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# DNA Probe for *Lactobacillus delbrueckii*

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**From a genomic DNA library of *Lactobacillus delbrueckii* subsp. *bulgaricus*, a clone was isolated which complements a leucine auxotrophy of an *Escherichia coli* strain (GE891). Subsequent analysis of the clone indicated that it could serve as a specific DNA probe. Dot-blot hybridizations with over 40 different *Lactobacillus* strains showed that this clone specifically recognizes *L. delbrueckii* subsp. *delbrueckii*, *bulgaricus*, and *lactis*. The sensitivity of the method was tested by using an α-$^{32}$P-labeled DNA probe.**

*Lactobacillus delbrueckii* subsp. *delbrueckii*, *bulgaricus*, and *lactis* are important organisms for food fermentation. *L. delbrueckii* subsp. *bulgaricus* and *lactis* are predominantly found in fermented milk products and are used as starter cultures for yogurt and cheese production, whereas *L. delbrueckii* subsp. *delbrueckii* is mainly found in vegetable fermentations. Fermentation and maturation of these food products, however, are not only due to growth of these bacteria but result from growth association and interaction of different lactobacillus strains with each other and with other bacteria, e.g., lactococci, lactic streptococci, propionibacteria, and others. Most of these different bacterial strains have very similar nutritional requirements and grow under similar environmental conditions. Therefore, a clear identification into species, especially within the genus lactobacillus, may sometimes be very difficult. Currently, identification of some of these species is tedious and involves criteria such as sugar fermentation patterns, bacteriophage resistance, and acid production.

Recently, DNA hybridization techniques with specific DNA probes for the identification of bacterial and viral strains have been developed. Up to now, the main emphasis in constructing such DNA probes was to have quick and reliable tools to identify pathogenic material in clinical diagnosis. Thus, DNA probes have already been used for the identification of *Plasmodium falciparum* (1), *Yersinia enterocolitica* (6), *Salmonella typhi* (14), *Bacillus subtilis* (7), *Haemophilus influenzae* (10), and other microorganisms and of DNA viruses (2, 16, 17) and RNA viruses (4, 9). For the genus *Lactobacillus*, a probe for *L. curvatus* (12), which is specifically associated with spoilage of vacuum-packed meats (13), has been reported. It certainly would be of use in the dairy industry to have a method to identify and classify rapidly and unambiguously relevant strains in fermentation processes. In this report, we describe the isolation of a specific DNA probe which can be used in hybridization procedures to specifically identify strains belonging to the *L. delbrueckii* species.

*Escherichia coli* strains used in this study were grown in Luria broth (8), lactobacilli and propionibacteria were grown in MRS broth (3), and lactococci were grown in M17 broth supplemented with 0.5% glucose (18). For plating, media were solidified by the addition of agar to a final concentration of 1.2% (wt/vol). Chromosomal DNA from lactobacillus, lactococcus, and propionibacteria was prepared as follows. Cells were diluted from overnight cultures into 10 ml of fresh medium supplemented with 1% lactose and grown to the mid-log phase at 43°C. They were then harvested by centrifugation, washed once in cold 1 M NaCl, incubated for 1 h at 37°C in the presence of proteinase K (250 μg/ml) and pronase E (500 μg/ml). Cells were washed in TE (10 mM Tris hydrochloride [pH 7.4], 1 mM EDTA) and treated with mutanolysin (200 μg/ml) in TE for 1 h at 37°C. Sodium dodecyl sulfate, EDTA, and proteinase K were added to final concentrations of 0.1%, 75 mM, and 200 μg/ml, respectively, and the mixture was incubated for 4 h at 65°C. The DNA was extracted with phenol, precipitated with ethanol, and spooled out on a sterile toothpick. Following this, the DNA was dissolved in TE in the presence of RNase A (200 μg/ml), chloroform extracted, reprecipitated in ethanol, and spooled again onto a toothpick. The purified DNA was dissolved in 100 μl of TE. Plasmid DNA from *E. coli* was isolated and purified on CsCl gradients (11). Chemicals were purchased from E. Merck Chemicals Inc. (Darmstadt, Federal Republic of Germany), and the enzymes were from Sigma Chemical Co. (St. Louis, Mo.).

A clone bank of *Eco*RI fragments of chromosomal DNA from *L. delbrueckii* subsp. *bulgaricus* type strain N123 was established in vector YRP17 (5). The *Eco*RI clone bank was transformed into the leucine-auxotrophic *E. coli* GE891 (G. Eggertsson, Institute of Biology, University of Iceland, Reykjavik [unpublished data]). One of the clones isolated in this way was pY85. This particular clone was found to complement the *leu-291* lesion of strain GE891.

Genomic Southern blots (15) of restriction enzyme-cleaved N123 DNA probed with the *Eco*RI fragment of pY85 showed that we had isolated a single-copy sequence (Fig. 1). We furthermore observed a restriction fragment length polymorphism between N123 and *L. delbrueckii* subsp. *delbrueckii* N8 with the enzyme *Hin*dIII. The observation that our probe did not hybridize with all the lactobacillus strains we used in the experiments prompted us to test further lactobacilli. We therefore tested pY85 against different representatives of the *Lactobacillus* genus and some other lactic acid bacteria with dot-blot hybridizations. Samples of 200 ng of chromosomal DNA in TE were denatured by heating for 5 min at 95°C. 20× SSC (1× SSC is 0.15 M NaCl plus 0.015 M sodium citrate) was added to the samples to give a final concentration of 16× SSC. The mixture was spotted onto 20× SSC-wetted GeneScreenPlus paper and rinsed once with 20× SSC. A Bio-Rad dot-blot apparatus was used. The filter was then ready for DNA hybridization, applying standard procedures for hybridization with 6× SSC at 65°C and a subsequent wash with 0.1× SSC at 65°C (11). The probe for these tests was the *Eco*RI fragment of pY85, labeled by

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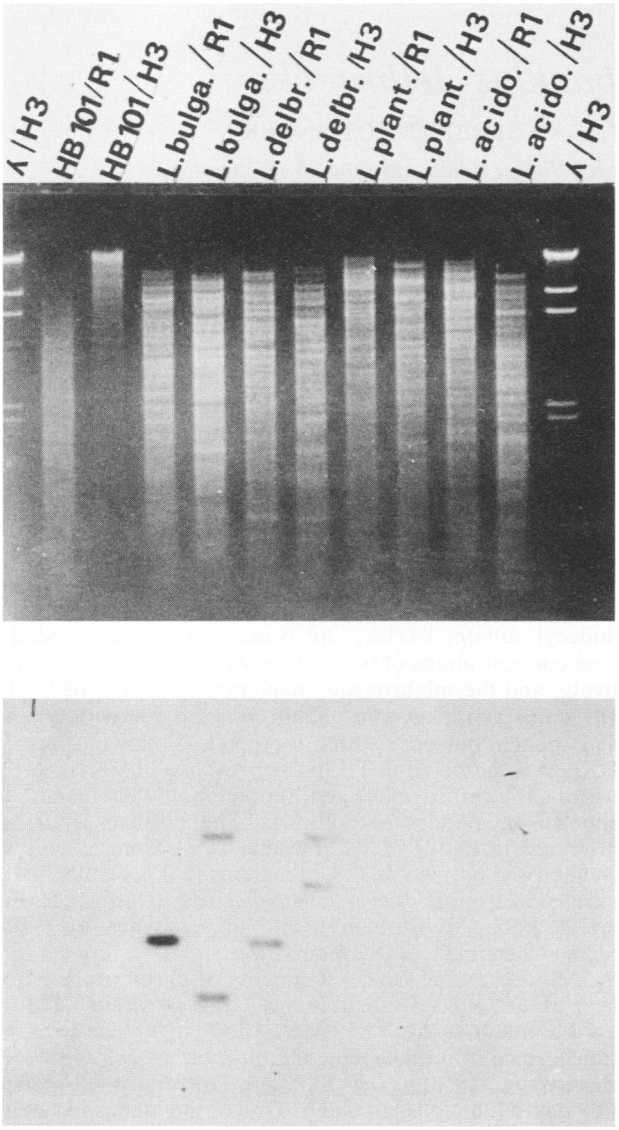


FIG. 1. Southern blot of genomic DNA. Upper panel shows ethidium bromide-stained 0.8% agarose gel of *Eco*RI (R1)- and *Hin*dIII (H3)- digested genomic DNA of *E. coli* HB101, *L. delbrueckii* subsp. *bulgaricus* N123, *L. delbrueckii* subsp. *delbrueckii* N8, *L. plantarum* N24, and *L. acidophilus* N12. Molecular weight marker is lambda *Hin*dIII digest. Lower panel shows Southern blot of above gel, using *Eco*RI fragment of pY85 as the probe.

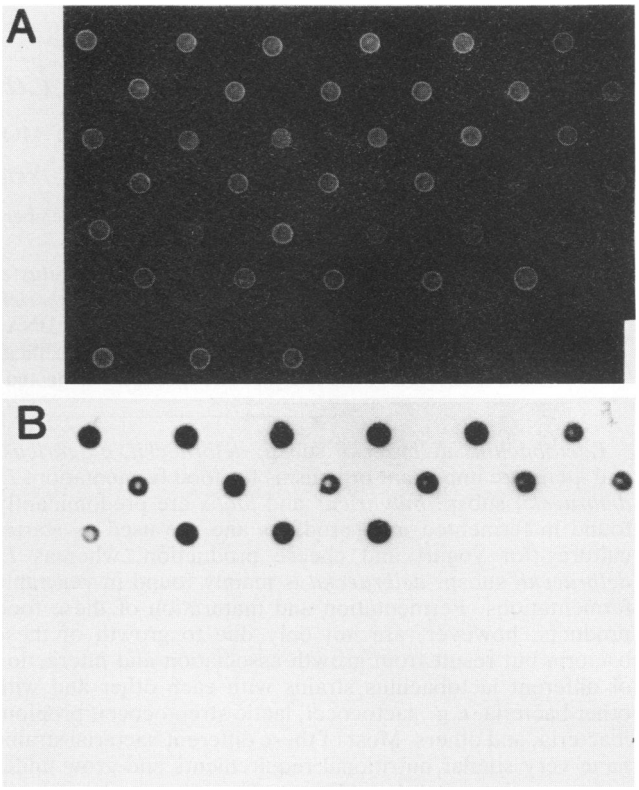


FIG. 2. Dot-blot hybridization. (A) Each 200 ng of purified chromosomal DNA was spotted onto a 1% agarose gel plate containing 0.5 μg of ethidium bromide per ml, and the DNA was visualized under UV light. (B) The same amount of DNA as in panel A was processed for dot-blot analysis. An $\alpha$-$^{32}$P-labeled *Eco*RI fragment of pY85 was used as a probe. Strains used were as follows (from left to right and top to bottom): N123, LB1, LB2, LB6, LB9, LB12, LB32, LB34, LB57.1, LB81.4, LB92.9, N9, N62, N8, N187, LD1, N2, N2, LB14, LB20, N7, N27, N6, N213, N25, N24, N207, N26, N12, N211, SL9, ST1, PP13, PP21, and HB101. The last line is a DNA concentration standard for the ethidium bromide agarose plate with 500, 250, 125, 62.5, 31.3, and 15.6 ng of pUC18 DNA, respectively.

fill-in replacement DNA synthesis with $^{32}$P. Some of these results are shown in Fig. 2, and a summary is presented in Table 1. In addition, we tested hybridization under less stringent washing conditions. After washing the filter in 2× SSC at 20°C, we were only able to detect a hybridization signal with DNA from the *L. delbrueckii* species.

Hybridization results showed that the insert of pY85 specifically hybridizes to DNA from the *L. delbrueckii* subsp. *delbrueckii*, *bulgaricus*, and *lactis* strains alone. All other strains tested from different species of lactobacillus, lactococcus, and propionibacteria were negative. Thus, the insert of pY85 proved to be a genetic probe specific for the *L. delbrueckii* species. It is interesting that four strains classified as *L. delbrueckii* subsp. *bulgaricus* by the American Type Culture Collection (Rockville, Md.) did not show up as belonging to the *L. delbrueckii* species with our test. Classical analysis of these four strains by using API test, acidification, and phage resistance indicates that these four strains may in fact be members of the *L. helveticus* species (R. Moreton and B. Marchesini, personal communication).

To test the sensitivity of our dot blots, we made serial dilutions of chromosomal DNA from some of the tested strains and hybridized them with the pY85 *Eco*RI fragment. We could easily detect a positive signal at 12 ng of total chromosomal DNA for *L. delbrueckii* subsp. *bulgaricus* and *lactis*. The probe hybridized less strongly to *L. delbrueckii* subsp. *delbrueckii* DNA. However, a clear positive signal could be observed at DNA levels of 50 ng per spot and higher. All other tested strains did not show hybridization at significantly higher DNA levels (Fig. 3).

The fragment isolated on pY85 originates from the genome of *L. delbrueckii* subsp. *bulgaricus* and was found to be well conserved among the different strains of the *L. delbrueckii* species. Further characterization shows that it presumably carries a structural gene, which is responsible for the complementation of the *leu* lesion in *E. coli* GE891 (unpublished

TABLE 1. Bacterial strains

| CRN[a] code | Source | Species | Signal[b] with pY85 |
|---|---|---|---|
| LB1 | Our collection | *Lactobacillus bulgaricus* | + |
| LB2 | Our collection | *Lactobacillus bulgaricus* | + |
| LB6 | Our collection | *Lactobacillus bulgaricus* | + |
| LB9 | Our collection | *Lactobacillus bulgaricus* | + |
| LB12 | Our collection | *Lactobacillus bulgaricus* | + |
| LB32 | Our collection | *Lactobacillus bulgaricus* | + |
| LB34 | Our collection | *Lactobacillus bulgaricus* | + |
| LB57.1 | Our collection | *Lactobacillus bulgaricus* | + |
| LB81.4 | Our collection | *Lactobacillus bulgaricus* | + |
| LB92.9 | Our collection | *Lactobacillus bulgaricus* | + |
| N52 | NCDO 1006 | *Lactobacillus bulgaricus* | + |
| N95 | NCDO B15 | *Lactobacillus bulgaricus* | + |
| N96 | NCDO B19 | *Lactobacillus bulgaricus* | + |
| N123 | NCDO 1489 | *Lactobacillus bulgaricus*[T][c] | + |
| N124 | ATCC 21815 | *Lactobacillus bulgaricus* | + |
| N141 | Piacenza CO14 | *Lactobacillus bulgaricus* | + |
| N5 | ATCC 12315 | *Lactobacillus lactis*[T] | + |
| N9 | Liebefeld 125 | *Lactobacillus lactis* | + |
| N62 | NCDO 270 | *Lactobacillus lactis* | + |
| N8 | NCIB 8130 | *Lactobacillus delbrueckii*[T] | + |
| N187 | ATCC 9649 | *Lactobacillus delbrueckii* | + |
| LD1 | Our collection | *Lactobacillus delbrueckii* | + |
| LB14 | Our collection | *Lactobacillus helveticus* | − |
| LB15 | Our collection | *Lactobacillus helveticus* | − |
| LB20 | Our collection | *Lactobacillus helveticus* | − |
| N2 | ATCC 15009 | *Lactobacillus helveticus*[T] | − |
| N6 | NCDO 87 | *Lactobacillus helveticus* | − |
| N106 | NCDO 2395 | *Lactobacillus helveticus* | − |
| N213 | Piacenza b50 | *Lactobacillus helveticus* | − |
| N122 | ATCC 11977 | ATCC: *Lactobacillus bulgaricus* | − |
| N125 | ATCC 27558 | ATCC: *Lactobacillus bulgaricus* | − |
| N126 | ATCC 33409 | ATCC: *Lactobacillus bulgaricus* | − |
| N7 | NCDO 1750 | *Lactobacillus fermentum* | − |
| N27 | ATCC 393 | *Lactobacillus casei*[T] | − |
| N25 | ATCC 4005 | *Lactobacillus buchneri* | − |
| N24 | ATCC 8041 | *Lactobacillus plantarum* | − |
| N207 | ATCC 27865 | *Lactobacillus maltaromicus* | − |
| N26 | ATCC 14869 | *Lactobacillus brevis* | − |
| N12 | ATCC 4356 | *Lactobacillus acidophilus* | − |
| N211 | DSM 20016 | *Lactobacillus reuteri*[T] | − |
| N50 | ATCC 12278 | *Lactobacillus* sp. | − |
| N51 | ATCC 13866 | *Lactobacillus* sp. | − |
| SL9 | Our collection | *Lactococcus lactis* | − |
| ST1 | Our collection | *Lactococcus thermophilus* | − |
| PP13 | Our collection | *Propionibacterium shermanii* | − |
| PP21 | Our collection | *Propionibacterium freudenreichii* | − |
| HB101 | Our collection | *Escherichia coli* | − |

[a] Nestlé Research Centre.
[b] By dot-blot hybridization.
[c] T, Type strain.

observation). Usually, essential protein-coding genes are well-conserved regions of DNA within a species. Codon redundancy and the observed differences in codon usage of species, however, result in very little DNA sequence conservation of such essential genes between species. This is presumably the reason why the pY85 insert is such a specific probe for the *L. delbrueckii* species. The different degree of hybridization of our DNA probe on the Southern blots and dot blots to *L. delbrueckii* subsp. *delbrueckii* in comparison with *L. delbrueckii* subsp. *bulgaricus* and *lactis* may be due to small variations between the target DNA sequence of the *L. delbrueckii* subsp. *delbrueckii* strains and pY85.

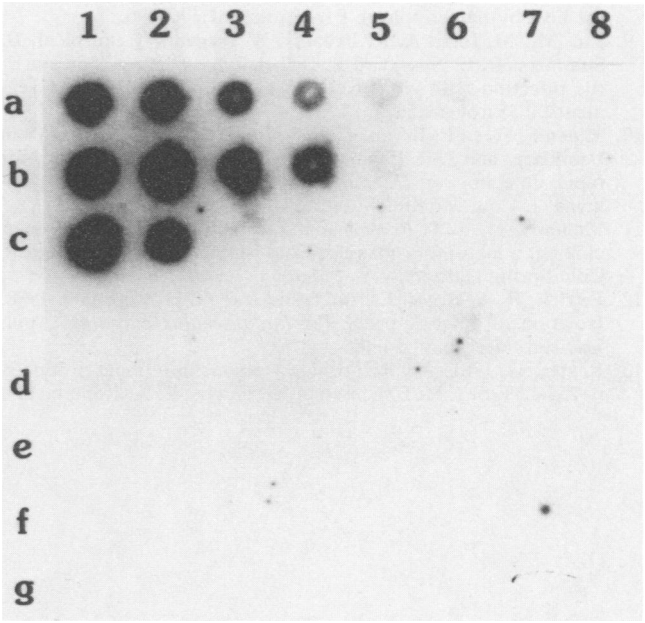


FIG. 3. Dot-blot hybridization. Serial dilutions of chromosomal DNA of N123 (a), N5 (b), N8 (c), N2 (d), N24(e), N12 (f), and N27 (g) were spotted onto GeneScreen paper. DNA quantities spotted were as follows: lines a, b and c, lanes 1 to 8, 100, 50, 25, 12.5, 6.25, 3.13, 1.56, and 0.78 ng, respectively; lines d, e, f, and g, lanes 1 to 8, 1,600, 800, 400, 200, 100, 50, 25, and 12.5 ng, respectively. Labeled *Eco*RI fragment of pY85 was used as a probe.

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