Evaluation of (GTG)₅-PCR fingerprinting for characterization of enterococci isolated from bryndza cheese

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Introduction

Enterococci are natural commensals inhabiting the gastrointestinal tract of different organisms, but they can be isolated even from the environment as well as from food. They are a common part of many dairy products and certain enterococcal strains are used as probiotics for their beneficial effect in the human digestive tract. Bryndza is a natural product representing a complex ecosystem containing bacteria, fungi and yeasts because no pasteurization or other thermal treatment is applied during its production. Enterococci together with lactococci and lactobacilli are the most abundant lactic acid bacteria representing an important part of bacterial microflora of this dairy product.

rep-PCR methods including (GTG)₅-PCR fingerprinting are easy to perform molecular typing tools that have been applied in taxonomical studies dealing with various bacterial groups. This short study focused on evaluation of the (GTG)₅-PCR fingerprinting for strain typing of enterococci isolated from Bryndza cheese.

Materials and methods

Bacterial strains representing E. faecium (n = 76), E. faecalis (n = 25) and E. durans (n = 10) were isolated from Bryndza cheese from two bryndza-processing manufactories (L and T) during three monthly samplings performed successively (April, May and June) in 2000 and 2001. Isolated strains were characterized by biotyping, ITS-PCR, dslB₅-PCR and dslJ₀-PCR assays and selected strains (assigned by asterisks in dendrograms) were characterized by whole-cell protein profile analysis by SDS-PAGE. Strain designation in dendrograms should be read as follows: strain T/A/9-01 = manufactory/sampled in month/year. Similarity between samples in the dendrograms was calculated using Pearson correlation coefficients using UPGMA clustering method.

Results and discussion

(GTG)₅-PCR fingerprinting was performed as described by Gevers et al. (2001; FEMS Microbiol. Lett. 205, 3136). Briefly, the (GTG)₅-PCR with whole genomic enterococcal DNAs and the (GTG)₅-PCR, primer (GTGTTGGTTGGTTGGTG)₃ was performed at the following amplification conditions: an initial denaturation step at 95°C for 7 min was followed by 30 cycles (1 min at 94°C + 1 min at 40°C + 8 min at 60°C) and final elongation for 16 min at 65°C. Obtained PCR products were separated in 1.5 % agarose gel electrophoresis in 1x TAE buffer at 55 V for 960 min and visualized after staining with ethidium bromide under UV light. Obtained fingerprints were digitalized and analysed using GelCompar II software (Applied Maths, Belgium). Numerical analysis of obtained patterns and dendrogram construction was calculated with Pearson correlation coefficients using UPGMA clustering method.

Conclusions

1. The (GTG)₅-PCR fingerprints revealed by E. faecium, E. faecalis and E. durans strains were clearly different and allowed us to separate individual taxa.
2. E. faecium strains were split into two clearly separated groups.
3. No clear correlation between the strain origin and the resulting (GTG)₅-PCR fingerprint was revealed.
4. Based on the obtained results it can be concluded that rep-PCR fingerprinting with the (GTG)₅-PCR primer is not a convenient tool for intraspecies typing of E. faecium, E. faecalis and E. durans.

A more exhaustive study dealing with evaluation of the (GTG)₅-PCR for identification of enterococci including representatives of all enterococcal species as well as enterococcal strains from different environments has been published recently (Gevers Microbiol. Lett. 205, 3136).

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*Fig. 1. Dendrogram based on cluster analysis of the (GTG)₅-PCR fingerprints obtained from E. faecium strain.

*Fig. 2. Dendrogram based on cluster analysis of the (GTG)₅-PCR fingerprints obtained from E. faecalis strain.

*Fig. 3. Dendrogram based on cluster analysis of the (GTG)₅-PCR fingerprints obtained from E. durans strain.