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Change to read:

▲*Lactacaseibacillus paracasei*▲ (USP 1-Aug-2022)

Change to read:

DEFINITION

▲*Lactacaseibacillus paracasei* (formerly *Lactobacillus paracasei*)▲ (USP 1-Aug-2022) is a lactic acid-producing, Gram-positive, rod-shaped, non-motile, non-spore-forming homofermentative bacterium. ▲▲ (USP 1-Aug-2022) 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 ▲the appropriate strain of *Lactacaseibacillus paracasei*.

- **Strain LPC-37:** *Lactacaseibacillus paracasei* LPC-37 (ATCC strain designation SD5275) is a pure, specific strain of *Lactacaseibacillus paracasei*. It is a non-D-lactate producing strain.

[NOTE—The nucleic acid-based identification method detailed in *Identification B* applies only to *Lactacaseibacillus paracasei* LPC-37.]▲ (USP 1-Aug-2022)

IDENTIFICATION

Add the following:

▲• A. MICROSCOPIC IDENTIFICATION

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

Acceptance criteria: Present as rods▲ (USP 1-Aug-2022)

Change to read:

• ▲B.▲ (USP 1-Aug-2022) NUCLEIC ACID-BASED IDENTIFICATION

[NOTE—In all cases for the *Identification* test, “sterile water” refers to sterile, nuclease-free water acceptable for use in molecular biology.¹]

Buffer: Use a molecular biology-grade 10 mM tris-hydrochloride, 1 mM EDTA sodium buffer.²

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') GTTTGTGGCGGCGTAACTTC and reverse primer sequence (5'–3') GGTGATCCTGAACGCGGTT.³ Primers should be diluted in *Buffer* to a stock concentration of 100 µM, then further diluted to 25 µM in *Buffer*, and stored at –20°. ▲▲ (USP 1-Aug-2022)

Polymerase chain reaction (PCR) sample preparation: 1 µL of the *Sample solution*, 10 µL of mastermix polymerase,⁴ 1 µL of diluted forward primer (25 µM), 1 µL of diluted reverse primer (25 µM), and 12 µL of sterile water.

PCR negative control: Prepare as directed for the *PCR sample preparation*, except replace the 1 µL of *Sample solution* with 1 µL of sterile water.

▲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 *L. paracasei*.▲ (USP 1-Aug-2022)

PCR amplification: Perform PCR on the *PCR sample preparation*, the *PCR negative control*, ▲and the *PCR positive control*▲ (USP 1-Aug-2022)

using an appropriate thermal cycler.⁵ Incubate at 95° for 7 min (step 1), 95° for 30 s (step 2), 57° for 30 s (step 3), and at 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*, the *PCR negative control*, ▲and the *PCR positive control*▲ (USP 1-Aug-2022) using an automated on-chip electrophoresis system with a DNA kit.⁶ Follow the manufacturer's instructions for analysis. Alternatively, analysis and visualization may be accomplished using gel electrophoresis. Prepare or use a commercially available ▲2%▲ (USP 1-Aug-2022) (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 de-stain 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 KB plus)⁷ suitable for determining the size of linear double-stranded DNA fragments

between 100 and 12,000 base pairs. 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 the *PCR positive control* must result in an amplification of specific base pairs as indicated in the acceptance criteria. If using species-specific universal primers as positive control, analysis of the *PCR positive control* must result in the amplification as found in published literature. ▲ (USP 1-Aug-2022) 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-Aug-2022) must be repeated, followed by *PCR amplification* and *Analysis*.

Acceptance criteria: The *PCR sample preparation* prepared with the *Primer set* gives an amplification product of 273 base pairs.

ASSAY

Change to read:

• ENUMERATION

▲ (See [Probiotic Tests \(64\)](#), [Enumeration](#), [Enumeration for Non-Spore-Forming Bacteria Strains](#).) ▲ (USP 1-Aug-2022)

Acceptance criteria: NLT 100% of the labeled viable cell count, in cfu/g

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.]

Delete the following:

▲ • [MICROBIAL ENUMERATION TESTS \(2021\)](#): The total combined molds and yeasts count does not exceed 10^2 cfu/g. ▲ (USP 1-Aug-2022)

Add the following:

▲ • [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. ▲ (USP 1-Aug-2022)

Delete the following:

▲ • **NON-LACTIC ACID BACTERIA:** ISO international standard number 13559 (IDF 153), available from the International Organization for Standardization (www.iso.org). The total non-lactic acid bacteria count is less than 5×10^3 cfu/g. ▲ (USP 1-Aug-2022)

• **ENTEROCOCCI:** (See *Food Chemicals Codex*, Appendix XV.) The total enterococci count is less than 10^2 cfu/g.

• **COLIFORMS:** (See *Food Chemicals Codex*, Appendix XV.) The total coliforms count is less than 10 cfu/g.

Change to read:

• [ABSENCE OF SPECIFIED MICROORGANISMS \(2022\)](#), [Test Procedures](#), [Test for Absence of *Staphylococcus aureus*](#) ▲ (USP 1-Aug-2022) : It meets the requirements of the test for absence of *Staphylococcus aureus*. ▲ (USP 1-Aug-2022)

Add the following:

▲ • [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. ▲ (USP 1-Aug-2022)

• **LISTERIA:** (See *Food Chemicals Codex*, Appendix XV.) It meets the requirements of the tests for absence of *Listeria* in 25 g, if the production lot of the strain has been in contact with any dairy-derived ingredients.

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in 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 names and with the formulated enumeration in cfu/g ▲ or dose/g. ▲ (USP 1-Aug-2022) This monograph applies only to ▲ *Lactobacillus paracasei* LPC-37. ▲ (USP 1-Aug-2022)

¹ Suitable PCR-Certified Waters, RNase and DNase Free, are available from www.teknova.com.

² Suitable buffers (e.g., TE Buffer 1X, Molecular Biology Grade) are available from www.promega.com.

³ DNA primers are commercially available (custom manufacture) from Integrated DNA Technologies (www.idtdna.com/pages) and other commercial sources.

⁴ ▲ AmpliTaq Gold 360 Master Mix from Applied Biosystems (www.thermofisher.com) or equivalent. ▲ (USP 1-Aug-2022)

⁵ Suitable thermal cyclers are available from Eppendorf (www.eppendorf.com).

⁶ Suitable automated on-chip electrophoresis systems with a DNA kit are available from Agilent (Agilent 2100 Bioanalyzer with Agilent 1000 Kit www.genomics.agilent.com).