Rye Agar B

M1855

Intended Use:

Recommended for sporulation of *Phytophthora infestans.*

Composition**

Ingredients	Gms / Litre
Rye	60.000
Sucrose	20.000
Beta-sitosterol	0.050
Agar	15.000

**Formula adjusted, standardized to suit performance parameters

Directions

Suspend 95.05 grams in 1000 ml purified / distilled water. Heat to boiling to dissolve the medium completely. Sterilize by autoclaving at 15 lbs pressure (121°C) for 20 minutes. Cool to 45-50°C. Mix well before pouring.

Principle And Interpretation

Phytophthora infestans is an oomycete that causes the serious potato disease known as late blight or potato blight. The organism can also infect tomatoes and some other members of the Solanaceae (8). *Phytophthora infestans* produces microscopic, asexual spores called sporangia. When the environment is highly conducive for disease, sporangia are airborne and spread for miles. The fungus will also survive in infected tubers that remain in soil from the previous season. Seed pieces can also be infected and harbor the pathogen (1,2,4).

Rye Agar A is suggested for the isolation of *Phytophthora infestans*. The appearance is flat, waxy when grown on agar medium. A study conducted to compare media for mycelial growth, sporangia, oospore production by isolation of *Phytophthora infestans* showed better growth on Rye Agar and V8 Juice Agar as compared to other media (7). Rye is a cereal grain which supplies manganese, tryptophan, phosphorous and magnesium to the pathogen. Sucrose is the carbohydrate source. Beta sitosterol helps in sporulation.

The optimum temperature for the growth of *Phytophthora infestans* was 18 to 24ºC and are able to growth between 10 to 25ºC (3).

Type of specimen

Plant samples - Seeds, vegetables.

Specimen Collection and Handling

For plant samples, follow appropriate techniques for sample collection, processing as per guidelines and local standards(8). After use, contaminated materials must be sterilized by autoclaving before discarding.

Warning and Precautions

Read the label before opening the container. Wear protective gloves/protective clothing/eye protection/ face protection. Follow good microbiological lab practices while handling specimens and culture. Standard precautions as per established guidelines should be followed while handling specimens. Safety guidelines may be referred in individual safety data sheets.

Limitations

1.Some strains may show poor growth due to nutritional variations.

2. Further biochemical and serological tests must be carried out for complete identification.

Performance and Evaluation

Performance of the medium is expected when used as per the direction on the label within the expiry period when stored at recommended temperature.

Quality Control

Appearance

Light yellow to light brown hygroscopic soft lumps which can be easily broken down to powder

Gelling

Firm, comparable with 1.5% Agar gel.

Colour and Clarity of prepared medium

Medium amber coloured opague gel forms in Petri plates

Cultural Response

Cultural characteristics observed after an incubation at 18-24°C for 2 weeks in dark.

Organism	Inoculum (CFU)	Growth
Phytophthora infestans	50-100	good

Storage and Shelf Life

Store dehydrated and the prepared medium at 2-8°C. Use before expiry date on the label. On opening, product should be properly stored dry, after tightly capping the bottle in order to prevent lump formation due to the hygroscopic nature of the product. Improper storage of the product may lead to lump formation. Store in dry ventilated area protected from extremes of temperature and sources of ignition. Seal the container tightly after use. Product performance is best if used within stated expiry period.

Disposal

User must ensure safe disposal by autoclaving and/or incineration of used or unusable preparations of this product. Follow established laboratory procedures in disposing of infectious materials and material that comes into contact with clinical sample must be decontaminated and disposed of in accordance with current laboratory techniques (5,6).

Reference

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8. Nowicki, Marcin et al. (17 August 2011), Potato and tomato late blight caused by *Phytophthora infestans*: An overview of pathology and resistance breeding, Plant Disease, ASP, doi:10.1094/PDIS-05-11-0458.

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