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Change to read:

▲Lacticaseibacillus rhamnosus▲ (USP 1-Dec-2023)

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DEFINITION

▲Lacticaseibacillus rhamnosus (formerly known as *Lactobacillus rhamnosus*)▲ (USP 1-Dec-2023) is a Gram-positive, rod-shaped, heterofermentative non-spore-forming bacterium. Cells are nonmotile rods often with square ends, occurring singly or in chains. Arginine is not hydrolyzed. 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 ▲Lacticaseibacillus rhamnosus.▲ (USP 1-Dec-2023)

- **Strain HN001:** *Lacticaseibacillus rhamnosus* HN001 is a pure, specific strain of ▲Lacticaseibacillus rhamnosus.▲ (USP 1-Dec-2023) It is a non-D-lactate producer strain. It contains plasmids.
- **Strain GG:** *Lacticaseibacillus rhamnosus* GG is a pure, specific strain of ▲Lacticaseibacillus rhamnosus.▲ (USP 1-Dec-2023) It is a non-D-lactate producer strain. It contains no plasmid.

▲[NOTE—The nucleic acid-based identification method detailed in *Identification B* applies only to *Lacticaseibacillus rhamnosus* HN001 and GG.]▲ (USP 1-Dec-2023)

IDENTIFICATION

• **A. MICROSCOPIC IDENTIFICATION**

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

Acceptance criteria

Strain HN001: Presents as rods of varying length, occurring in short chains.

Strain GG: Presents as small uniform rods, occurring in chains.

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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 sets: See [Table 1](#).

Table 1. Primer Sets and Acceptance Criteria

Strain	Primer Set ^a	Forward Primer Sequence	Reverse Primer Sequence	Acceptance Criteria ^b
HN001	1	(5'–3') CACCTGCGATCAAAGCG AAAC	(5'–3') GCTCCCACCGGCACATT A	Amplification product of 340 base pairs.
GG	1	(5'–3') GTGATCGGAACATCTTT GGCCACTTGTTTG	(5'–3') ACTTAATCAAACAAGGC AAGCTGGACGATG	Amplification product of 552 base pairs; it may result in different amplicons in other strains.
	2	(5'–3') CTTCCACATTGCGCCAA ACA	(5'–3') ACAACCTTCACCACGAC CAA	Amplification product of 388 base pairs with 1 single nucleotide polymorphism (SNP); it

Strain	Primer Set ^a	Forward Primer Sequence	Reverse Primer Sequence	Acceptance Criteria ^b
				may result in a similar amplicon in other strains. The SNP sequence is identified as (underlined in the following 21 base pair sequence) ACCAATGTAA <u>g</u> CATACC CATC and the location is 250 base pairs from the 3' end of the forward primer described in <i>Primer set 2</i> . The sequence of the amplicon should be determined by validated, standard sequencing technologies such as Sanger sequencing.
				Amplification product of 293 base pairs with 2 SNPs; it may result in a similar amplicon in other strains. The first SNP is identified as (underlined in the following 21 base pair sequence) TCTTCCCGAC <u>t</u> TTAGCA GGGA and the location is 71 base pairs from the 3' end of the forward primer described in <i>Primer set 3</i> . The second SNP is identified as (underlined in the following 21 base pair sequence) TCAGGCGCA <u>t</u> TATGAAG ATGG and the location is 98 base pairs from the 3' end of the forward primer described in <i>Primer set 3</i> . The sequence of the amplicon should be determined by validated, standard sequencing technologies such as Sanger sequencing.
	3	(5'–3') GCCGGTTGTCAGTGAC CATA	(5'–3') AGACAAATGCCTGGATG CCT	
	4	(5'–3') TGCAACACATCAAGCGA TTGG	(5'–3') TCATAACCAGCATACGC CGG	Amplification product of 490 base pairs. There should be no amplification product of 367 base pairs; it may result in a similar

