Original Research

Rapid Molecular Diagnosis of Lactobacillus Bacteremia by Terminal Restriction Fragment Length Polymorphism Analysis of the 16S rRNA Gene

Jeffrey E. Christensen, PhD, Cory E. Reynolds, MS, Sanjay K. Shukla, PhD, Kurt D. Reed, MD

ABSTRACT

OBJECTIVE

Bacteremia due to lactobacilli is uncommon, yet it is increasing in frequency, especially among immunosuppressed patients. In the clinical laboratory, lactobacilli must be subcultured from positive blood cultures before identification by traditional biochemical methods. Delays in diagnosis are significant because the organisms are inherently resistant to vancomycin, a drug frequently prescribed for empiric therapy for gram-positive bacteremia. Recently, we developed a rapid terminal-restriction fragment length polymorphism (T-RFLP) diagnostic assay based on species-specific variations in the bacterial 16S rRNA gene. We sought to apply this technique to the identification of Lactobacillus spp. from three cases of bacteremia.

DESIGN

The results of the T-RFLP analysis are compared with two standard biochemical identification methods.

METHODS

Lactobacillus strains were isolated from positive clinical blood cultures. Initial suspect cultures were subcultured and characterized using an automated substrate hydrolysis system and Lactobacillus carbohydrate fermentation profiles. Further biochemical and molecular analyses were performed from isolates propagated in Lactobacillus MRS broth. DNA was extracted and the 16S rRNA gene sequenced. Two sets of fluorescent labeled primers targeting the 16S rRNA gene were used for polymerase chain reaction (PCR) with chromosomal preparations from reference strains and blood isolates. The PCR products were digested with restriction enzymes and terminal-restriction fragment profile analysis performed.

RESULTS

T-RFLP analysis correctly identified the Lactobacillus species in each case. T-RFLP analysis could be completed within 8 hours of obtaining a positive blood culture as compared to more than the 24 to 48 hours required for traditional culturing and biochemical characterizations.

CONCLUSION

T-RFLP analysis allows for rapid identification of Lactobacillus directly from positive blood cultures and circumvents the requirement for subculture. Reduced diagnostic time has implications for duration of infection, the cost of patient care, length of hospitalization, development of broad-spectrum antibiotic resistance, and mortality due to bacteremia. T-RFLP profiling represents a highly reproducible and predictive source for identification of many organisms associated with bacteremia.

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INTRODUCTION

The detection and identification of bacterial infections of the bloodstream is one of the most important functions of the clinical microbiology laboratory. Bacteremia results in significant morbidity and mortality, especially among patient populations that are immunocompromised. One of the current limitations of processing blood cultures in the laboratory is the requirement to subculture isolates in order to perform biochemical or other tests needed for bacterial identification. This often results in one or more days of delay during which it is often necessary to administer broad-spectrum antimicrobial therapy. This practice contributes to the continued emergence of drug-resistant strains of pathogenic bacteria. In addition, some species of bacteria associated with bacteremia are inherently resistant to commonly prescribed antimicrobial agents and may not be effectively treated with empiric therapy.

Lactobacilli belong to a diverse group of bacteria that are gram-positive or gram-variable, facultatively anaerobic, non-sporing, and produce lactic acid as a major product of carbohydrate fermentation. These bacteria are ubiquitous in the environment and propagate in ecological niches, such as plant surfaces and decaying plant material. In humans they colonize the oral cavity, gastrointestinal tract, and vagina. L. casei appears to be the most frequently isolated species within the genus, although L. paracasei and L. rhamnosus are also encountered in clinical situations. However, isolates are often only identified by genus and most automated identification systems are not capable of accurate differentiation of Lactobacillus species. In general, lactobacilli are avirulent or of low virulence, but are recognized as opportunistic pathogens involved in bacteremia, as well as other significant infections such as amnionitis, abscesses, and endocarditis. Lactobacilli are also considered emerging pathogens in patients with chemotherapy induced neutropenia.

One important clinical feature of lactobacilli is their inherent resistance to vancomycin, a drug frequently given as empiric therapy for bacteremia due to gram-positive bacteria. Therefore, rapid identification of lactobacilli from blood cultures would allow a more timely prescription of appropriate antimicrobial therapy. We recently described a rapid identification procedure for positive blood cultures that is based on terminal-restriction fragment length polymorphism (T-RFLP) profile analysis of the 16S rRNA gene. This molecular diagnostic procedure is applied directly to positive blood culture bottles and circumvents the need to subculture the isolate prior to identification. The assay is robust and is applicable to a broad range of bacterial pathogens. In this report we demonstrate the utility of T-RFLP in three cases of Lactobacillus bacteremia and compare the results with two conventional methods of bacterial identification.

MATERIALS AND METHODS

Bacterial strains and isolation

The Lactobacillus strains characterized in this study were isolated from positive blood cultures at Marshfield Laboratories as part of routine diagnostic testing. Marshfield Laboratories uses the BacT/Alert blood culturing system (Organon Teknika Corp., Durham, NC) and performs Gram staining for all positive blood cultures. Positive blood bottles were cultured on trypticase soy–5% sheep blood or chocolate agar at 37°C under the appropriate atmospheric conditions. Blood bottle isolates were initially characterized using the automated VITEK system (bioMérieux Vitek Inc., Hazelwood, MO) and standard biochemical methods. Additional biochemical and molecular analyses described below were performed from isolates propagated in Lactobacillus MRS broth (Difco Laboratories, Detroit, MI).

Anaerobe identification card

The VITEK anaerobe identification card was used in conjunction with the VITEK system for the automated identification of each strain by characterizing the ability to hydrolyze 28 substrates. Following initial isolation and identification of each strain, cultures were propagated through MRS broth and plated on blood agar at 37°C under anaerobic conditions. Identification of isolates was repeated with the anaerobe identification cards as per the manufacturer’s protocol.

Lactobacillus carbohydrate fermentation profile

The API 50 CH test kit and the API CHL medium (bioMérieux Vitek Inc.) were used to determine the species of each Lactobacillus isolate by characterizing the ability to ferment 49 carbohydrates. Strains were grown for 18 hours to stationary phase in MRS broth and 1 mL of culture was harvested by centrifugation at 20,000 g for 1 minute. The cell pellets were washed with 1 mL sterile saline, suspended in 0.5 mL API CHL medium, and then further diluted 100-fold in API CHL medium. The culture dilutions were then loaded to the API 50 CH test strips as per the manufacturer’s protocol, the capsules were covered with mineral oil, and the test strips were incubated at 37°C for up to 48 hours. Test strips information was read and recorded at 16 and 40 hours. Carbohydrate fermentation profiling was performed in duplicate for each strain. Species identification was made by comparison of the results from API 50 CH test strips with known carbohydrate fermentation profiles of lactobacilli as indicated in Bergey’s Manual of Systematic Bacteriology.

DNA extraction

DNA was extracted from cultured colonies using a modification of the tissue protocol for the QI Amp DNA Mini Kit (Qiagen, Inc., Valencia, CA). Briefly, 200 µL of a cell suspension in H2O (containing ~5 to 10 mg cells) was mixed with 200 µL of QIAmp Buffer AL and 10 µL of proteinase K (20 mg/mL). Samples were incubated for 15 minutes at 56°C and then for 15 minutes at 95°C. Two hundred microliters of ethanol was added and mixed by
vortexing. The samples were loaded, washed, and eluted in 200 µL from QIAGen spin columns, as indicated in the remainder of the manufacturer's protocol.

**Sequencing of the 16S rRNA gene**

The template DNA for sequencing of the 16S rRNA gene was amplified using primers FD1 (5'-AGA GTT TGA TCC TGG CTC AG) and RD1 (5'-AAG GAG GTG ATC CAG CC), resulting in PCR products of ~1500 bp. The reaction mixtures contained 1 x polymerase chain reaction (PCR) buffer, 200 µM each deoxynucleoside triphosphate, 3.0 mM MgCl2, 0.5 µM each primer species, 1.0 U Taq DNA polymerase, and 1.0 µL chromosomal preparation per 15 µL reaction. The PCR products were used for cycle sequencing reactions without further purification.

Cycle sequence reactions were prepared with BigDye Terminator mix (Applied Biosystems, Foster City, CA) as per supplier's protocol using primer FD1 (see above), RD1 (see above), 515F (5'-TGC CAG CAG CCG CGG TAA), 806R (5'-GGA CTA CCA GGG TAT CTA AT), 91E (5'-TCA AAK GAA TTG ACG GGG GC), or 13B (5'-AGG CCC GGG AAC GTA TTC AC). Sequence determination was done on an Applied Biosystems model 377XL automated DNA sequencing instrument. DNA sequences were aligned using Lasergene99 software (DNASTAR Inc., Madison, WI). The nucleotide sequences, determined from a minimum of 4-fold coverage, were deposited in GenBank under the accession numbers AY299486, AY299487, and AY299488. Sequence analysis was performed using the BLAST program for database searches at the National Center for Biotechnology Information Web site and the sequence match program at the Ribosomal Database Project (RDP-II) Web site. Multiple sequence alignments were performed using the ClustalW program.

**PCR primers for determining T-RFLP**

Independent reactions with two sets of fluorescent labeled primers targeting the 16S rRNA gene were used for PCR with chromosomal preparations from reference strains and blood isolates. The primers were chosen as described in Christensen et al.18 based on evaluations of domain specific primers and maximization of matches received from queries using the TAP T-RFLP on-line analysis of RDP-II. As indicated, primers were labeled at the 5’ end with the dyes 6-carboxyfluorescein (6-FAM), 4,7,2',4',5',7'-hexachloro-6-carboxyfluorescein (HEX), or N,N,N',N'-tetramethyl-6-carboxyrhodamine (TAMRA).

The 5’ region (encompassing hypervariable regions V1-V3) of the 16S rRNA gene was amplified with S-D-Bact-0045-b-S-20 and S-D-Bact-1371-a-A-20 (amplifying the 5’ region of the 16S rRNA gene) was only digested with HhaI. Restriction digestions were performed in a model 9600 Perkin-Elmer thermal cycler programmed for a 1 hour incubation at 37°C followed by 20 minutes at 65°C for enzyme inactivation. The restriction digest products were purified with DyeEx 96-well spin columns (Qiagen) as per manufacturer's protocol.

**PCR conditions for T-RFLP**

Fluorescent dye labeled PCR products for T-RFLP were prepared as described previously.18 The reaction mixtures for PCR contained 1 x PCR buffer, 200 µM each deoxynucleoside triphosphate, 3.0 mM MgCl2, 0.5 µM each primer species, 1.0 U Taq DNA polymerase, and 1.0 µL chromosomal preparation per 15 µL reaction. Due to approximately 4-fold higher detection sensitivity of the fluorescence emission from the 6-FAM dye relative to HEX dye, a ratio of 1 part 6-FAM labeled primer (0.125 µM) to 3 parts unlabeled primer (0.375 µM) was used for primers S-D-Bact-0045-b-S-20 and S-D-Bact-0785-a-S-19. DNA was amplified using a model 9600 thermal cycler (Perkin-Elmer, Norwalk, CT) with the following program: 15 minutes at 94°C for denaturation and Taq activation, 35 cycles consisting of denaturation (45 seconds at 94°C), annealing (30 seconds at 52°C), extension (60 seconds at 72°C), and a final extension for 5 minutes at 72°C. All PCR reactions were analyzed by gel electrophoresis and stained with ethidium bromide to assure product formation prior to restriction digest.

**Restriction digests of PCR products**

PCR products were digested with restriction enzymes (Invitrogen, Carlsbad, CA and New England BioLabs, Beverly, MA) without further purification. Each 10 µL restriction digest contained 1.0 µL of PCR product, 0.5 µL of restriction enzyme, and 8.5 µL of prepared stock containing 2.5 ng/µL each appropriate size/digest control DNA in 1 x buffer. The PCR product of 6-FAM labeled S-D-Bact-0045-b-S-20 and HEX labeled S-D-Bact-0785-a-A-19 (amplifying the 5’ region of the 16S rRNA gene) was digested in separate reactions with AluI, HhaI, MspI, and RsaI. The PCR product of 6-FAM labeled S-D-Bact-0785-a-S-19 and HEX labeled S-D-Bact-1371-a-A-20 (amplifying the 3’ region of the 16S rRNA gene) was only digested with HhaI. Restriction digestions were performed in a model 9600 Perkin-Elmer thermal cycler programmed for a 1 hour incubation at 37°C followed by 20 minutes at 65°C for enzyme inactivation. The restriction digest products were purified with DyeEx 96-well spin columns (Qiagen) as per manufacturer's protocol.

**Terminal restriction fragment (TRF) profile analysis and organism identification**

Samples were prepared for electrophoresis by combining 1.0 µL of restriction digest product, 0.50 µL of X-rhodamine MapMarker 1000 XL (BioVenture, Inc., Muffreeboro, TN), and 14 µL of Hi-Di formamide (Applied Biosystems Instruments). The size standard contains single strands of DNA with a single ROX fluorophore at 50, 75, 100, 125, 150, 200, 250, 300, 350, 400, 450, 475, 500, 550, 600, 650, 700, 750, 800, 850, 900, 950, and 1000 bases. Samples were mixed by pipetting, denatured at 94°C, and immediately
cooled to 4°C. The lengths of TRFs derived from amplified DNA products were determined with an Applied Biosystems model 3100 capillary DNA sequencing instrument operating in GeneScan™ mode. The samples were loaded to a 50 cm capillary with an injection voltage of 1 kV for 22 seconds and run at 12.2 kV for 2.2 hours at 50°C. The sizes of control and sample TRFs were calculated by comparison with the internal standard using the local Southern method. Fragment lengths \( \geq 20 \) but \(< 50 \) bases were calculated by linear extrapolation from the migration times of the 50 and 75 base standards.

Identification of bacteria was accomplished through the use of a program for calculating terminal fragment sizes and a searchable terminal restriction fragment profile database (TRFPD). Briefly, the maximum area 6-FAM and HEX peak data for each sample are identified and organized according to the respective primer set and restriction digest combination. The program is then used to compare the sample TRF profile with 2435 TRF profiles representing 921 different species of potential relevance to bacteremia in the TRFPD. The results from a Gram stain can also be selected in the data input window to limit the search. The

![Figure 1](image_url)

**Figure 1.** Representative TRF profile of the PCR products from the 16S rRNA gene of *Lactobacillus rhamnosus* MCRF-412. Each panel shows a PCR product/restriction digest electropherogram and the applicable range of the standard curve (50-850 bases). The single major 6-FAM (blue peaks) and HEX (green peaks) fragment of each digest are identified by their respective nucleotide size. TRFs from PCR products obtained with (6-FAM)-S-D-Bact-0045-b-S-20 and (HEX)-S-D-Bact-0785-a-A-19 are shown in panels A (\( Alu I \)), B (\( Hha I \)), C (\( Msp I \)), and D (\( Rsa I \)). TRFs from PCR products obtained with (6-FAM)-S-D-Bact-0785-a-S-19 and (HEX)-S-D-Bact-1371-a-A-20 are shown in panel E (\( Hha I \)). The presence of a single peak size \( \geq 700 \) bases for both the 6-FAM and HEX fragments in panel D indicates the PCR product was not digested with \( Rsa I \).
result of a search includes the number of matching fragment lengths (within chosen bp windows), ordering of closest matches, and links to best match TRF profiles. In addition, a sum of differences (SOD) score was determined for each TRF profile relative to the predicted fragments from the database. Specifically, the difference between the size of the experimentally determined TRF and the sequence predicted fragment size was calculated for each fragment/digest combination, and then the sum of these differences was calculated. A smaller SOD score is taken to represent a closer match to a given organism in the database.

### RESULTS

**Characterization of organisms from positive blood bottles**

The three organisms described in this manuscript were isolated from positive anaerobic blood bottles in Marshfield Laboratories. Each culture was determined to contain either Gram positive or Gram variable rod-shaped cells. Isolates grew in 24 hours on trypticase soy–5% sheep blood or chocolate agar at 37°C under anaerobic conditions. Isolates were used for the identification of each organism using the following biochemical tests.

#### Table 1. VITEK anaerobe identification.

<table>
<thead>
<tr>
<th>Substrate</th>
<th>MCRF-271 &amp; MCRF-412</th>
<th>MCRF-284</th>
</tr>
</thead>
<tbody>
<tr>
<td>p-nitrophenyl phosphate</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>p-nitrophenyl phosphate choline</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>p-nitrophenyl-β-D-galactopyranoside</td>
<td>Positive</td>
<td>Positive</td>
</tr>
<tr>
<td>p-nitrophenyl-α-D-galactopyranoside</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>p-nitrophenyl-β-D-glucomyranoside</td>
<td>Positive</td>
<td>Positive</td>
</tr>
<tr>
<td>p-nitrophenyl-α-D-glucomyranoside</td>
<td>Positive</td>
<td>Positive</td>
</tr>
<tr>
<td>p-nitrophenyl-β-D-glucuronide</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>p-nitrophenyl-β-D-lactoside</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>p-nitrophenyl-α-D-mannopyranoside</td>
<td>Weak</td>
<td>-</td>
</tr>
<tr>
<td>p-nitrophenyl-α-L-fucopyranoside</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>p-nitrophenyl-β-D-xlyopyranoside</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>p-nitrophenyl-α-L-arabinofuranoside</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>p-nitrophenyl-N-acetylglucosaminide</td>
<td>Positive</td>
<td>Positive</td>
</tr>
<tr>
<td>N-benzoyl-DL-arginine p-nitroanilide</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>L-leucine p-nitroanilide</td>
<td>Positive</td>
<td>Positive</td>
</tr>
<tr>
<td>L-proline p-nitroanilide</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>L-alanine p-nitroanilide</td>
<td>Positive</td>
<td>Positive</td>
</tr>
<tr>
<td>L-lysine p-nitroanilide</td>
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<td>Positive</td>
</tr>
<tr>
<td>γ-glutamyl p-nitroanilide</td>
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<td>-</td>
</tr>
<tr>
<td>Triphenyl tetrazolium</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Arginine</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Urea</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Glucose</td>
<td>Positive</td>
<td>Positive</td>
</tr>
<tr>
<td>Trehalose</td>
<td>Positive</td>
<td>Positive</td>
</tr>
<tr>
<td>Arabinose</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Raffinose</td>
<td>-</td>
<td>-</td>
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<tr>
<td>Xylose</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

**VITEK Identification**

<table>
<thead>
<tr>
<th>L. casei</th>
<th>L. casei</th>
</tr>
</thead>
</table>

*Symbols for MCRF isolate results from the VITEK (VITEK (bioMérieux Vitek Inc., Hazelwood, MO) anaerobe identification: positive, positive for hydrolysis; weak, limited for hydrolysis; -, no detectable hydrolysis.*
VITEK anaerobe identification

The substrate hydrolysis results from the anaerobe identification card are shown in table 1. The single difference found between the strains was the inability of MCRF-284 to use \( \rho \)-nitrophenyl-\( \alpha \)-D-mannopyranoside, whereas strains MCRF-271 and MCRF-412 provided a weak reaction. All three strains were identified as \( L. \) casei on the basis of the results from Gram reaction, lack of indole production, and substrate hydrolysis on the anaerobic identification card.

Identification of \( L. \) casei by carbohydrate fermentation profile

Table 2 shows the API 50 CH results and the predicted carbohydrate fermentation profiles for \( L. \) casei and \( L. \) rhamnosus with 22 carbohydrates necessary for species differentiation and identification of most heterofermentative lactobacilli. Strains of \( L. \) casei and \( L. \) rhamnosus were differentiated by the ability of the latter isolates to ferment arabinose and rhamnose. In addition to the data shown in table 2, all of the blood isolates were positive for fermentation of arbutine, \( \beta \)-gentiobiose, D-tagatose, inositol, N-acetyl-glucosamine, and saccharose. Also, strains MCRF-271 and MCRF-412 were positive for fermentation of L-fucose and L-sorbose, while no reaction was found with MCRF-284. Conversely, MCRF-284 was positive for fermentation of adonitol, D-turanose, and inuline, while no reaction was found with MCRF-271 and MCRF-412.

Identification of species by sequence determination of \( 16S \) rRNA gene

The nucleotide sequence was determined for a 1480 base region of the \( 16S \) rRNA gene from each of the blood isolates (GenBank accession numbers AY299486, AY299487, and AY299488). The sequences determined for MCRF-271 and MCRF-412 were identical for all 1480 bases. The BLAST analysis of MCRF-271 and MCRF-412 nucleotide sequence indicated the highest identities with a \( L. \) casei (99.5%) and \( L. \) rhamnosus (99.2%) strain. The similarity scores from the

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Table 2. Identification by carbohydrate fermentation profile.

<table>
<thead>
<tr>
<th>Carbohydrate</th>
<th>( L. ) rhamnosus(^a)</th>
<th>MCRF-271 &amp; MCRF-412(^b)</th>
<th>( L. ) casei(^a)</th>
<th>MCRF-284(^b)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amygdaline</td>
<td>Positive</td>
<td>Positive</td>
<td>Positive</td>
<td>Positive</td>
</tr>
<tr>
<td>D-arabinose</td>
<td>C</td>
<td>Weak</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Cellobiose</td>
<td>Positive</td>
<td>Positive</td>
<td>Positive</td>
<td>Positive</td>
</tr>
<tr>
<td>Esculine</td>
<td>Positive</td>
<td>-</td>
<td>Positive</td>
<td>-</td>
</tr>
<tr>
<td>D-fructose</td>
<td>Positive</td>
<td>Positive</td>
<td>Positive</td>
<td>Positive</td>
</tr>
<tr>
<td>Galactose</td>
<td>Positive</td>
<td>Positive</td>
<td>Positive</td>
<td>Positive</td>
</tr>
<tr>
<td>D-glucose</td>
<td>Positive</td>
<td>Positive</td>
<td>Positive</td>
<td>Positive</td>
</tr>
<tr>
<td>Glucuronate</td>
<td>Positive</td>
<td>Weak</td>
<td>Positive</td>
<td>Weak</td>
</tr>
<tr>
<td>Lactose</td>
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<td>Positive</td>
<td>C</td>
<td>Positive</td>
</tr>
<tr>
<td>Maltose</td>
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<td>-</td>
<td>Positive</td>
<td>Positive</td>
</tr>
<tr>
<td>Mannitol</td>
<td>Positive</td>
<td>Positive</td>
<td>Positive</td>
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<td>D-mannose</td>
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<td>Melezitose</td>
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<td>Melibiose</td>
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<td>-</td>
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<tr>
<td>D-raffinose</td>
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<td>-</td>
<td>-</td>
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<tr>
<td>Rhamnose</td>
<td>Positive</td>
<td>Positive</td>
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<tr>
<td>Ribose</td>
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<td>Salicin</td>
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<td>Sorbitol</td>
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<td>Trehalose</td>
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<td>Positive</td>
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<td>Positive</td>
</tr>
<tr>
<td>D-xylose</td>
<td>-</td>
<td>-</td>
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<td>-</td>
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<tr>
<td>L-xylose</td>
<td>-</td>
<td>-</td>
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</tbody>
</table>

\(^a\) Symbols for indicated reference organism profile from Bergey's Manual\(^19\): positive, 90% or more strains positive for fermentation of the indicated carbohydrate; -, 90% or more strains negative; C, 11-89% strains positive.

\(^b\) Symbols for MCRF isolate results using the API 50 CH source strips: positive, positive for fermentation of indicated carbohydrate; weak, limited for fermentation; -, no detectable fermentation.
The BLAST analysis of MCRF-284 nucleotide sequence indicated the highest identities with two L. rhamnosus strains. The RDP-II sequence match analysis of MCRF-284 nucleotide sequence matched closely to those predicted from their respective rRNA gene PCR products for each of the blood isolates and having a SOD score of 13-15 bases. The next closest matches were for three strains of L. paracasei, again matching all 10 fragments within 5 bases, but with a slightly greater SOD score (16 base difference).

**DISCUSSION**

This study describes the use of TRF profiling for identification of Lactobacillus species from blood cultures and comparison of this technique with other methods of identification. In a previous study, it was shown that TRF profiling was effective in differentiating many organisms responsible for bacteremia.

Biochemical characterization of the blood culture isolates using the VITEK anaerobe system resulted in identification of L. casei for all three strains, although the substrates available in the panel tests are not expected to differentiate all Lactobacillus species. To attempt more accurate identification of the blood culture isolates we tested their ability to ferment 49 carbohydrates in a commercially available kit for Lactobacillus speciation, which resulted in differentiation of the strains to L. rhamnosus and L. casei (table 2). This was primarily due to the ability of the L. rhamnosus strains

<table>
<thead>
<tr>
<th>Isolate or organism</th>
<th>Strain</th>
</tr>
</thead>
<tbody>
<tr>
<td>L. rhamnosus</td>
<td>DSM 20021</td>
</tr>
<tr>
<td>L. rhamnosus</td>
<td>JCM 1136</td>
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<tr>
<td>L. plantarum</td>
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<td>L. manihotivorans</td>
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<td>L. rhamnosus</td>
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**Table 3. TRF profiles for blood isolates and SOD scores for TRF database matches.**

<table>
<thead>
<tr>
<th>Isolate or organism</th>
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UD: Undigested PCR product (6-FAM and HEX fragment sizes were both >700 bases).

Organisms are presented in ascending order of the SOD scores relative to the TRF results for each MCRF strain.

Value calculated from the differences between the size of the experimentally determined TRF and the sequence predicted fragment sizes for a restriction digest of organisms in the TRF database. The SOD for each organism was then calculated.

Each TRF size reported was determined from the average of at least two digests.

The TRF profile for MCRF-284 was closest to those for three L. casei strains and one L. zeae strain in the TRFPD, matching all 10 fragments within 5 bases for all four strains and having a SOD score of 13-15 bases. The next closest matches were for three strains of L. paracasei, again matching all 10 fragments within 5 bases, but with a slightly greater SOD score (16 base difference).
to ferment rhamnose and D-arabinose. Although the lack of differentiation of these strains using the VITEK anaerobe system is likely of limited clinical significance, the primary disadvantage of both biochemical identification systems is the requirement for isolated colonies to be grown out from the positive blood culture bottle before analysis.

The analysis of DNA sequence data from the 16S rRNA gene of the blood isolates clearly indicated all three strains were lactobacilli. However, differentiation of species was less obvious due to the high degree of BLAST identity scores and RDP-II similarity scores between the 16S rRNA sequences of some \textit{L. casei} and \textit{L. paracasei} strains, and also between \textit{S. rhamnosus} and \textit{L. casei} strains. Since Genbank is a public database, there may be some ambiguity as a result of phenotypic misidentification of the \textit{Lactobacillus} species for which 16S rRNA gene sequence have been submitted.

The blood culture isolates were clearly identified as lactobacilli using TRF profiling. The MCRLF-271 and MCRLF-412 TRF profile had a conclusive SOD score that identified them as \textit{L. rhamnosus}. However, similar to identification by 16S rRNA gene sequencing, determination of species for MCRLF-284 was not as obvious. The MCRLF-284 TRF profile had similar SOD scores with \textit{L. casei} and \textit{L. zeae}, with a slightly greater difference from \textit{L. paracasei}.

Commercially available biochemical identification systems often require isolation of a microorganism after growth in a blood culture bottle and generally require an additional 1 to 3 days before organism identification. Due to the fact that bacterial DNA can be obtained directly by extraction from positive blood culture bottles for the PCR step of TRF profiling, significant delays that result from the need for isolated colonies for biochemical identification methods are avoided. Also, TRF profiling for bacterial identification is more rapid than sequencing of the 16S rRNA gene, primarily because it does not require a second thermal cycler reaction to produce dye terminated products for sequencing. The analysis of 16S rRNA genes by TRF profiling allows the identification process to be completed within ~8 hours after obtaining a positive blood bottle sample. Reduced diagnostic time has implications for duration of infection, the cost of patient care, length of hospitalization, development of broad-spectrum antibiotic resistance, and mortality due to bacteremia. TRF profiling represents a highly reproducible and predictive source for identification of many organisms associated with bacteremia.

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