7994

APPLIED AND ENVIRONMENTAL MICROBIOLOGY, Aug. 1998, p. 3096–3098 Vol. 64, No. 8
0099-2240/98/$04.00+0
Copyright © 1998, American Society for Microbiology. All Rights Reserved.

Differentiation of Dextran-Producing *Leuconostoc* Strains by a Modified Randomly Amplified Polymorphic DNA Protocol

SCOTT M. HOLT[1]* AND GREGORY L. COTE[2]

Department of Biological Sciences, Western Illinois University, Macomb,[1] and Biopolymer Research Unit, National Center for Agricultural Utilization Research, USDA Agricultural Research Service, Peoria,[2] Illinois

Received 26 January 1998/Accepted 1 June 1998

Seven dextran-producing *Leuconostoc* strains were differentiated by using a modified randomly amplified polymorphic DNA (RAPD) protocol that incorporated specific primers designed from conserved regions of dextransucrase genes. RAPD profiles showed intraspecies differences among the *Leuconostoc mesenteroides* strains tested. This modified RAPD protocol will aid in the differentiation of polymer-producing leuconostocs, which are currently distinguished by time-consuming analyses of the dextrans they synthesize.

Leuconostocs are heterofermentative lactic acid bacteria that can produce extracellular polymers such as alternan, dextran, and levan from sucrose metabolism (2, 4). Dextrans are high-molecular-weight homopolymers composed of α-D-glucose with predominantly α-(1→6) linkages and have been used in a variety of commercial applications (2). Dextrans are structurally diverse and are characterized according to the percentage, nature, and distribution of their non-α(1→6) linkages (5). The exact molecular structure of a dextran is determined by the specific *Leuconostoc* strain that synthesizes the polymer (2, 5). Many *Leuconostoc* strains can produce more than one type of dextran, and the proportions of each polymer synthesized can vary depending on the culture conditions used (5, 19). Since dextran-producing leuconostocs are physiologically similar (4), differentiation of specific strains is often accomplished through unreliable colonial morphological comparisons of the microorganism and structural analyses of the specific polymer produced. Differences in dextran structure are determined by time-consuming and elaborate methods such as gas chromatography-mass spectrometry of methylated dextran derivatives (15), ^{1}H and ^{13}C nuclear magnetic resonance spectrometry (14), or Fourier-transform infrared spectroscopy (13). The development of a convenient method to aid differentiation and identification of dextran-producing *Leuconostoc* strains would assist both basic and applied research.

Several DNA-based methods for the differentiation of *Leuconostoc mesenteroides* strains have been developed (6, 17); however, the protocols used either were time-consuming or did not significantly differentiate between strains. Random amplification of polymorphic DNA (RAPD) is a PCR method that incorporates a single arbitrarily designed oligonucleotide primer in the amplification reaction to generate DNA fragment polymorphisms (18, 21). The DNA fragment polymorphisms, or RAPD profiles, have been used to distinguish between closely related bacterial species (1, 11). The RAPD technique is simple and quick, and thus it would serve as a convenient alternative protocol to complement the methods currently used to distinguish dextran-producing *Leuconostoc* strains. The objective of this study was to determine if dextran-producing *Leuconostoc* strains could be differentiated by a modified RAPD protocol using primers designed from conserved regions of dextransucrase genes.

All *Leuconostoc* strains used in this study (NRRL B-512F, B-742, B-1118, B-1142, B-1149, B-1299, and B-1355) were obtained from the Agricultural Research Service culture collection (Peoria, Ill.) and are designated *L. mesenteroides* except for B-742, which is considered *L. citreum* (16). These *Leuconostoc* strains were chosen for this study because they are commonly used in research and for commercial applications (2, 7, 8, 10). *Leuconostoc* strains were grown in MRS (3) broth containing 2% glucose, and the genomic DNA was isolated according to the method of Pitcher et al. (12). Three oligonucleotide primers were used for RAPD analyses and were designed from conserved sequences of dextransucrase genes from *Leuconostoc* B-512F and B-1299 (9, 20). Two primers were designed from the N-terminal region of the B-512F dextransucrase gene (9, 20) and were designated 512Fa (5′-GATGCA GTCGACAATGTGGATGCT-3′) and 512Fb (5′-GTCATAA GGATCCTGTGAATGCATA-3′ [9]). Primers 512Fa and 512Fb were originally designed and used by Monchois et al. (9) to clone a dextransucrase gene from *L. mesenteroides* NRRL B-1299. One primer was designed from the C-terminal region of the B-1299 dextransucrase gene (9) and was designated 1299 [5′-(AGCT)CC(AG)TC(CT)TG(AGCT)CC(AG)AA(AG) TA(AGCT)ACCCA-3′]. (Degenerate positions within primer 1299 are indicated by parentheses.) Primer 1299 was designed degenerate in order to increase the number of DNA fragments detected. The typical 20-μl reaction mixture for RAPD PCR analysis of the *Leuconostoc* strains contained 1× *Pfu* buffer (Stratagene, La Jolla, Calif.), 250 μM (each) dATP, dCTP, dGTP, and dTTP (Stratagene), 1 μg of template DNA, 0.25 μg of a single oligonucleotide primer, and 1.5 U of *Pfu* DNA polymerase (Stratagene). A denaturation step of 95°C for 1 min was performed with the reaction mixture before PCR cycling. The RAPD PCR cycling program consisted of 40 cycles of denaturation at 95°C for 1 min, annealing at 30°C for 1 min, and primer extension at 72°C for 5 min. After the cycling program was finished, the samples were held at 72°C for 10 min to complete the extension of products. PCR amplification was performed in a Progene thermocycler (Techne Incorporated, Princeton, N.J.). The DNA banding patterns were examined by

* Corresponding author. Mailing address: Western Illinois University, Department of Biological Sciences, 1 University Circle, Waggoner Hall 316, Macomb, IL 61455. Phone: (309) 298-1484. Fax: (309) 298-2270. E-mail: smholt@ccmail.wiu.edu.

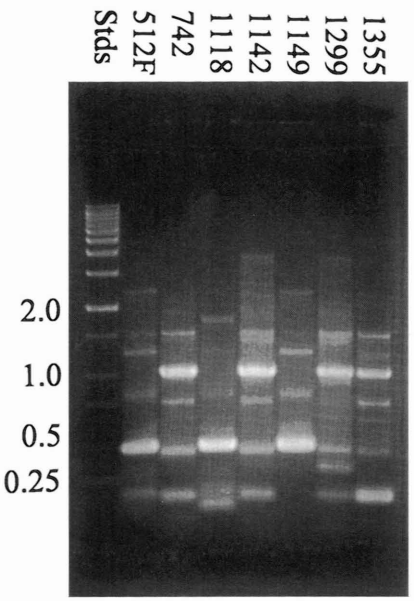

FIG. 1. RAPD analysis of dextran-producing *Leuconostoc* strains using primer 512Fa. RAPD profiles were examined by using 1.5% agarose gel electrophoresis. Stds, DNA standards.

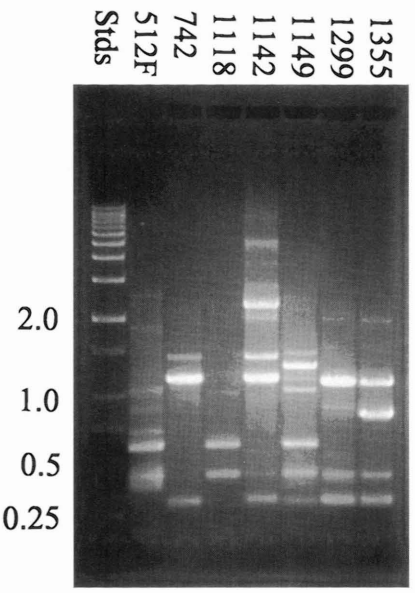

FIG. 3. RAPD analysis of dextran-producing *Leuconostoc* strains using primer 1299. RAPD profiles were examined by using 1.5% agarose gel electrophoresis. Stds, DNA standards.

using 1.5% agarose gel electrophoresis, and a DNA ladder (0.25 to 12 kb) was used as size standards (Stratagene).

Results from the modified RAPD analysis using each primer are illustrated in Fig. 1 through 3. All seven *Leuconostoc* strains that were examined by RAPD analysis could be differentiated based on DNA banding patterns when all three oligonucleotide primers were used in separate tests. Many of the strains shared common DNA band sizes (for example, strains B-512F, B-1118, and B-1149 in Fig. 1, strains B-1142 and B-1299 in Fig. 2, and strains B-742 and B-1142 in Fig. 3), which is not unexpected, since all the strains except B-742 are designated *L. mesenteroides*. All the strains tested showed reproducible RAPD profiles based on DNA band size, number, and intensity after three trials.

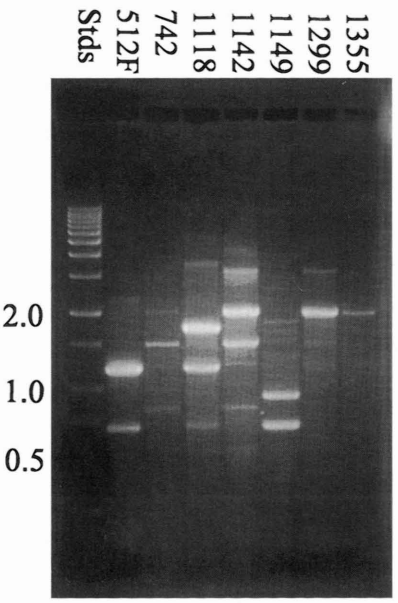

FIG. 2. RAPD analysis of dextran-producing *Leuconostoc* strains using primer 512Fb. RAPD profiles were examined by using 1.5% agarose gel electrophoresis. Stds, DNA standards.

All the dextran-producing *Leuconostoc* strains tested could be differentiated from each other by RAPD PCR amplification using a combination of primers that were designed from conserved regions of dextransucrase genes. RAPD analysis showed intraspecies differences between the dextran-producing strains examined. Use of all three primers for RAPD analysis would ensure accurate differentiation of dextran-producing *Leuconostoc* strains when control strains are used. It is not clear why long (24-mer), specific primers produced polymorphic DNA banding patterns, since short, arbitrary primers are usually used in RAPD PCRs. Only two *Leuconostoc* dextransucrase genes have been cloned (from B-512F [20] and B-1299 [9]), and these displayed 55% amino acid sequence similarity (9). Evidence has indicated that many polymer-producing *Leuconostoc* strains produce more than one type of dextransucrase (2), which may account for some of the DNA polymorphisms observed during RAPD analysis. *L. mesenteroides* B-512F, however, has only one type of dextransucrase (2) but still displays DNA polymorphism when the modified RAPD protocol is followed. The presence of multiple dextransucrase genes may not account for all the DNA polymorphism observed when the modified RAPD protocol is used. Although the exact molecular mechanism defining the modified RAPD protocol is unknown, this should not preclude its use as a tool to differentiate polymer-producing *Leuconostoc* strains.

RAPD analysis was proven to be a simple and reproducible method for differentiation of the dextran-producing *Leuconostoc* strains compared to other strain characterization methods which involve polymer structural analyses. This study provides a basis for molecular differentiation of dextran-producing *Leuconostoc* strains that are used in basic and applied research.

We thank Richard Greene, Jeffrey Ahlgren, and Timothy Leathers for their contributions.

REFERENCES

1. **Cocconcelli, P. S., D. Porro, S. Galandini, and L. Senini.** 1995. Development of RAPD protocol for typing of strains of lactic acid bacteria and enterococci. Lett. Appl. Microbiol. **21:**376–379.
2. **Cote, G. L., and J. A. Ahlgren.** 1995. Microbial polysaccharides, p. 578–611. *In* Kirk-Othmer encyclopedia of chemical technology, 4th ed., vol. 16. John Wiley & Sons, Inc., New York, N.Y.
3. **deMan, J. C., M. Rogosa, and M. Sharpe.** 1960. A medium for the cultivation of lactobacilli. J. Appl. Bacteriol. **23:**130–135.
4. **Holzapfel, W. H., and U. Schillinger.** 1992. The genus *Leuconostoc*, p. 1500–1535. *In* A. Balows, H. Truper, M. Dworkin, W. Harder, and K.-H. Schleifer (ed.), The prokaryotes, 2nd ed. Springer-Verlag, New York, N.Y.
5. **Jeanes, A., W. C. Haynes, C. A. Wilham, J. C. Rankin, E. H. Melvin, M. J. Austin, J. E. Cluskey, B. E. Fisher, H. M. Tsuchiya, and C. E. Rist.** 1954. Characterization and classification of dextrans from ninety-six strains of bacteria. J. Am. Chem. Soc. **76:**5041–5052.
6. **Kelly, W. J., R. V. Asmundson, G. L. Harrison, and C. M. Huang.** 1995. Differentiation of dextran-producing *Leuconostoc* strains from fermented rice cake (*puto*) using pulsed-field gel electrophoresis. Int. J. Food Microbiol. **26:**345–352.
7. **Kim, D., and J. F. Robyt.** 1994. Production and selection of mutants of *Leuconostoc mesenteroides* constitutive for glucansucrases. Enzyme Microb. Technol. **16:**659–664.
8. **Martinez-Murcia, A. J., and M. D. Collins.** 1990. A phylogenetic analysis of the genus *Leuconostoc* based on reverse transcriptase sequencing of 16S rRNA. FEMS Microbiol. Lett. **70:**73–84.
9. **Monchois, V., R.-M. Willemot, M. Remaud-Simeon, C. Croux, and P. Monsan.** 1996. Cloning and sequencing of a gene for a novel dextransucrase from *Leuconostoc mesenteroides* NRRL-B-1299 synthesizing only $\alpha(1\rightarrow6)$ and $\alpha(1\rightarrow3)$ linkages. Gene **182:**23–32.
10. **Monsan, P., and F. Paul.** 1995. Enzymatic synthesis of oligosaccharides. FEMS Microbiol. Rev. **16:**187–192.
11. **Permaul, K., D. Pillay, and B. Pillay.** 1996. Random-amplified polymorphic DNA (RAPD) analysis shows intraspecies differences among *Xanthomonas albilineans* strains. Lett. Appl. Microbiol. **23:**307–311.
12. **Pitcher, D. G., N. A. Saunders, and R. J. Owen.** 1989. Rapid extraction of bacterial genomic DNA with guanidium thiocyanate. Lett. Appl. Microbiol. **8:**151–156.
13. **Seymour, F. R., and R. L. Julian.** 1979. Fourier-transform, infrared difference spectrometry for structural analysis of dextrans. Carbohydr. Res. **74:**63–75.
14. **Seymour, F. R., R. D. Knapp, and B. L. Lamberts.** 1980. Structural analysis of soluble D-glucans from strains of *Streptococcus mutans* by ^{13}C-nuclear magnetic resonance spectrometry. Carbohydr. Res. **84:**187–195.
15. **Seymour, F. R., M. E. Slodki, R. D. Plattner, and A. Jeanes.** 1977. Six unusual dextrans: methylation structural analysis by combined G.L.C.-M.S. of per-*o*-acetyl-aldononitriles. Carbohydr. Res. **53:**153–166.
16. **Takahashi, M., S. Okada, T. Uchimura, and M. Kozaki.** 1992. *Leuconostoc amelibiosum* Schillinger, Holzapfel, and Kandler 1989 is a later subjective synonym of *Leuconostoc citreum* Farrow, Facklam, and Collins 1989. Int. J. Syst. Bacteriol. **42:**649–651.
17. **Villani, F., G. Moschetti, G. Blaiotta, and S. Coppola.** 1997. Characterization of strains of *Leuconostoc mesenteroides* by analysis of soluble whole-cell protein pattern, DNA fingerprinting and restriction of ribosomal DNA. J. Appl. Microbiol. **82:**578–588.
18. **Welsh, J., and M. McClelland.** 1990. Fingerprinting genomes using PCR with arbitrary primers. Nucleic Acids Res. **18:**7213–7218.
19. **Wilham, C. A., B. H. Alexander, and A. Jeanes.** 1955. Heterogeneity in dextran preparations. Arch. Biochem. Biophys. **59:**61–75.
20. **Wilke-Douglas, M., J. T. Perchorowicz, C. M. Houck, and B. R. Thomas.** December 1989. Methods and compositions for altering physical characteristics of fruit and fruit products. Patent WO 89/12386.
21. **Williams, J. G. K., A. R. Kubelik, K. J. Livak, J. A. Rafalski, and S. V. Tingey.** 1990. DNA polymorphisms amplified by arbitrary primers are useful as genetic markers. Nucleic Acids Res. **18:**6531–6535.

Supplied by U.S. Dept. of Agriculture
National Center for Agricultural
Utilization Research, Peoria, Illinois