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Add the following:

^Streptococcus thermophilus

DEFINITION

Streptococcus thermophilus (formerly known as *Streptococcus salivarius* subsp. *thermophilus*) is a lactic acid-producing, Gram-positive, cocci-shaped, non-spore-forming, facultatively anaerobic bacterium that is homofermentative. Cells are non-motile, commonly found in linear chains. 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 *Streptococcus thermophilus*.

- **Strain St-21:** *Streptococcus thermophilus* St-21 (ATCC SD-5207) is a pure, specific strain of *Streptococcus thermophilus*. It is a non-lactate producer strain.

[NOTE—The nucleic acid-based method detailed in *Identification B* applies only to *Streptococcus thermophilus* St-21.]

IDENTIFICATION

• A. MICROSCOPIC IDENTIFICATION

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

Acceptance criteria: Cocci-shaped bacterium found in linear chains

• B. NUCLEIC ACID-BASED IDENTIFICATION

[NOTE—In all cases for *Identification B*, “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 1: Use a primer set comprised of forward primer sequence (5'-3') AAAACTCCAAAATGATCAGCAACTT and reverse primer sequence (5'-3') TTCATTAGTTGATTGTGTCAAAGGG.³ Dilute primers with *Buffer* to a stock concentration of 100 µM, further dilute with *Buffer* to 25 µM, and store at -20°.

Primer set 2: Use a primer set comprised of forward primer sequence (5'-3') GCCACACGGACTCCTTGTTA and reverse primer sequence (5'-3') GCGAACCCTGTGACATCAACC. 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*, 12.5 µL of master mix polymerase,⁴ 1 µL of diluted forward primer (25 µM), 1 µL of diluted reverse primer (25 µM), and 9.5 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 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 *S. thermophilus*.

PCR amplification: Perform PCR on the *PCR sample preparation*, *PCR negative control*, and *PCR positive control* using an appropriate thermal cycler.⁵ Incubate at 95° for 5 min (step 1), 95° for 30 s (step 2), 54° 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 negative control*, and *PCR positive control* 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% (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 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 217 base pairs.

Primer set 2: The *PCR sample preparation* prepared with *Primer set 2* produces an amplification product of 179 base pairs with 1 single nucleotide polymorphism (SNP). The SNP sequence is identified as (underlined in the following 11 base pair sequence) GCAATaTACGA and the location is 103 base pairs from the 5' end of the forward primer. The sequence of the amplicon should be determined by validated, standard sequencing technologies.

ASSAY

• ENUMERATION

Elliker broth: Prepare as follows (see [Table 1](#)) or use a suitable commercially available agar.⁸

Table 1. Elliker Broth Preparation

Reagent	Quantity (g)
Casein enzymic hydrolysate	20.0
Yeast extract	5.0
Gelatin	2.5
Dextrose	5.0
Lactose	5.0
Saccharose	5.0
Sodium chloride	4.0
Sodium acetate	1.5
Ascorbic acid	0.5
Final pH (25°)	6.8 ± 0.2

Elliker agar medium: Suspend *Elliker broth* in 1 L of purified water in an appropriately sized conical flask or beaker (sufficiently large enough to prevent boiling over) and add 15-g/L agar.⁹ Cover the flask or beaker with aluminum foil and mix the ingredients for 15 min on a stir plate. Heat the mixture at 100° for 30 min to allow the agar to dissolve completely. Mix for another 15 min for a homogenous solution, and then autoclave the solution at 121° for 15 min. *Elliker agar medium* also may be aseptically transferred to individual media bottles in 100- or 200-mL aliquots before sterilizing, and then autoclaved and stored for later use. [NOTE—*Elliker agar medium* can be stored at 4° (heat gently to 45° to melt the medium before use). Do not store prepared *Elliker agar medium* at 4° for more than 3 months.]

Buffered peptone water: Prepare as follows (see [Table 2](#)) or use a suitable commercially available buffered peptone water.¹⁰

Table 2. Buffered Peptone Water Preparation

Reagent	Quantity (g)
Peptone ^a	10.0
Sodium chloride	5.0



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