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Preparation of polygalacturonic acid (PGA) to study virulence in Erwinia/Pectobacterium

Pol Nadal Jimenez[1], Rita S. Valente[2]

[1]University of Liverpool; [2]Instituto Gulbenkian de Ciencia

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Pol Nadal-Jimenez

ABSTRACT

This medium is used for the growth of Erwinia/Pectobacterium species to ensure that virulence genes are turned ON. These bacteria turn on virulence (plant cell-wall degrading enzymes = PCWDEs) in response to various environmental signals. One of these signals is of plant origin, and it is thought to allow the bacterium to determine whether it has located a potential plant host. These plant signals are sensed by the bacterial sensor/regulator KdgR.

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KEYWORDS

Erwinia, kdgr, PCWDE, plant pathogen, pectate

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GUIDELINES

The 2% PGA solution is to be used on the day that it is prepared.

MATERIALS TEXT

Polygalacturonic acid sodium salt from citrus fruit, ≥75% (titration) (Sigma Aldrich P3850, **CAS Number:** 9049-37-0).

Water

Magnet

Magnetic stirrer

Microwave

SAFETY WARNINGS

Stir/move the PGA solution slowly when it's warm to avoid getting burned.

BEFORE STARTING

Make sure to have and use protective gloves and googles to avoid burns.

2% PGA stock solution preparation

1 Weight **0.2 g** of PGA (Sigma, P3850) using a precision scale.

2 Transfer the powder solution to a **100 mL** (low-thermal-expansion borosilicate glass) beaker.

3 Add **10 mL** of demineralised water and a (microwave resistant) magnet.

4 Place the beaker containing the PGA, the water, and the magnet into a microwave and turn the microwave ON at the maximum power.

5 ⚠

Stare at the beaker while it's being heated and stop the microwave every time that the solution starts bubbling (be careful, this will happen very sudden and it's easy that you loose the whole mixture if the content overflows the beaker and you don't stop it at the right time).

6 Using some heat-protective gloves remove the beaker form the microwave and place it on a magnetic stirrer. Let the sample cool down while it is being mixed by the magnet.

7 Repeat the process multiple times till the PGA is fully solved in water. The solution will be transparent with a light yellow coloration.

8 Place some sterile aluminium foil on the top of the beaker and keep the solution at room temperature. This solution tends to go off easily, as it has not been properly sterilised. Use it on the same day.

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9 Use PGA at a final concentration of [M]**0.4 % (v/v)** (1 part of PGA stock solution + 4 parts of growth medium)

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