RANDOMLY AMPLIFIED POLYMORPHIC DNA (RAPD) FOR RAPID TYPING OF LACTIC ACID STARTER CULTURES APPLIED IN FERMENTED SAUSAGES.

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BACKGROUND

Starter cultures from lactic acid bacteria play an important role in meat industry to ensure high quality, reduce variability and enhance organoleptic characteristics in sausage production. In order to be able to follow these different strains during fermentation and curing and distinguish one from another, it is very important to find a rapid way to easily identify each strain. RAPD is a PCR-based method and it has been used to compare intra- and interspecific differences in bacteria (Welsh and McClelland, 1990). By reducing the stringency of the primer annealing step, a primer that has no known homology to a genome can anneal at sites for which the match is imperfect and allow certain regions of the genome to be amplified, giving rise to artificial fingerprinting when resolving on agarose gels.

OBJECTIVE

The aim of this study was to evaluate the typing potential of RAPD on lactic acid bacteria isolated from fermented sausages and used as starter cultures.

MATERIAL AND METHODS

Strains

The strains used in this assay were: *Lactobacillus sakei* CTC41; CTC232; CTC232 (pNZ12); CTC284; CTC287; CTC329; CTC335; CTC372; CTC423; CTC494; CTC430; CTC460; *Lact.curvatus* CTC243; CTC435; CTC435 (pNZ12); CTC371; *Lact.plantarum* CTC305, *Enterococcus .faecium* CTC492; *Pediococcus spp.* CTC706 (CTC-IRTA collection, Meat Technology Center, Monells, Spain); *Lact.curvatus* LTH1174; *Lact.sakei* LTH673; LTH2799 (LTH collection, Hohenheim University. Institut für Lebensmitteltechnologie. Stutgart. Germany); *Lact.sakei* Lb706 (Schillinger and Lücke, 1991) and *Lact.sakei* MI401 (Larsen et al., 1993). Apart from MI401, all the strains were isolated from fermented sausages (Spanish or German). Two of them CTC706 and LTH2799 are comercial starter cultures. Cultures were aerobically grown overnight at 30ºC in MRS.

DNA preparation

Total DNA was isolated from overnight cultures according to Anderson and McKay (1983).

Crude extracts

Crude extracts from agar colonies or directly from a filtered sausage sample diluted 1:10 in ID (peptone, 1.0 $g.l^{-1}$ and NaCl, 8.5 $g.l^{-1}$) were obtained after treatment with 50% triton X-100, heated to 100ºC 10 min and cooled to room temperature.

PCR amplification

Samples of 25 µl containing either 20 ng of purified DNA or 1 µl of the crude extract were carried out in a thermal cycler (Perkin Elmer 2400). Six different primers were used at a concentration of 25 pmol: Primer 1: 5'-d[GGTGCGGGAA], primer 2: 5'-d[GTTTCGCTCC], primer 3: 5'-d[GTAGACCCGT], primer 4: 5'-d[AAGAGCCCGT], primer 5: 5'-d[AACGCGCAAC], primer 6: 5'-d[CCCGTCAGCA]. Pharmacia Ready to go RAPD-Pharmacia Kit containing 1.5 mM $MgCl_2$ in the buffer solution and a mix of Taq polimerase and Stoffel fragment was used. For effectivity comparison the Gibco PCR mix was also used, containing the same buffer but only 0.5 U Taq polimerase. Reaction mix was cycled through the following temperature profile: 1 cycle at 94ºC 5 min, 40 cycles at 94ºC 30s, 36.5ºC 30 s and 72ºC 30 s. The PCR was terminated at 72ºC for 7 min and cooled to 4ºC.

Gel electrophoresis

2 µl of each reaction sample was runned in an 2% agarose horizontal gel during 3 hours at 150V. 1Kb marker was used as a molecular weight marker (Gibco-BRL).

Patterns comparison and numerical analysis

Electrophoresis band patterns were collected by the Gelstation (TDI). The data were normalized and processed using the Lane Manager 2.1 program (TDI). The densitometric traces were analysed by the RAPD coefficient (Clark and Lanigan, 1993) and the unweighed pair group method with arithmetic averages (UPGMA, Romersburg, 1984) and compilated in a dendogram (Sneath and Sokal, 1973)

RESULTS AND DISCUSSION

Purified DNA amplified with a mix of Taq polimerase and Stoffel fragment (Ready to go RAPD kit, Pharmacia) gave larger number of bands when compared with Taq DNA polimerase alone (Gibco PCR supermix). With Gibco PCR supermix only the strongest band was kept (data not shown). Thus the rest of the experiments were done with Ready to go RAPD Kit (Pharmacia). Stoffel fragment is said to favour the creation of smaller PCR products with enhanced reproducibility (Sobral and Honeycutt, 1993).

Crude extract from overnight colonies or sausage samples treated with TritonX100 gave different PCR patterns when compared with purified DNA according to Johansson et al (1995). Future assays are in progress in order to test its reproducibility.

In strain *Lb.sakei* CTC494 the different primers gave patterns with a number of bands ranging from 6 to 12. We selected primer 5 for its ability to amplify larger number of bands while diferenciating the different strains tested. Primer 3, 4, 6 had not enough discriminatory power.

The different electrophoretic patterns were numerically analyzed in a dendogram using RAPD distance index. Two different clusters with an homology of 65.3% were easily made diferentiating the *Enterococcus* and the *Pediococcus* strains (group 2) from the rest of the *Lactobacillus* tested (group 1). In the group I different subclusters with an homology of 84% could be made, subcluster 1a included several strains of *Lact sakei* and one *Lact. curvatus* and subcluster 1b, the rest of *Lact.sakei, Lact.curvatus* and the strain of *Lb. plantarum*. The allocation of species in each cluster agrees with the close phylogenetic relationship between species of *Lact.curvatus* and *Lact.sakei*. Only one strain, CTC460 was not able to be amplified (figure 3).
As a control of reproducibility two different samples of CTC494 were run in two different gels (figure 1 and 2). The differences were minimal, only a 0.07% of error. Considering this minimal error sample, all the strains assayed were able to be differentiate one from another with the exception of the following pairs: CTC329 from CTC287, CTC232 from CTC41 and CTC435 from its pNZ12 transformant (figure 3).

CONCLUSIONS

RAPD technique, when using the conditions described in this study, is a rapid way to generate fingerprinting that practically allowed typing of all the lactic acid bacteria strains studied.

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Fig 1. RAPD band patterns obtained from: CTC41 (lane 1), CTC232 (2), CTC232 (pNZ 12) (3), CTC243 (4), CTC284 (5), CTC287 (6), CTC305 (7), CTC329 (8), CTC335 (9), CTC371 (10), CTC372 (11), CTC423 (12), CTC435 (13), CTC435 (pNZ12) (14), CTC494 (15), 1Kb marker (16).

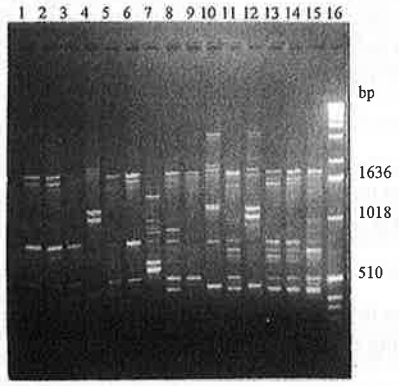

Fig 2. RAPD band patterns obtained from: CTC430 (lane 1), CTC460 (2), CTC492 (3), MI401 (4), LTH673 (5), LTH1174 (6), LTH2799 (7), Lb706 (8), CTC706 (9), CTC494 (10), 1Kb marker (11).

Fig 3. Dendogram obtained by using RAPD on purified DNA from lactic acid bacteria strains followed by evaluation using RAPD distance index.