

**PSEUDOMONAS AERUGINOSA
CONFIRMATION METHOD**

PRINCIPLE

Pseudomonas aeruginosa is isolated from samples by using an enrichment procedure. It is confirmed through use of selective and differential media. Caution must be exercised when applying the method since several species of *Pseudomonas* are opportunistic pathogens.

SCOPE

The method is applicable to the starches, sugars, syrups and most co-products of the corn wet milling industry.

MEDIA AND REAGENTS

1. Glycerol
2. Tryptic Soy Broth (TSB). Prepare medium according to the manufacturer's directions. Dispense 100 mL in 250 mL Erlenmeyer flasks. Sterilize by autoclaving at 121°C for 15 minutes. Cool to room temperature.
3. Cetrimide Agar (CEA). Prepare medium according to the manufacturer's directions. Add 10 mL glycerol per liter, dispense in bottles and sterilize by autoclaving at 121°C for 15 minutes. Temper agar to 45°C before pouring into petri dishes.
4. *Pseudomonas* Agar F-PAF. Prepare medium according to manufacturer's directions. Add 10 mL glycerol per liter. Sterilize by autoclaving. Dispense 15 mL of sterile medium into petri dishes, and solidify.
5. *Pseudomonas* Agar P-PAP. Prepare medium according to manufacturer's directions. Add 10 mL glycerol per liter. Sterilize by autoclaving. Dispense 15 mL of sterile media into petri dishes, and solidify.

PSEUDOMONAS AERUGINOSA
CULTURE METHOD — continued

PROCEDURE

Aseptically weigh 10 g of sample into 100 mL Tryptic soy broth, mix well and incubate for 48 hrs. at 35-37°C. Using a 3 mm inoculating loop streak from TSB onto a CEA plate. Cover, invert and incubate for 48 hrs. at 35-37°C. After incubation, examine plates for growth. Select one colony of each morphological type present. Streak each of these colonies onto separate PAF and PAP plates.

Incubate the plates at 35-37°C for 18-24 hours and examine the isolated colonies for pigment formation. Confirmed strains of *Pseudomonas aeruginosa* produce a fluorescent yellow and green pigment, which diffuses into PAF agar or a blue pigment in PAP.

CALCULATION

Report positive *Pseudomonas aeruginosa* per 10 g of sample if confirmed colonies are present.

REFERENCE

Compendium of Methods for the Microbiological Examination of Foods, Current Edition, American Public Health Association.