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Lactobacillus acidophilus

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DEFINITION

Lactobacillus acidophilus is a lactic acid-producing, Gram-positive, non-motile, non-spore-forming homofermentative bacterium presenting as rods, sometimes in short chains. 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 specified strain of *Lactobacillus acidophilus*.

- **Strain La-14:** *Lactobacillus acidophilus* La-14 (ATCC strain designation SD5212) is a pure, specific strain of *Lactobacillus acidophilus*. It produces 60% L-lactate and 40% D-lactate.
- **Strain NCFM:** *Lactobacillus acidophilus* NCFM (ATCC strain designation SD5221) is a pure, specific strain of *Lactobacillus acidophilus*. It produces 66% L-lactate and 34% D-lactate.

▲ (IRA 1-Mar-2024)

IDENTIFICATION

• A. MICROSCOPIC IDENTIFICATION

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

Acceptance criteria

- Strain La-14:** Presents as rods
Strain NCFM: Presents as rods in short chains

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• B. NUCLEIC ACID-BASED IDENTIFICATION

▲[NOTE—This nucleic acid-based identification method applies only to *Lactobacillus acidophilus* La-14 and NCFM.]▲ (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 sets: See [Table 1](#).

Table 1. Primer Sets and Acceptance Criteria

Strain	Primer Set ^a	Primer Sequence	Acceptance Criteria ^b
La-14	1	Forward (5'–3') AAACTGCAATTTAAGATTATGA GTTTC Reverse (5'–3') GGTACCGTCTTGATTATTAGTG TA The annealing temperature for primer set is 51.0°	Amplification product of 184 base pairs. There should be no amplification product of 600 base pairs.
NCFM	1	Forward (5'–3') TCGGCAAACCTTAGTTGCAGAA GG Reverse (5'–3') GGTGCTAAGCTAGGTATGGAAC The annealing temperature for primer set is 57.0°	Amplification product of 365 base pairs with two single nucleotide polymorphisms (SNPs). The first SNP is identified as GAGTAAATCCATCA (underlined in the 15 base pair sequence), and the location is 50 base pairs from the 5' end of the forward primer. The second

Strain	Primer Set ^a	Primer Sequence	Acceptance Criteria ^b
			SNP is identified as TTCAATTGAAGTTTG (underlined in the 15 base pair sequence), and the location is 242 base pairs from the 5' end of the forward primer. The sequence of the amplicon should be determined by validated, standard sequencing technologies, such as Sanger sequencing.
	2	Forward (5'–3') GCTGACGGAGATTATTCAAGCC A Reverse (5'–3') GCAGAACACATACGCTACCAG A The annealing temperature for primer set is 57.0°	Amplification product of 433 base pairs with two SNPs. The first SNP is identified as AAGCATTCAATACAC (underlined in the 15 base pair sequence), and the location is 72 base pairs from the 5' end of the forward primer. The second SNP is identified as ATATGGTCTTACCT (underlined in the 15 base pair sequence), and the location is 215 base pairs from the 5' end of the forward primer. The sequence of the amplicon should be determined by validated, standard sequencing technologies, such as Sanger sequencing.
	3	Forward (5'–3') AAACTGCAATTTAAGATTATGA GTTTC Reverse (5'–3') GGTACCGTCTTGATTATTAGTG T The annealing temperature for primer set is 51.0°	Amplification product of 600 base pairs. There should be no amplification product of 184 base pairs.

^a DNA primers are commercially available (custom manufacture) from Integrated DNA Technologies (<https://www.idtdna.com/pages>) and other commercial sources.

^b Acceptance criteria for each primer set must be met to prove strain identity.

Primer dilution: Dilute primers with *Buffer* to a stock concentration of 100 µM, further dilute with *Buffer* to 25 µM, and store at –20°.

Polymerase chain reaction (PCR) sample preparation: 1 µL of the *Sample solution*, 10 µL of mastermix polymerase,¹ 1 µL of diluted forward primer (25 µM), 1 µL of diluted reverse primer (25 µM), and 12 µL of ▲[water, DNase/RNase free](#) ▲ (IRA 1-Mar-2024)

PCR negative control: Prepare as directed for the *PCR sample preparation*, except replace 1 µL of the *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 *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. acidophilus*.

PCR amplification: See [Table 2](#). For each primer set, perform PCR on the *PCR sample preparation*, *PCR negative control*, and *PCR positive control* using a thermal cycler.²

Table 2. PCR Amplification