PSEUDOMONAS SPECIES PRESUMPTIVE MEMBRANE FILTER METHOD AND CONFIRMATION OF *PSEUDOMONAS AERUGINOSA*

PRINCIPLE

Presumptive *Pseudomonas* bacteria are quantitated by a membrane filter technique, using a selective isolation medium, followed by differential media. Confirmation of *Pseudomonas aeruginosa* is accomplished by differential media. Use of the membrane filter technique allows accurate quantification of *Pseudomonas* when low counts are anticipated. Caution must be exercised when applying the method, since several species of *Pseudomonas* are opportunistic pathogens.

SCOPE

The method is applicable to sugars and syrups of the corn wet milling industry.

SPECIAL APPARATUS

1. Smooth tipped stainless steel forceps

2. 47 mm grid marked, white sterile 0.45 micron membranes (Millipore or equivalent)

3. Autoclavable 47 mm filtration systems with holder base, funnel assembly and receiver flask (Millipore or equivalent)

4. Vacuum pump capable of 22-27 inches of vacuum

5. Sterile 47 mm plastic petri dishes, tight sealing

MEDIA AND REAGENTS

1. *Pseudomonas* Isolation Agar-PIA. Prepare medium according to manufacturer's directions. Add 20 mL glycerol per liter. Dispense in bottles and sterilize by autoclaving.

PSEUDOMONAS SPECIES
MEMBRANE FILTER METHOD — continued

2. *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.

3. *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.

4. Glycerol

5. Butterfield's Phosphate Diluent

   Stock solution: Dissolve 34 g of potassium dihydrogen phosphate (KH_2PO_4) in 500 mL of purified water, adjust to pH 7.2 with about 175 mL of 1*N* NaOH solution and dilute to 1 L volume. Store under refrigeration.

   Diluent: Dilute 1.25 mL of stock solution of 1 L volume with purified water. Prepare dilution blanks using this solution.

6. Dilution Blanks

   Dilution Blanks: Fill dilution bottles to 50 mL with Butterfield's phosphate diluent. Cap bottles and sterilize at 121°C at 15 pounds of pressure for 15 minutes in a steam autoclave.

7. 47 mm petri dishes plus medium: Prepare 47 mm petri dishes by pouring approximately 2-3 mL of sterile and properly prepared PIA medium into each of the needed number of dishes. Allow the media to solidify.

PROCEDURE

A. Quantitative Method for Total *Pseudomonas*

   1. Aseptically weigh 25 g of the sample into a sterile 50 mL phosphate buffer diluent blank and homogenize.

PSEUDOMONAS SPECIES
MEMBRANE FILTER METHOD — continued

2. Assemble a sterile 47 mm filtration system with a 47 mm gridded white 0.45 micron pore size membrane (Note 1). Connect the receiver flask to the vacuum pump using the vacuum hose.

3. Aseptically pour the homogenized sample solution into the filter funnel and then cover the funnel top opening. Apply vacuum and filter the sample solution through the membrane filter. If desired, sterile phosphate buffer can be used for rinsing of the funnel.

4. Using sterile forceps transfer the membrane to a petri dish containing PIA medium. Roll the filter onto the medium in such a manner to avoid air bubbles forming between the membrane and the medium surface.

5. Invert and incubate the petri dish containing the membrane at 35-37°C for 18-24 hours. Using a light magnifier, count all blue, green and non-pigmented colonies. Record the results and calculate the number of Total *Pseudomonas* per gram of sample. If poor or no growth, re-incubate dish for 24 hours at 35-37°C (Note 2).

B. Quantitative Method for *Pseudomonas aeruginosa*

1. Aseptically weigh 25 g of the sample into a sterile 50 mL phosphate buffer diluent blank and homogenize.

2. Assemble a sterile 47 mm filtration system with a 47 mm gridded white 0.45 micron pore size membrane (Note 1). Connect the receiver flask to the vacuum pump using the vacuum hose.

3. Aseptically pour the homogenized sample solution into the filter funnel and then cover the funnel top opening. Apply vacuum and filter the sample solution through the membrane filter. If desired, sterile phosphate buffer can be used for rinsing of the funnel.

4. Using sterile forceps transfer the membrane to a petri dish containing PAF or PAP medium. Roll the filter onto the medium in

PSEUDOMONAS SPECIES
MEMBRANE FILTER METHOD — continued

such a manner to avoid air bubbles forming between the membrane and the medium surface.

5. Invert and incubate the petri dish containing the membrane at 35-37°C for 18-24 hours. Using a light magnifier, count all blue, green and non-pigmented colonies. Record the results and calculate the number of *Pseudomonas aeruginosa* per gram of sample. If poor or no growth, re-incubate dish for 24 hours at 35-37°C (Note 2).

C. Confirmation of *Pseudomonas aeruginosa*

Select two or more typical colonies from the membrane and streak for isolation onto a plate of PAF and PAP agars. Incubate the plates at 35-37°C for 18-24 hours and examine the isolated colonies for pigment formation. Confirmed *P. aeruginosa* colonies produce a fluorescent yellow green pigment which diffuses into PAF agar or a blue pigment in PAP (Note 2).

CALCULATION

The number of *Pseudomonas* can be reported either as is or on a dry solids basis:

1. As is basis (per g) $= \dfrac{\text{Total } \textit{Pseudomonas} \text{ Count}}{25}$

2. Percent Confirmed *Pseudomonas aeruginosa* $= \dfrac{\text{Number of Confirmed } \textit{P. aeruginosa} \text{ Colonies x } 100}{\text{Total } \textit{Pseudomonas} \text{ Count}}$

NOTES AND PRECAUTIONS

1. The membrane can only be handled with sterile forceps. Sterilize the forceps by keeping the forcep blades in ½ inch of ethanol and then igniting the alcohol to burn itself out just prior to handling the membrane.

2. To confirm presumptive (+) colonies as positive *Pseudomonas* or *Pseudomonas aeruginosa* the appropriate morphological and biochemical tests must be performed on representative colonies by an experienced microbiological laboratory.

PSEUDOMONAS SPECIES
MEMBRANE FILTER METHOD — continued

REFERENCE

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