[1] - Paula Teixeira[2]

[1]Department of Microbiology and Biotechnology, Corvinus University of Budapest
[2]CBQF-Centro de Biotecnologia e Química Fina – Laboratório Associado, Escola Superior de Biotecnologia, Universidade Católica Portuguesa/Porto

Introduction

Nowadays, the application of lactic acid bacteria (LAB) and their bacteriocins are widely used in food industry. Bacteriocin produced by LAB cultures are commonly used as biopreservatives in fermented food products; thus the food industry is interested in stable cultures with stable bacteriocin activity. *Pediococcus acidilactici* is usually isolated from different fermented meat products.

The aim of our study was to investigate how the *P. acidilactici* HA-6111-2 (isolated from alheira) (Albano et al., 2007) produce bacteriocins under different stress conditions: pH, NaCl and temperature.

Methods

Screening of different stress conditions was performed previously in order to select the adequate stress conditions for *P. acidilactici* and for proceeding the further experiments (data not shown).

MRS broth (150 ml) was set up to the chosen stress conditions and inoculated with 1% (v/v) of an overnight culture of *P. acidilactici* and incubated at different temperatures. Changes in pH were recorded in every hour, the cell count was determined in every 3 h for 42 h. Bacteriocin activity (AU/ml) in the cell-free supernatant was recorded in every 3 h for 48 h, as described by Van Reenen et al. (1998). *Listeria monocytogenes* 1486/1 (serogroup IIb, from cheese), *L. monocytogenes* 1604/2 (serogroup IVb, from cheese), *L. monocytogenes* 971 (serogroup IIb, from hamburger) and *L. innocua* NCTC 11288 were used as target strains.

Results and Discussion

Pediococcus acidilactici HA-6111-2 could not grew well at low pH (Fig. 1), the pH and the cell count did not change during 48 h. However, *P. acidilactici* HA-6111-2 could produce moderate amount of PA-1 bacteriocin. The highest activity was 6400 AU/ml. In case of pH 8.5 (Fig. 2) the alkaline adaptation was clearly observed (~ 28 hours). During the adaptation, the pH slightly decreased; at pH 7 the exponential phase started, and the pH rapidly dropped until levels of pH ~ 4.3. The highest bacteriocin production (25600 AU/ml) was observed in the stationary phase.

Previous examinations showed that several LAB strains can rapidly adapt to temperature downshift to about 20 °C below the optimal growth temperature. In contrast, our results showed that at 10 °C, *P. acidilactici* HA-6111-2 could not recover (Fig. 3). However, it was able to produce a low amount of bacteriocin. At 50 °C (Fig. 4), the cells were damaged; after 24 hours we could not detect viable cells, although the bacteriocin activity was detected at low levels until 40 hours. The response of *P. acidilactici* HA6111-2 to elevated temperature can be similar to cold shock response. In our study, we demonstrated that *P. acidilactici* HA6111-2 could not recover and produce low amount of bacteriocins under both cold and heat stresses.

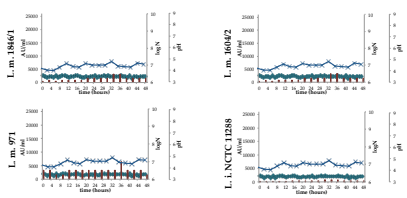

Fig. 1 Production of PA-1 bacteriocin *Ped. acidilactici* in MRS broth (pH 3.5) at 30 °C against *Listeria* strains. Antimicrobial activity of cell-free supernatants showed in AU/ml (bars), logN (X) and pH (■) also indicated.

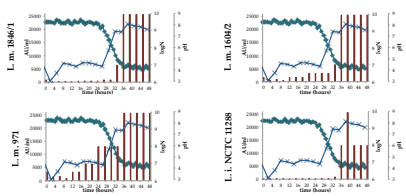

Fig. 2 Production of PA-1 bacteriocin *Ped. acidilactici* in MRS broth (pH 8.5) at 30 °C against *Listeria* strains. Antimicrobial activity of cell-free supernatants showed in AU/ml (bars), logN (X) and pH (■) also indicated.

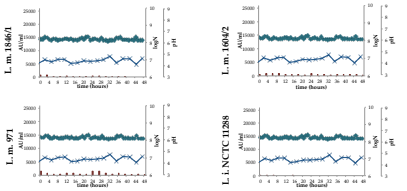

Fig. 3 Production of PA-1 bacteriocin *Ped. acidilactici* in MRS broth at 10 °C against *Listeria* strains. Antimicrobial activity of cell-free supernatants showed in AU/ml (bars), logN (X) and pH (■) also indicated.

Under 7.5% NaCl (Fig. 5), viable cell number of *P. acidilactici* HA-6111-2 was slightly increased and the pH had a low decrease after 20-24 hours. *P. acidilactici* HA-6111-2 was able to show the highest bacteriocin activity (6400 AU/ml) after 30-33 hours. Adaptation of bacteria to osmotic stress can be explained by accumulation, synthesis and transport of certain solutes to restore turgor.

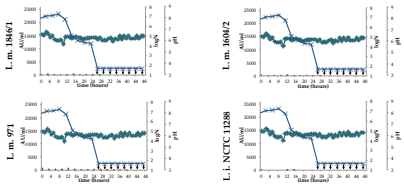

Fig. 4 Production of PA-1 bacteriocin *Ped. acidilactici* in MRS broth at 50 °C against *Listeria* strains. Antimicrobial activity of cell-free supernatants showed in AU/ml (bars), logN (X) and pH (■) also indicated. Arrows indicate values below the detection limit.

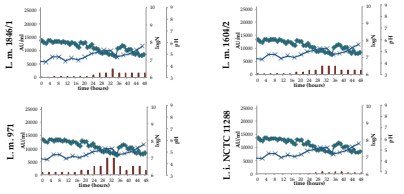

Fig. 5 Production of PA-1 bacteriocin *Ped. acidilactici* in MRS broth supplemented with 7.5% NaCl at 30 °C against *Listeria* strains. Antimicrobial activity of cell-free supernatants showed in AU/ml (bars), logN (X) and pH (■) also indicated.

Conclusions

All of the results indicated that *L. monocytogenes* 971 (serogroup IIb, from hamburger) was more sensitive to PA-1 bacteriocin than *L. innocua* NCTC 11288. It was demonstrated that under stress conditions, *P. acidilactici* HA-6111-2 could produce antilisterial PA-1 bacteriocin. The bacteriocin activity was dependent on the different stress conditions. Further studies will investigate why *P. acidilactici* HA-6111-2 can not recover and produce PA-1 bacteriocin under cold and heat stresses. For the use of *P. acidilactici* HA-6111-2 as starter culture in the fermented meat industry, it would be also interesting to determine the response to high hydrostatic pressure.

References

Albano, H., Todorov, S.D., van Reenen, C.A., Hogg, T., Dicks, L.M.T. and Teixeira P. (2007): Characterization of a bacteriocin produced *by Pediococcus acidilactici* isolated from "Alheira", a fermented sausage traditionally produced in Portugal. *International Journal of Food Microbiology* 116, 239–247.

Van Reenen, C.A., Dicks, L.M.T. and Chikindas, M.L. (1998): Isolation, purification and partial characterization of plantaricin 423, a bacteriocin produced by *Lactobacillus plantarum*. *Journal of Applied Microbiology* 84, 1131–1137.

Acknowledgements

This work was supported by National Funds from FCT – Fundação para a Ciência e a Tecnologia through projects PEst-OE/EQB/LA0016/2011 and SFRH/BPD/47826/2008 Post-doc grant to Helena Albano. The work of T. Engelhardt was supported by the Campus Hungary program in the frame of TÁMOP 4.2.4.B/1-11/1 and TÁMOP 4.2.4.B/2-11/1 project