Isolation and Identification of Indonesian *Lactobacillus reuteri* Strain from the Saliva of Young Adults

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### ABSTRACT

**Background:** Biofilms are involved in a wide variety of microbial infections, including dental caries and periodontitis. The use of probiotics has been a promising prevention and treatment modality with which to combat biofilm-related diseases in the oral cavity. The probiotic *Lactobacillus reuteri* has been proven to reduce gingivitis and plaque index inside the oral cavity. These bacteria can be found in the digestive system and also in the human oral cavity. **Objective:** The aim of this study was to identify *L. reuteri* in the saliva of Indonesian young adults. **Methods:** Forty saliva samples were collected from 18–24 year-old Indonesian subjects. DNA extraction was performed, and then, the identification of *L. reuteri* was accomplished using PCR. Six subjects showed positive results. The positive samples were cultured in Rogosa Agar for 24 hours at 37°C anaerobically. Several single colonies were further cultured separately in broth medium before DNA extraction and PCR identification were performed. The four thickest bands were selected for DNA sequencing. **Results:** An analysis performed using BLAST showed that two of the *L. reuteri* strains obtained from the Indonesian saliva isolates had 96% (isolate 3.11) and 95% (isolate 5.14) values. This confirmed the presence of new strains based on average nucleotide identity (ANI). The isolate strains of 3.11 and 5.14 have been registered at DDBJ/EMBL/GenBank under the accession number LC382415 and LC382416, respectively. **Conclusion:** *L. reuteri* novel strain can be isolated from the saliva of Indonesian young adults. Further studies involving biochemical tests and phenotypic analysis are needed to better understand these new *L. reuteri* strains.

Keywords: bacterial isolation, *Lactobacillus reuteri*, novel strains, PCR identification, probiotic

### Background

Biofilms are involved in a wide variety of microbial infections (by one estimate, 80% of all infections). These include dental caries and periodontitis. There are several preventive strategies for dental caries, such as plaque removal, adequate oral hygiene, diet modification including reduced sugar intake, and the use of fluorides
and tooth sealants. Although such methods have improved the oral health of the global population, problems remain in communities all over the world.2,3 One effective therapeutic alternative is the use of probiotics (non-pathogenic bacteria). The use of probiotics has become a promising prevention and treatment to combat biofilm-related diseases in the oral cavity.4 In addition, some evidence has suggested that probiotics can improve the oral conditions, such as preventing dental caries and periodontal diseases.5,6,7

A probiotic is a product that consists of specific strains of bacteria that have potential health benefits.8 According to reports, the most commonly consumed probiotics is yogurt, which is a fermentation of milk mostly by *Lactobacillus* or *Bifidobacterium*. Consuming *Lactobacillus* and *Bifidobacterium* also has beneficial effects on the intestinal system. These bacteria are regarded as part of the normal human microbiota.9 The *Lactobacillus* genus has long been known to include hetero-fermentative bacteria that can produce organic acids (mostly lactic acid, as well as other organic acids, such as propionic acid, acetic acid, and phenyllactic acid), thus causing a decrease in the pH of the environment in which they grow.10 This may be considered the primary antimicrobial activity of the *Lactobacillus* genus. It is also known that lactobacilli can interact with host cells and have the ability to affect specific mechanisms of the innate immune system through many signaling pathways, including Toll-like receptors (TLRs).11

The *Lactobacillus* genus consists of Gram-positive, non-spore forming rods or coccobacilli. They can be strictly fermentative, aero-tolerant, or anaerobic, as well as aciduric or acidophilic, and they have complex nutritional requirements.12 Many studies have reported that the dominant species in both adult and childhood caries include *L. fermentum*, *L. rhamnosus*, *L. gasseri*, *L. casei*/*paracasei*, *L. salivarius*, *L. plantarum*, and, to a lesser degree, *L. oris* and *L. vaginalis*. Less common species include *L. mucosae*, *L. crispatus*, *L. ultunesis*, *L. reuteri*, *L. gastricus*, and *L. parabuchneri*. Most *Lactobacillus* species found in caries lesions cohabitate with other lactobacilli; only *L. fermentum*, *L. casei*/*paracasei*, and *L. salivarius* were found as single *Lactobacillus* occupants of caries lesion.13

Among those species of the genus *Lactobacillus*, *L. reuteri* can eliminate the growth of pathogenic bacteria, such as *Streptococcus mutans*, in the oral cavity.14 *L. reuteri* is a probiotic bacteria that normally colonizes the digestive system and the oral cavity. Probiotic *L. reuteri* has been proven to reduce gingivitis and the plaque index inside the oral cavity.15 The consumption of yogurt or chewing tablets containing *L. reuteri* can reduce the colonization of *S. mutans* and the number of *Lactobacilli* present in the oral cavity.16,17 *L. reuteri* produces reuterin, a broad-spectrum antimicrobial substance that can affect oral pH. Reuterin is resistant to proteolytic and lipolytic enzymes and acts on both Gram-positive and Gram-negative bacteria.18

As an easily collectable biological material, saliva is suitable for use in medical investigations. Several health and disease-associated factors are reflected in the saliva.19 Therefore, the aim of this study was to identify *L. reuteri* in the saliva of Indonesian young adults. We next compared the results obtained in this study with those of previous reports to find novel strains. Furthermore, this study sought to perform a DNA sequence analysis of *L. reuteri* strains based on the complete 16S rRNA gene sequences.

**Materials and Methods**

**Ethics Statement**

This study received approval from the Ethics and Research Committee of the Faculty of Dentistry, Trisakti University, Jakarta, Indonesia, under process number 118/KE/FKG/12/2014 in accordance with the Helsinki Declaration. The participants were made aware of the objectives and procedures of the study and agreed to participate by providing written informed consent.

**Subjects and Sample Collection**

Forty subjects who ranged in age from 18 to 24 years were randomly selected. All subjects were registered as patients at the Dental Hospital of the University of Trisakti, Jakarta, Indonesia (35% male, 65% female). They had no systemic diseases and had received no antibiotic or probiotic therapy in the previous 6 months.
All subjects received a clinical oral examination and were evaluated using decayed/missing/filled teeth (DMFT) and papilla bleeding index (PBI) scores. Among all subjects, 20 subjects were caries-free and periodontitis-free, ten subjects had significant caries activity experience (DMFT scores > 2), and ten subjects had periodontal disease (PBI score > 2).

Human Saliva Samples

After the oral examination, stimulated saliva samples were collected according to a standard protocol. Each subject was instructed to chew 1 gram of paraffin gum for 1 minute until a solid bolus was formed, which was collected in a 15 ml sterile macro-centrifugal tube. Saliva samples of approximately 2 ml were collected in each tube, and the tubes were then stored at -80°C until analysis.

DNA Extraction

The genomic DNA was extracted from the saliva samples using a Wizard Genomic DNA Purification Kit (Promega, Wisconsin, USA) according to the manufacturer’s protocol. The DNA concentration was determined using a NanoPhotometer (IMPLEN P-Class, Ontario, Canada) at A260/A280 nm.

Identification of L. reuteri from Saliva Samples Using PCR

A master mix was made as follows: water 2 µL, GoTaq Green Master Mix (Promega, Wisconsin, USA) 10 µL, F primer 2 µL, R primer 2 µL, and 4 µL DNA sample. Then, the mix was added to PCR tubes and then arranged in a SimpliAmp Thermal Cycler (Applied Biosystems, California, USA). To identify L. reuteri, one primer pair, 16S rRNA-F (5'-ACCTGATTGACGATGGATCACCAGT-3') and 16S rRNA-R (5'-CCACCTTCCTCGGTTTTGCTCA-3'), was used. The amplified fragmental DNA was subjected to preheating at 94°C for 2 mins; followed by 35 cycles of 94°C for 30 secs, annealing at 51°C for 40 secs, and extension at 72°C for 1 min, with a final extension at 72°C for 1 min. The bacterial strain of L. reuteri known as ATCC 55730 was used as a positive control. The PCR products of the positive control and saliva samples were applied to 1.5% agarose gel. After electrophoresis was performed using a Mupid-eXu Submarine Electrophoresis System (Mupid, Japan), the gel was placed in a UV Trans Illuminator (Pacific Image Electronics, New Delhi, India) to observe the DNA bands of L. reuteri (1,100 bp).

Culture Conditions

From the samples with positive PCR results, aliquots (100 µL) of ten-fold diluted samples were inoculated on Rogosa Agar™ (Oxoid, Hampshire, United Kingdom). After inoculation, all media were incubated under anaerobic conditions using a gaspack jar system at 37°C for 24 hours. After bacterial colonization, single colonies were selected and inoculated in Rogosa broth selective media and incubated under anaerobic conditions at 37°C for 24 hours.

Extraction DNA of L. reuteri Colonies from Rogosa Broth with Heat Shock Method

A one hundred µL bacterial culture in Rogosa broth was centrifuged at 1,000×g for 10 min. The supernatant was discarded, and the bacterial pellet was washed using 1 mL of phosphate buffered saline (PBS) and then centrifuged again at 10,000×g for 10 min. The supernatant was removed. Meanwhile, 100 µL of dd H2O were added to the pellet. The 1.5 mL micro-centrifugal tube was closed with a Sherlock cap before incubation in a water bath for 20 min at 100°C using a floating boat. The tube was immediately transferred to ice for 10 min. During this process, called heat shock, the cells were broken, allowing the DNA to diffuse from the cells into the supernatant. Samples were homogenized with a vortexer and centrifuged for 2 min at 10,000×g. After centrifugation, the supernatant was transferred to a new 1.5 mL micro-centrifugal tube. These DNA samples were stored for 24 hours at 4°C, followed by storage at -20°C until further analysis.

Identification of L. reuteri from Cultured Colonies Using PCR

Species-specific 16S rRNA primers (the same as above) were used for the identification of L. reuteri in the cultured colonies. The amplification of fragmental DNA was performed via preheating to 94°C for 2 mins, followed by 35 cycles of 20-sec denaturation at the same
temperature, 40-secs annealing at 51°C, and extension at 68°C for a few seconds. Final extension was done for 7 mins at 68°C. The PCR results were visualized via electrophoresis using 1.5% agarose gel.

**Sequencing DNA from PCR Results and BLASTn**

Based on the PCR results of this study, six healthy subjects were positive for *L. reuteri* due to the presence of a 1,100 bp fragment. Four of these samples (3.11, 4.14, 5.15, and 6.14) were chosen as the representative strains (Fig. 3) because they had the thickest bands. The genomic DNA was extracted using a Wizard Genomic DNA Purification Kit (Promega, Wisconsin, USA) from the pure bacterial culture of the samples isolated from the Rogosa broth (Oxoid, Hampshire, UK) and then purified using a Column First Base (Singapore) according to the manufacturer’s protocols. Nucleotide sequences from *L. reuteri* ATCC 55730 were determined via a whole-genome shotgun with small genomic DNA (2 kb) and large genomic DNA (5 kb). A DNA template for sequencing was prepared via DNA amplification using PCR with Ex-Taq (Takara Bio Inc, Shiga, Japan). The nucleotide sequences were analyzed using the Basic Local Alignment Search Tool (BLAST), with a sequence of the 16S rRNA *L. reuteri* ATCC 55730 gene as a control. The average nucleotide identity (ANI) was used to determine the evolutionary distance between the bacterial strains.

**Result**

This study included examinations of 40 subjects, who consisted of 20 healthy subjects, 10 subjects with significant caries activity, and 10 subjects with periodontal disease, using a conventional PCR method. The results of this study showed that 6 among 20 healthy subjects had positive bands for *L. reuteri* 16S rRNA (Fig. 1, 2). However, single colonies of *L. reuteri* had not yet been isolated from these six subjects. Therefore, saliva samples from them were cultured in Rogosa agar for 24 hrs. Fourteen colonies typical of *L. reuteri*24,25 were chosen from each plate and numbered from 1.1 to 1.14, from 2.1 to 2.14, from 3.1 to 3.14, from 4.1 to 4.14, from 5.1 to 5.14, and from 6.1 to 6.14.

After incubation, the DNA was extracted to perform the PCR identification of *L. reuteri*. Some positive bands (1,100 bp) were detected (Fig. 3). Four thickest bands from four different subjects were sequenced for further examination. The result of DNA sequencing of four isolates and *L. reuteri* ATCC 55730 as a control were shown in supplementary data 1.

**Figure 1.** Result of the electrophoresis PCR products from ten caries-free subjects; in subject no. 3 and 9, there were 1,100 bp DNA bands of 16S rRNA of *L. reuteri*. 
Figure 2. Electrophoresis results for PCR products from ten periodontal disease-free subjects: in subject no. 1, 2, 4, and 5, there were 1,100 bp bands of L. reuteri 16S rRNA.

Figure 3. Electrophoresis gel image of L. reuteri 16S rRNA amplicon bands; Lane 1: L. reuteri 4.11; Lane 2: L. reuteri 4.12; Lane 3: L. reuteri 4.13; Lane 4: L. reuteri 4.14; Lane 5: L. reuteri 3.11; Lane 6: L. reuteri 3.12; Lane 7: L. reuteri 5.14; Lane 8: L. reuteri control 1; Lane M: marker (1,100 bp); Lane 9: L. reuteri control 2; Lane 10: L. reuteri 6.11; Lane 11: L. reuteri 6.13; Lane 12: L. reuteri 6.14.

Basic Local Alignment Search Tool (BLAST)

The results of the DNA sequencing were obtained from the DNA isolation of L. reuteri, using alignment with L. reuteri ATCC 55730 DNA as a control. Then, BLAST was used to determine the similarity of the L. reuteri strains.

The DNA sequence alignments of L. reuteri isolate 3.11 (L. reuteri LC382415), 4.14, 5.14 (L. reuteri LC382416) and 6.14 with the L. reuteri ATCC 55730 control were 96%, 99%, 95%, and 99%, respectively.
Table 1. Identification of the *L. reuteri* isolates from Indonesian subjects using BLAST, with *L. reuteri* ATCC 55730 as a control.

<table>
<thead>
<tr>
<th>Isolate No</th>
<th>Identified as</th>
<th>Probability (%) Based on BLAST Analysis as Compared to <em>L. reuteri</em> ATCC 55730</th>
</tr>
</thead>
<tbody>
<tr>
<td>3.11</td>
<td><em>L. reuteri</em> LC382415</td>
<td>96%</td>
</tr>
<tr>
<td>4.14</td>
<td><em>L. reuteri</em></td>
<td>99%</td>
</tr>
<tr>
<td>5.14</td>
<td><em>L. reuteri</em> LC382416</td>
<td>95%</td>
</tr>
<tr>
<td>6.14</td>
<td><em>L. reuteri</em></td>
<td>99%</td>
</tr>
</tbody>
</table>

Discussion

Probiotics work mainly by stimulating the intestinal immune system and by promoting a normal, healthy balance of microbiota in the digestive system.\(^{26,27}\) Initially, probiotics were used in prevention and treatment of gastro-intestinal related disorders, such as diarrhea, intestinal irritation and atopic dermatitis in the children.\(^{28}\) Generally, the microorganism that considered as probiotics bacteria are lactate acid bacteria (LAB), especially the genus of *Lactobacillus* and *Bifidobacterium*. They are normal flora in the human digestive system that can produce high-molecular-mass compounds called bacteriocins to fight against selective pathogenic bacteria. Probiotics bacteria also produce organic acids, such as lactic acid, acetic acid, propionic acid, and phenyllactic acid, as well as other low-molecular-mass compounds, such as hydrogen peroxide, carbon dioxide, ethanol, diacetyl and acetaldehyde, lactoperoxide, lipopolysaccharides, and other antimicrobial agents.\(^{11,29}\) According to the study, from these two main probiotics bacteria, *L. reuteri* has an anti-inflammatory effect through modulation of the cytokine production and host immune response such as interleukin and human beta defensin-2.\(^{30}\)

Not all probiotics can reside in the oral cavity, because every bacterial strain has affinities toward certain tissues.\(^{28}\) Moreover, bacteria also produce bacteriocins to eliminate their competitors and thus obtain optimum nutrients from the environment.\(^{31}\) Currently, in dentistry, probiotics are used as a preventive therapy for dental caries, *Candida albicans* infection, and halitosis. *Streptococcus thermophilus* and *Lactococcus lactis* can weaken dental plaque biofilm formation. Probiotic *Lactobacillus*, which is obtained from the intestine, can limit the growth of *Streptococcus mutans*, the main pioneer pathogen of dental caries.\(^{28}\)

The results of this study support the finding of other studies demonstrating that the isolate of genus *Lactobacillus* can not be found in any patients with periodontitis.\(^ {32}\) Positive results for *L. reuteri* DNA were found in 6 healthy subjects. This suggests that *L. reuteri* represents normal flora in the oral cavity. In this study, isolates 3.11, 4.14, 5.14, and 6.14 (Fig. 3) were selected for DNA sequencing because they showed the thickest 16S rRNA gene bands. After PCR, the sequences of all the isolates, including the control, were aligned via nucleotide-nucleotide BLAST (BLASTn) on the National Center for Biotechnology Information (NCBI) website. The results of the sequencing and BLASTn confirmed that the isolates had similar DNA sequences to that of the *L. reuteri* ATCC 55730 control, i.e., *L. reuteri* 3.11 at 96%, *L. reuteri* 4.14 at 99%, *L. reuteri* 5.14 at 95%, and *L. reuteri* 6.14 at 99%.
The evolutionary distance between two bacterial strains can be calculated using the average nucleotide identity (ANI) of their genes via BLAST, with ANI values of 98–99% indicating that both of these bacteria are almost identical at the nucleotide level. DNA alignment showing a DNA reassociation value > 70% indicates different species and even different genera. Differences between bacterial strains indicate a large number of nucleotide changes, which are indicated by ANI values of 94–97%. In this research project, bacterial strains showing ANI values > 97% were included in the same species, in accordance with re-associated DNA-DNA standards. Thus, it was concluded that two of the four strains of *L. reuteri* isolates were different from *L. reuteri* ATCC 55730 because their ANI values were 96% (isolate 3.11) and 95% (isolate 5.14). This genome sequences of strain 3.11 and 5.14 have been registered at DDBJ/EMBL/GenBank under the accession number LC382412 and LC382416 respectively.

Biochemical methods can be used to identify microorganisms. However, biochemical identification is not accurate enough to distinguish microorganisms phenotypically. BLAST is one of the most rapid and accurate methods of identifying bacteria, determining a new strain of bacteria, or analyzing such a bacteria’s phylogenetic relationships. Basic Local Alignment Search Tool (BLAST) is a method to compare genetic information, such as amino acid sequences from different proteins or nucleotide sequences from DNA. Also, BLAST can compare the sequences of samples listed in a database and identify similarity within certain thresholds using equations. The BLAST results for isolates 3.11, 4.14, 5.14, and 6.14 in this study were 96%, 99%, 95%, and 99%, respectively. This indicates that isolates 3.11 and 5.14 can be considered as new strains and not *L. reuteri* ATCC 55730.

The primers used in this study were 16S rRNA gene-specific for *L. reuteri*. As a control, *L. reuteri* ATCC 55730 was obtained and cultured from chewing gum (BioGaia, Kalbe). *L. reuteri* ATCC 55730 has been widely used in dairy products. The bacteria were first introduced in Sweden in 1991, before being sold in several other countries, such as the US, England, Finland, Spain, Japan, and Korea. In 2000, chewing gum containing *L. reuteri* ATCC 55730 was first introduced in the United States. Then, it continued to make its way to other countries in Europe and Asia. The DNA sequence of *L. reuteri* ATCC 55730 was obtained from GeneBank, with accession number EU394679.2.

This study demonstrates several limitations. First, only salivary samples were collected. The oral microbiome includes supra- and sub-gingival plaque might be more relevant to dental caries and periodontal disease. In this study, only *L. reuteri* were identified from saliva. Therefore, the composition of other *Lactobacillus* species can not be evaluated. Accordingly, these factors should be considered in future studies.

**Conclusion**

*L. reuteri* is a normal oral microbiota that can be isolated from the saliva of healthy subjects. The present study also showed that two of the *L. reuteri* strains obtained from Indonesian saliva isolates had 96% and 95% similarity values compared to *L. reuteri* ATCC 55730, which confirmed the presence of new strains based on their average nucleotide identity (ANI). The isolate strains of 3.11 and 5.14 have been registered at DDBJ/EMBL/GenBank under the accession number LC382415 and LC382416, respectively. Further studies involving biochemical tests and phenotypic analysis are needed to better understand these new *L. reuteri* strains.

**Conflict of interest**

The authors declare that there are no conflicts of interest.

**Acknowledgment**

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References

**Lactobacillus reuteri** strain isolate 3.1.16 ribosomal RNA gene complete sequence

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AGAGTTTGATCCTGACGACAGCCGGGGGTGGTCCATATTACATGGCAACTGGCAGGCCTTGCCCTGTAAATACATGCAAGTCGTACGCACTGGCCCAACTGATTGATGGTGCTTGCACC
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**Lactobacillus reuteri** strain isolate 4.1.16 ribosomal RNA gene complete sequence

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GGGAGTCGGCGGTAGTATCCGGCTACGACAGCCGGGGGTGGTCCATATTACATGGCAACTGGCAGGCCTTGCCCTGTAAATACATGCAAGTCGTACGCACTGGCCCAACTGATTGATGGTGCTTGCACC
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**Lactobacillus reuteri** strain isolate 5.1.16 ribosomal RNA gene complete sequence

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GGGGGGCGCGGCTGAGTACACGTAGGTACCTGCCCCGGAGCGGGGGATAACATTTGGAAACAGATGCTAATACCGCATAACAACAAAACGGGCGATGGCTTTTGAAGATGCTTCCATATTACATGGCAACTGGCAGGCCTTGCCCTGTAAATACATGCAAGTCGTACGCACTGGCCCAACTGATTGATGGTGCTTGCACC
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**The Results of DNA Sequencing of Four Lactobacillus reuteri Clinical Isolates (3,11, 4,14, 5,15 & 6,14) and Lactobacillus reuteri Strain ATCC 55730**

**Lactobacillus reuteri** strain isolate 3.1.16 ribosomal RNA gene complete sequence

```
AGAGTTTGATCCTGACGACAGCCGGGGGTGGTCCATATTACATGGCAACTGGCAGGCCTTGCCCTGTAAATACATGCAAGTCGTACGCACTGGCCCAACTGATTGATGGTGCTTGCACC
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**Lactobacillus reuteri** strain isolate 4.1.16 ribosomal RNA gene complete sequence

```
GGGAGTCGGCGGTAGTATCCGGCTACGACAGCCGGGGGTGGTCCATATTACATGGCAACTGGCAGGCCTTGCCCTGTAAATACATGCAAGTCGTACGCACTGGCCCAACTGATTGATGGTGCTTGCACC
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**Lactobacillus reuteri** strain isolate 5.1.16 ribosomal RNA gene complete sequence

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GGGGGGCGCGGCTGAGTACACGTAGGTACCTGCCCCGGAGCGGGGGATAACATTTGGAAACAGATGCTAATACCGCATAACAACAAAACGGGCGATGGCTTTTGAAGATGCTTCCATATTACATGGCAACTGGCAGGCCTTGCCCTGTAAATACATGCAAGTCGTACGCACTGGCCCAACTGATTGATGGTGCTTGCACC
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**Lactobacillus reuteri** strain isolate 6.1.16 ribosomal RNA gene complete sequence

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**Lactobacillus reuteri** strain isolate 7.1.16 ribosomal RNA gene complete sequence

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**Lactobacillus reuteri** strain isolate 8.1.16 ribosomal RNA gene complete sequence

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**Lactobacillus reuteri** strain isolate 9.1.16 ribosomal RNA gene complete sequence

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**Lactobacillus reuteri** strain isolate 10.1.16 ribosomal RNA gene complete sequence

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**Lactobacillus reuteri** strain isolate 11.1.16 ribosomal RNA gene complete sequence

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**Lactobacillus reuteri** strain isolate 12.1.16 ribosomal RNA gene complete sequence

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**Lactobacillus reuteri** strain isolate 13.1.16 ribosomal RNA gene complete sequence

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**Lactobacillus reuteri** strain isolate 14.1.16 ribosomal RNA gene complete sequence

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**Lactobacillus reuteri** strain isolate 15.1.16 ribosomal RNA gene complete sequence

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**Lactobacillus reuteri** strain isolate 16.1.16 ribosomal RNA gene complete sequence

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**Lactobacillus reuteri** strain isolate 17.1.16 ribosomal RNA gene complete sequence

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**Lactobacillus reuteri** strain isolate 18.1.16 ribosomal RNA gene complete sequence

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**Lactobacillus reuteri** strain isolate 19.1.16 ribosomal RNA gene complete sequence

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**Lactobacillus reuteri** strain isolate 20.1.16 ribosomal RNA gene complete sequence

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**Lactobacillus reuteri** strain isolate 21.1.16 ribosomal RNA gene complete sequence

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**Lactobacillus reuteri** strain isolate 22.1.16 ribosomal RNA gene complete sequence

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**Lactobacillus reuteri** strain isolate 23.1.16 ribosomal RNA gene complete sequence

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**Lactobacillus reuteri** strain isolate 24.1.16 ribosomal RNA gene complete sequence

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**Lactobacillus reuteri** strain isolate 25.1.16 ribosomal RNA gene complete sequence

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**Lactobacillus reuteri** strain isolate 26.1.16 ribosomal RNA gene complete sequence

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**Lactobacillus reuteri** strain isolate 27.1.16 ribosomal RNA gene complete sequence

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**Lactobacillus reuteri** strain isolate 28.1.16 ribosomal RNA gene complete sequence

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**Lactobacillus reuteri** strain isolate 29.1.16 ribosomal RNA gene complete sequence

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**Lactobacillus reuteri** strain isolate 30.1.16 ribosomal RNA gene complete sequence

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**Lactobacillus reuteri** strain isolate 32.1.16 ribosomal RNA gene complete sequence

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**Lactobacillus reuteri** strain isolate 33.1.16 ribosomal RNA gene complete sequence

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**Lactobacillus reuteri** strain isolate 34.1.16 ribosomal RNA gene complete sequence

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**Lactobacillus reuteri** strain isolate 35.1.16 ribosomal RNA gene complete sequence

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**Lactobacillus reuteri** strain isolate 36.1.16 ribosomal RNA gene complete sequence

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**Lactobacillus reuteri** strain isolate 37.1.16 ribosomal RNA gene complete sequence

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**Lactobacillus reuteri** strain isolate 38.1.16 ribosomal RNA gene complete sequence

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**Lactobacillus reuteri** strain isolate 39.1.16 ribosomal RNA gene complete sequence

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**Lactobacillus reuteri** strain isolate 40.1.16 ribosomal RNA gene complete sequence

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**Lactobacillus reuteri** strain isolate 6.14 16S ribosomal RNA gene complete sequence

GGATTTGATCCTGGCTCAGGATGAACGCCGGCGGTGTGCCTAATACATGCAAGTCGTACGCACTGGCCCAACTGATTGATGGTGCTTGCACCTGATTGACGATGGATCACCAGTGAGTGGCGGACGGGTGAGTAACACGTAGGTAACCTGCCCCGGAGCGGGGGATAACATTTGGAAACAGATGCTAATACCGCATAACAACAAAAGCCACATGGCTTTTGTTTGAAAGATGGCTTTGGCTATCACTCTGGGATGGACCTGCGGTGCATTAGCTAGTTGGTAAGGTAACGGCTTACCAAGGCGATGATGCATAGCCGAGTTGAGAGACTGATCGGCCACAATGGAACTGAGACACGGTCCATACTCTACGGGAGGCAGCAGTAGGGAATCTTCCACAATGGGCGCAAGCCTGATGGAGCAACACCGCGTGAGTGAAGAAGGGTTTCGGCTCGTAAAGCTCTGTTGTTGGAGAAGAACGTGCGTGAGAGTAACTGTTCACGCAGTGACGGTATCCAACCAGAAAGTCACGGCTAACTACGTGCCAGCAGCCGCGGTAATACGTAGGTGGCAAGCGTTATCCGGATTTATTGGGCGTAAAGCGAGCGCAGGCGGTTGCTTAGGTCTGATGTGAAAGCCTTCGGCTTAACCGAAGAAGTGCATCGGAAACCGGGCGACTTGAGTGCAGAAGAGGACAGTGGAACTCCATGTGTAGCGGTGGAATGCGTAGATATATGGAAGAACACCAGTGGCGAAGGCGGCTGTCTGGTCTGCAACTGACGCTGAGGCTCGAAAGCATGGGTAGCGAACAGGATTAGATACCCTGGTAGTCCATGCCGTAAACGATGAGTGCTAGGTGTTGGAGGGTTTCCGCCCTTCAGTGCCGGAGCTAACGCATTAAGCACTCCGCCTGGGGAGTACGACCGCAAGGTTGAAACTCAAAGGAATTGACGGGGGCCCGCACAAGCGGTGGAGCATGTGGTTTAATTCGAAGCTACGCGAAGAACCTTACCAGGTCTTGACATCTTGCGCTAACCTTAGAGATAAGGCGTTCCCTTCGGGGACGTAATGACAGGTGGTGCATGGTCGTCGTCAGCTCGTGTCGTGAGATGTTGGGTTAAGTCCCGCAACGAGCGCAACCCTTGTTACTAGTTGCCAGCATTAAGTTGGGCACTCTAGTGAGACTGCCGGTGACACCGGAGGAAGGTGGGGACGACGTCAGATCATCATGCCCCTTATGACCTGGGCTACACACGTGCTACAATGGACGGTACAACGAGTCGCAAGCTCGCGAGAGTAAGCTAATCTCTTAAAGCCGTTCTCAGTTCGGACTGTAGGCTGCAACTCGCCTACACGAAGTCGGAATCGCTAGTAATCGCGGATCAGCATGCCGCGGTGAATACGTTCCCGGGCCTTGTACACACCGCCCGTCACACCATGGGAGTTTGTAACGCCCAAAGTCGGTGGCCTAACCTTTATGGAGGGAGCCGCCTAAGGCGGGACAGATGACTGGGGTGAAGTCGTAACAAGGTAGCCGTAGGAGAACCTGCGGCTGGATCACCTCCTTTCT

**Lactobacillus reuteri** strain ATCC 55730 16S ribosomal RNA gene complete sequence

AGAGTTTGATCCTGGCTCAGGATGAACGCCGGCGGTGTGCCTAATACATGCAAGTCGTACGCACTGGCCCAACTGATTGATGGTGCTTGCACCTGATTGACGATGGATCACCAGTGAGTGGCGGACGGGTGAGTAACACGTAGGTAACCTGCCCCGGAGCGGGGGATAACATTTGGAAACAGATGCTAATACCGCATAACAACAAAAGCCACATGGCTTTTGTTTGAAAGATGGCTTTGGCTATCACTCTGGGATGGACCTGCGGTGCATTAGCTAGTTGGTAAGGTAACGGCTTACCAAGGCGATGATGCATAGCCGAGTTGAGAGACTGATCGGCCACAATGGAACTGAGACACGGTCCATACTCTACGGGAGGCAGCAGTAGGGAATCTTCCACAATGGGCGCAAGCCTGATGGAGCAACACCGCGTGAGTGAAGAAGGGTTTCGGCTCGTAAAGCTCTGTTGTTGGAGAAGAACGTGCGTGAGAGTAACTGTTCACGCAGTGACGGTATCCAACCAGAAAGTCACGGCTAACTACGTGCCAGCAGCCGCGGTAATACGTAGGTGGCAAGCGTTATCCGGATTTATTGGGCGTAAAGCGAGCGCAGGCGGTTGCTTAGGTCTGATGTGAAAGCCTTCGGCTTAACCGAAGAAGTGCATCGGAAACCGGGCGACTTGAGTGCAGAAGAGGACAGTGGAACTCCATGTGTAGCGGTGGAATGCGTAGATATATGGAAGAACACCAGTGGCGAAGGCGGCTGTCTGGTCTGCAACTGACGCTGAGGCTCGAAAGCATGGGTAGCGAACAGGATTAGATACCCTGGTAGTCCATGCCGTAAACGATGAGTGCTAGGTGTTGGAGGGTTTCCGCCCTTCAGTGCCGGAGCTAACGCATTAAGCACTCCGCCTGGGGAGTACGACCGCAAGGTTGAAACTCAAAGGAATTGACGGGGGCCCGCACAAGCGGTGGAGCATGTGGTTTAATTCGAAGCTACGCGAAGAACCTTACCAGGTCTTGACATCTTGCGCTAACCTTAGAGATAAGGCGTTCCCTTCGGGGACGTAATGACAGGTGGTGCATGGTCGTCGTCAGCTCGTGTCGTGAGATGTTGGGTTAAGTCCCGCAACGAGCGCAACCCTTGTTACTAGTTGCCAGCATTAAGTTGGGCACTCTAGTGAGACTGCCGGTGACACCGGAGGAAGGTGGGGACGACGTCAGATCATCATGCCCCTTATGACCTGGGCTACACACGTGCTACAATGGACGGTACAACGAGTCGCAAGCTCGCGAGAGTAAGCTAATCTCTTAAAGCCGTTCTCAGTTCGGACTGTAGGCTGCAACTCGCCTACACGAAGTCGGAATCGCTAGTAATCGCGGATCAGCATGCCGCGGTGAATACGTTCCCGGGCCTTGTACACACCGCCCGTCACACCATGGGAGTTTGTAACGCCCAAAGTCGGTGGCCTAACCTTTATGGAGGGAGCCGCCTAAGGCGGGACAGATGACTGGGGTGAAGTCGTAACAAGGTAGCCGTAGGAGAACCTGCGGCTGGATCACCTCCTTTCT

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