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Bacillus coagulans Capsules

DEFINITION

Bacillus coagulans Capsules contain NLT 100% of the labeled viable cell count of the *Bacillus coagulans* GBI-30, 6086 strain.

IDENTIFICATION

• NUCLEIC ACID-BASED IDENTIFICATION

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

Sample preparation reagent: Use PrepMan™ Ultra Sample Preparation Reagent.²

Sample: The preparation of the *Sample* requires isolation of colonies grown on plates in the test for *Enumeration*. Surface colonies grown in the *Enumeration* test should be carefully removed from incubated *BC agar medium* plates following counting of the colonies as described in the *Assay* as follows. Dispense 100 µL of *Sample preparation reagent* into a 2.0-mL microcentrifuge tube with a screw cap closure. [NOTE—Dispense the *Sample preparation reagent* according to the manufacturer's instructions.] Using a sterile loop, carefully remove the cells from a single surface colony and suspend the cells in the microcentrifuge tube. Cap the tube, and vortex for 10–30 s or until the cells are homogeneously suspended in the *Sample preparation reagent*. Heat the mixture for 10 min in a water bath held at 100°, then centrifuge the mixture for 3 min at 16,000 × g. Carefully transfer the supernatant to a new microcentrifuge tube. This solution contains the *Bacillus coagulans* GBI-30, 6086 genomic DNA. Before analysis, prepare the *Sample* by diluting an aliquot of this solution with sterile water (1:100, v/v).

Primer set 301: Use a set of 301_Left, primer sequence (5'–3') AAGAAGTGGATGTGGGCCGTTC and 301_Right, primer sequence (5'–3') CTTTGGCCATGCCAGCATCC.³ Primers should be diluted to 2 µg/mL in sterile water and stored between –20° and –80°. Immediately before use, dilute an aliquot of each primer with sterile water (1:5, v/v). The annealing temperature for *Primer set 301* is 65°.

Primer set 210243: Use a set of 210243_Left, primer sequence (5'–3') GCCTGATGCAGGGCTTTTCCT and 210243_Right, primer sequence (5'–3') CCGTCCGCTTCCGTTAAGCCG. Primers should be diluted to 2 µg/mL in sterile water and stored between –20° and –80°. Immediately before use, dilute an aliquot of each primer with sterile water (1:5, v/v). The annealing temperature for *Primer set 210243* is 70°.

Primer set 31125: Use a set of 31125_Left, primer sequence (5'–3') TTATAGGCGGTAGCAGGGATC and 31125_Right, primer sequence (5'–3') CGATTGTTTTCCGAAGCA. Primers should be diluted to 2 µg/mL in sterile water and stored between –20° and –80°. Immediately before use, dilute an aliquot of each primer with sterile water (1:5, v/v). The annealing temperature for *Primer set 31125* is 61°.

Polymerase chain reaction (PCR) sample preparations: For each primer set, prepare a PCR sample preparation that contains 7.5 µL of polymerase master mix,⁴ 1 µL of diluted primer left (approximately 400 ng), 1 µL of diluted primer right (approximately 400 ng), 1 µL of diluted *Sample* containing template DNA (approximately 10–100 ng), and 4.5 µL of sterile water.

PCR negative control: Prepare as directed for the *PCR sample preparations*, replacing the 1 µL of diluted *Sample* with 1 µL of nuclease-free water.

PCR amplification: Perform PCR on each PCR sample preparation and the *PCR negative control* using a thermal cycler.⁵ Incubate at 98° for 30 s (1 cycle), followed by 30 cycles at 98° for 10 s, annealing temperature for 10 s, and 1 cycle at 72° for 45 s, followed by a final incubation at 72° for 5 min with a hold at 4°.

Analysis: Analyze the products of the *PCR amplification* for each PCR sample preparation and for the *PCR negative control* using an automated on-chip electrophoresis system with a DNA kit.⁶ Follow the manufacturer's instructions for analysis. Analysis of the *PCR negative control* must result in the absence of any amplification products or the preparation of the *PCR sample preparations* and the *PCR negative control* must be repeated, followed by *PCR amplification* and *Analysis*.

Acceptance criteria

Primer set 301: The PCR sample preparation prepared with *Primer set 301* produces an amplification product of 376–399 base pairs.

Primer set 210243: The PCR sample preparation prepared with *Primer set 210243* does not produce the expected amplification product of 1253–1331 base pairs.

Primer set 31125: The PCR sample preparation prepared with *Primer set 31125* produces an amplification product of 329–339 base pairs.

ASSAY

Change to read:

• ENUMERATION

Peptone diluent: Prepare a solution of 0.1% peptone⁷ in water (w/v) and adjust with a solution of lactic acid to a pH of 7.0. Using an autoclave, steam sterilize the solution at 121° for NLT 15 min, then allow to cool in the unopened autoclave. Dispense into sterile containers as needed for preparing samples.

▲Trace mineral solution:▲ (ERR 1-Jun-2019) Prepare a solution containing the mineral concentrations shown in [Table 1](#) in deionized water.

Table 1. Preparation of Trace Mineral Solution

Reagent	Quantity (mg/mL)
Sodium chloride	10
Iron(II) sulfate, heptahydrate	18
Manganese(II) sulfate, monohydrate	16
Zinc sulfate, heptahydrate	1.6
Copper(II) sulfate, pentahydrate	1.6
Cobalt(II) sulfate, heptahydrate	1.6

[NOTE—The solution will be slightly pink in color. It may be refrigerated for up to 2 months. In the case of hydrated salts, users may substitute other hydration forms so long as the mineral salt concentration is maintained in the final solution.]

BC agar medium: Prepare as shown in [Table 2](#).

Table 2. Preparation of Glucose Yeast Extract BC Agar Medium

Reagent	Quantity
Yeast extract powder	5.0 g
Peptone	5.0 g
D-Glucose	5.0 g
Dibasic potassium phosphate (K_2HPO_4)	0.5 g
Monobasic potassium phosphate (KH_2PO_4)	0.5 g
Magnesium sulfate	0.3 g
Trace mineral solution	1.0 mL
Water	1000.0 mL

Adjust the mixture with a solution of lactic acid to a pH of 6.3. Transfer the mixture to a large conical flask, add 15.0 g of bacteriological agar to the flask, cover the flask with aluminum foil, and bring to boiling on a hot plate with stirring. Allow the mixture to boil until the agar has completely dissolved, then sterilize in an autoclave at 121° for NLT 15 min. Once the autoclave can safely be opened, remove the flask and incubate in a water bath at 50° until needed for plating. [NOTE—Can be stored at 4° (allow the agar to come to room temperature before use).]

Sample preparation: Weigh the contents of NLT 20 Capsules and determine the average weight. Empty the Capsules and transfer 1.00 g of the Capsule contents into a sterile stomacher bag. Add 199 mL of previously sterilized *Peptone diluent* to the bag and mix at about 150–200 rpm for 5 min in a stomacher. Check the pH of the suspension. If the pH is below 7.0, adjust with 5 N sodium hydroxide solution to a pH of 8.5 ± 0.2 . If the pH is above 8.7, adjust with 5 N lactic acid solution to a pH of 8.5 ± 0.2 . Transfer 20–30 mL of the homogenized suspension to a sterile 50-mL conical centrifuge tube with a cap. Incubate the tube in a water bath held at 75° for exactly 30 min, then immediately cool to below 45°. Transfer 1.0 mL of the cooled suspension into a sterile test tube containing 9 mL of *Peptone diluent*, previously prepared. Mix thoroughly by vortexing. This suspension represents a 0.5×10^{-3} dilution of the sample. Repeat dilution in a succession of test tubes until the final dilution is expected to contain about 30 cfu/mL. The final three dilutions will be used in the *Analysis*. [NOTE—The *Sample preparation* should be performed in duplicate. Take care to plate the *Sample preparation* dilutions within 10–20 min of preparation.]