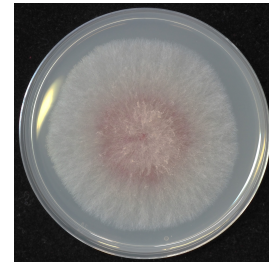


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## Leucine auxotrophy test of Fusarium graminearum strains

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## Abstract

This protocol outlines the methodology for preparing Leucine dropout medium, essential for assessing Leucine auxotrophy in *Fusarium graminearum* strains. The procedure involves the step-by-step preparation of the medium to selectively evaluate the growth characteristics of these strains. Understanding the Leucine auxotrophy status in *Fusarium graminearum* strains holds significance in elucidating their metabolic pathways or assign phenotypes to mutant strains.

## 1 Testing *Fusarium graminearum* strains for Leucine Auxotrophy

### Media and supplements:

#### 1. Biotin (Supplier: Fluka/Merck)

Biotin is essential for robust growth, particularly beneficial for vivid photography on minimal medium.

##### *Procedure:*

- Dissolve in water to achieve a 0.05% solution.
- Add 20 µl of the 0.05% Biotin stock to 400 ml agar.

Biotin, while not strictly necessary for growth, leads to more vigorous growth, facilitating better photography of the mycelic growth. There might be a role for biotin in the initial steps of glucose metabolism (see references: STRIGINI, P., MORPURGO, G. (1961) and Wu, P.-C., et al. (2020)).

#### 2. Yeast Nitrogen Base (Formedium, CYN0202)

This complete medium is used for routine growth of all strains before the auxotrophy test.

#### 3. Synthetic complete (Kaiser) Drop-out: -LEU (supplier: Formedium, Ref no: DSCK052)

This is the test medium.

#### 4. L-Leucine (supplier Formedium, Ref no: DOC0155)

##### *Procedure:*

- Dissolve 1.312 g L-Leucine in 1 M HCL to produce a 1 M stock solution of L-Leucine.

## 2 Preparation for 400 ml YNB agar and the Kaiser Drop-out agar with and without Leucine

### Medium Composition:

- Yeast Nitrogen Base (YNB): 1.76 g (contains all essential aminoacids)
- Kaiser Drop-out: -LEU: 2.76 g (contains all aminoacids but not Leucine)
- 2% agar

##### *Procedure:*

- Weigh YNB and Kaiser Drop-out medium into two 500 ml Schott glass media bottles,

and add 8 g agar.

- Add 400 ml deionised water and autoclave for 15 minutes.
- After autoclaving, cool agar to 50 C.
- Pour YNB medium into agar plates
- For the Kaiser Drop-out medium, add 20 ul 0.05% Biotin stock to 400 ml agar and mix.
- Divide the 400 ml agar into two 200 ml aliquots using sterile Schott glas bottles.
- Pour one aliquot into agar plates to produce the Leucine drop-out medium.
- For the remaining 200 ml aliquot, add 400 ul 1M L-Leucine to 200 ml agar to produce a medium containing 2 mM Leucine.

### 3 **Growth test of *Fusarium graminearum* strains**

*F. graminearum* strains are first grown on YNB agar. Small agar plugs ( $3 \times 3 \text{ mm}^2$ ) taken from the actively growing end of the colony are then transferred on agar plates with Kaiser Drop-out medium with and without added Leucine. Plates are incubated at 25 C and growth is assessed after 5 days.