

Status: Currently Official on 15-Feb-2025
Official Date: Official as of 01-Mar-2024
Document Type: Dietary Supplement Monographs
DocId: GUID-8A12BD5E-E9D9-4298-BAD1-23DC7841CC85_3_en-US
DOI: https://doi.org/10.31003/USPNF_M15341_03_01
DOI Ref: 7t8y5

© 2025 USPC
Do not distribute

Ligilactobacillus salivarius

Change to read:

DEFINITION

Ligilactobacillus salivarius (formerly known as *Lactobacillus salivarius*) is a lactic acid-producing, Gram-positive, rod-shaped, non-spore-forming, facultatively anaerobic bacterium that is homofermentative. Suitable cryoprotectants may be added to the concentrated bacteria following fermentation, after which the product is frozen and 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 specified strain of *Ligilactobacillus salivarius*.

- **Strain Ls-33:** *Ligilactobacillus salivarius* Ls-33 (ATCC SD-5208) is a pure, specific strain of *Ligilactobacillus salivarius*. It is a non-D-lactate producer strain.

▲▲ (IRA 1-Mar-2024)

IDENTIFICATION

• A. MICROSCOPIC IDENTIFICATION

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

Acceptance criteria: Rod-shaped bacterium

Change to read:

• B. NUCLEIC ACID-BASED IDENTIFICATION

▲[NOTE—This nucleic acid-based method applies only to *Ligilactobacillus salivarius* Ls-33.]▲ (IRA 1-Mar-2024)

Buffer: Use a molecular biology-grade ▲[tris-EDTA buffer, pH 8.0 TS](#).▲ (IRA 1-Mar-2024)

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

Primer set 1: Use a primer set comprised of forward primer sequence (5'–3') TCATTGCAACACTCCTTTATTCC and reverse primer sequence (5'–3') ACAGTATGGAGGGTGCTAATC.¹ Dilute primers in *Buffer* to a stock concentration of 100 µM, further dilute to 25 µM in *Buffer*, and store at –20°.

Primer set 2: Use a primer set comprised of forward primer sequence (5'–3') CTTACGTAATCTCTTCCAGTTGA and reverse primer sequence (5'–3') TTATCTGGTGACCACATCTATAAGAT. Dilute primers in *Buffer* to a stock concentration of 100 µM, further dilute to 25 µM in *Buffer*, and store at –20°.

Polymerase chain reaction (PCR) sample preparation: 1 µL of the *Sample solution*, 12.5 µL of mastermix polymerase,² 1 µL of diluted forward primer (25 µM), 1 µL of diluted reverse primer (25 µM), and 9.5 µL of ▲[water, DNase/RNase free](#).▲ (IRA 1-Mar-2024)

PCR negative control: Prepare as directed for the *PCR sample preparation*, except replace the 1 µL of *Sample solution* with 1 µL of ▲[water, DNase/RNase free](#).▲ (IRA 1-Mar-2024)

PCR positive control: Prepare as directed for the *PCR sample preparation*, except either replace the 1 µL of *Sample solution* with 1 µL of genomic DNA of the reference strain or replace the strain-specific primers with species-specific universal primers for *L. salivarius*.

PCR amplification: Perform PCR on the *PCR sample preparation*, *PCR positive control*, and *PCR negative control* using an appropriate thermal cycler.³ Incubate at 95° for 5 min (step 1); 95° for 30 s (step 2); 53° for 30 s (step 3); and at 72° for 1 min (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 positive control*, and *PCR negative control* ▲with suitable methods, which include an automated on-chip electrophoresis system, an automated capillary electrophoresis system, or equivalent.▲ (IRA 1-Mar-2024) ⁴ Follow the manufacturer's instructions for analysis. Alternatively, analysis and visualization may be accomplished 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 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)⁵ suitable for determining the size of linear double-stranded DNA fragments amplified with the specified *Primer set*. The ladder standard should be used in the first and last lanes on the gel to allow for proper comparison of amplicons. Analysis of the *PCR positive control* must result in the amplification of specific base pairs as indicated in the *Acceptance criteria* if using genomic DNA of the strain or must result in the amplification of specific base pairs as found in the published literature if

using species-specific universal primers. Analysis of the *PCR negative control* must result in the absence of any amplification products. If the results are not as expected, preparation of the *PCR sample preparation*, *PCR positive control*, and *PCR negative control* must be repeated, followed by *PCR amplification* and *Analysis*.

Acceptance criteria

Primer set 1: The *PCR sample preparation* prepared with *Primer set 1* produces an amplification product of 159 base pairs.

Primer set 2: The *PCR sample preparation* prepared with *Primer set 2* produces an amplification product of 241 base pairs with 1 single nucleotide polymorphism (SNP). The SNP sequence is identified as (underlined in the following 11 base pair sequence) AATTCcGAAAC and the location is 121 base pairs from the 5' end of the forward primer. The sequence of the amplicon should be determined by validated, standard sequencing technologies.

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. Immediately before use, add 1 mL of sterile 5% (w/v) [cysteine hydrochloride](#) solution to each 100 mL for a final cysteine hydrochloride concentration of 0.05%. ▲ (IRA 1-Mar-2024)

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

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 10² cfu/g. The total non-lactic acid bacteria count is less than 5 × 10³ cfu/g.
- [PROBIOTIC TESTS \(64\)](#), [Contaminants](#), [Specified Microorganisms](#): It meets the requirements of the tests for the absence of *Escherichia coli* in 10 g and for the 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 high-barrier foil laminate bags ▲ (IRA 1-Mar-2024) and store at or below 4°.
- **LABELING:** This ingredient should be labeled with the genus, species, and strain names and with the formulated enumeration in cfu/g. This monograph applies only to *Ligilactobacillus salivarius* strain Ls-33.

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

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

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

⁴ ▲ Systems include E-Gel Precast Agarose Electrophoresis System available from Thermo Fisher Scientific (www.thermofisher.com), 4200 TapeStation System from Agilent (www.agilent.com), and SeqStudio Genetic Analyzer from Thermo Fisher Scientific (www.thermofisher.com), as well as similar products from other manufacturers. ▲ (IRA 1-Mar-2024)

⁵ Suitable 1 KB plus DNA ladders are available from www.lifetechnologies.com.

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

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

Chromatographic Database Information: [Chromatographic Database](#)

Most Recently Appeared In:

Pharmacopeial Forum: Volume No. 49(5)

Current DocID: GUID-8A12BD5E-E9D9-4298-BAD1-23DC7841CC85_3_en-US

DOI: https://doi.org/10.31003/USPNF_M15341_03_01