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Change to read:

▲*Limosilactobacillus reuteri*▲ (USP 1-Dec-2023)

Change to read:

DEFINITION

▲*Limosilactobacillus reuteri* (formerly known as *Lactobacillus reuteri*)▲ (USP 1-Dec-2023) is a lactic acid-producing, Gram-positive, rod-shaped, non-spore-forming heterofermentative bacterium. Cells are nonmotile, slightly irregular, bent rods with rounded ends, occurring singly, in pairs, and in small clusters. Suitable cryoprotectants may be added to the concentrated bacteria following fermentation, after which the product is frozen and then freeze-dried. The formulated product may be blended with suitable diluents and/or bulking agents. It contains NLT 100% of the labeled viable cell count of ▲*Limosilactobacillus reuteri*.▲ (USP 1-Dec-2023)

- **Strain 1E1:** *Limosilactobacillus reuteri* 1E1 is a pure, specific strain of ▲*Limosilactobacillus reuteri*.▲ (USP 1-Dec-2023) It produces 41% D-lactate and 59% L-lactate.

▲[NOTE—The nucleic acid-based identification method detailed in *Identification B* applies only to *Limosilactobacillus reuteri* 1E1.]▲ (USP 1-Dec-2023)

IDENTIFICATION

• A. MICROSCOPIC IDENTIFICATION

Analysis: Proceed as directed in the Assay and perform microscopic examination.

Acceptance criteria: Rod-shaped, slightly irregular, bent rods with rounded ends, occurring singly, in pairs, and in small clusters.

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• B. NUCLEIC ACID-BASED IDENTIFICATION

▲ (USP 1-Dec-2023)

Buffer: Use a molecular biology-grade ▲[tris-EDTA buffer, pH 8.0 TS](#).▲ (USP 1-Dec-2023)

Sample solution: 100 mg/mL of the freeze-dried probiotic powder in *Buffer*

Primer set: Use a primer set comprised of forward primer sequence (5'–3') GTCGAAAGTCTTGACAA and reverse primer sequence (5'–3') ACATTGTTCTAGCCACTTGA.¹ Primers should be diluted with *Buffer* to a stock concentration of 100 µM, then further diluted with *Buffer* to 25 µM and stored at –20°. A positive test for this *Primer set* is expected to give an amplification product of about 170 base pairs.

Polymerase chain reaction (PCR) sample preparation: 1 µL of the *Sample solution*, 12 µL of mastermix polymerase,² 1 µL of diluted forward primer (25 µM), 1 µL of diluted reverse primer (25 µM), and 10 µL of ▲[water, DNase/RNase free](#).▲ (USP 1-Dec-2023)

PCR negative control: Prepare as directed for the *PCR sample preparation*, except replace the 1 µL of the *Sample solution* with 1 µL of ▲[water, DNase/RNase free](#).▲ (USP 1-Dec-2023)

PCR positive control: Prepare as directed for the *PCR sample preparation*, except either replace the *Sample solution* with 1 µL of the genomic DNA of the reference strain or replace the strain-specific primers with species-specific universal primers for ▲*Limosilactobacillus reuteri*.▲ (USP 1-Dec-2023)

PCR amplification: Perform PCR on the *PCR sample preparation*, *PCR negative control*, ▲and *PCR positive control*▲ (USP 1-Dec-2023) using an appropriate thermal cycler.³ Incubate at 95° for 7 min (step 1), 95° for 30 s (step 2), ▲52°▲ (USP 1-Dec-2023) for 30 s (step 3), and 72° for 30 s (step 4). Repeat steps 2–4 for 34 cycles, then incubate at 72° for 5 min, and hold at 4°.

Analysis: Analyze the products of the *PCR amplification* for the *PCR sample preparation*, *PCR negative control*, and *PCR positive control* using an automated on-chip electrophoresis system with a DNA kit.⁴ Follow the manufacturer's instructions for analysis. Alternatively, analysis and visualization may be accomplished by using gel electrophoresis. Prepare or use a commercially available 2% (w/v) [agarose](#) gel in a 1X tris-acetic acid–EDTA buffer (40 mM [tris-hydrochloride](#), 1% glacial acetic acid, and 1 mM [EDTA](#)). Stain the gel with 0.5 mg/mL of [ethidium bromide](#) in water and destain with deionized water. [CAUTION—Ethidium bromide is considered a toxic substance and a potential mutagen. Use appropriate personal protective equipment, including nitrile gloves, when handling this reagent.] Use a DNA ladder standard (1 kilobase plus)⁵ suitable for determining the size of linear double-stranded DNA fragments amplified with the *Primer set*. The ladder standard should be used in the first and last lanes on the gel to allow for proper comparison of amplicons.

If using genomic DNA of the strains as positive control, analysis of *PCR positive control* must result in the amplification of specific base pairs as indicated in the acceptance criteria. If using species-specific universal primers as positive control, analysis of *PCR positive control* must result in the amplification of specific base pairs as found in published literature. Analysis of the *PCR negative control* must result in the absence of any amplification products, or the preparation of the *PCR sample preparation*, *PCR negative control*, [▲]and *PCR positive control* [▲](USP 1-Dec-2023) must be repeated, followed by *PCR amplification* and *Analysis*.

Acceptance criteria: The *PCR sample preparation* prepared with the *Primer set* provides an amplification product of about 170 base pairs.

ASSAY

Change to read:

• ENUMERATION

(See [Probiotic Tests \(64\)](#), [Enumeration](#), [Enumeration for Non-Spore-Forming Bacteria Strains](#).)

▲ Agar medium: Prepare *Lactobacilli MRS agar* as directed in the chapter with addition of 1 mL of sterile 5% (w/v) [cysteine hydrochloride](#) solution to each 100 mL for a final cysteine hydrochloride concentration of 0.05% immediately before use. [▲](USP 1-Dec-2023)

Acceptance criteria: NLT 100% of the labeled viable cell count, in cfu/g, of the specified strain of *Limosilactobacillus reuteri*

CONTAMINANTS

[NOTE—The methods of microbial analysis included in this section as examples represent currently accepted methods commonly used in industry. Users may substitute other validated test methods for the methods in this section.]

• **PROBIOTIC TESTS (64), Contaminants, Contaminant Microorganisms:** The total combined molds and yeasts count does not exceed 10^2 cfu/g. The total non-lactic acid bacterial count is less than 5×10^3 cfu/g.

• **PROBIOTIC TESTS (64), Contaminants, Specified Microorganisms:** It meets the requirements of the test for absence of *Escherichia coli* in 10 g. It meets the requirements of the test for absence of *Salmonella* species in 40 g.

• **LISTERIA:** (See *Food Chemicals Codex*, Appendix XV.) It meets the requirements of the test for absence of *Listeria* in 25 g.

ADDITIONAL REQUIREMENTS

Change to read:

• **PACKAGING AND STORAGE:** [▲]Protect from moisture using [▲](USP 1-Dec-2023) high barrier foil laminate bags and store at or below 4°.

Change to read:

• **LABELING:** This ingredient should be labeled with the genus, species, and strain name and with the formulated enumeration in cfu/g [▲](USP 1-Dec-2023) · This monograph applies only to [▲]*Limosilactobacillus reuteri* [▲](USP 1-Dec-2023) strain 1E1.

¹ DNA primers are commercially available (custom manufacture) from Integrated DNA Technologies (www.idtdna.com/pages) and other commercial sources.

² AmpliTaq Gold 360 Master Mix from Applied Biosystems (www.thermofisher.com) or equivalent.

³ Suitable thermal cyclers are available from Eppendorf (www.eppendorf.com).

⁴ Suitable automated on-chip electrophoresis systems with DNA kits (e.g., Agilent 2100 Bioanalyzer with Agilent DNA 1000 Kit) are available from Agilent (www.genomics.agilent.com).

⁵ Suitable 1-kilobase-plus DNA ladders are available from Life Technologies (<https://www.thermofisher.com/us/en/home.html>).

Auxiliary Information - Please [check for your question in the FAQs](#) before contacting USP.

Topic/Question	Contact	Expert Committee
LACTOBACILLUS REUTERI	Binu Koshy Scientific Liaison	NBDS2020 Non-botanical Dietary Supplements

Chromatographic Database Information: [Chromatographic Database](#)

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