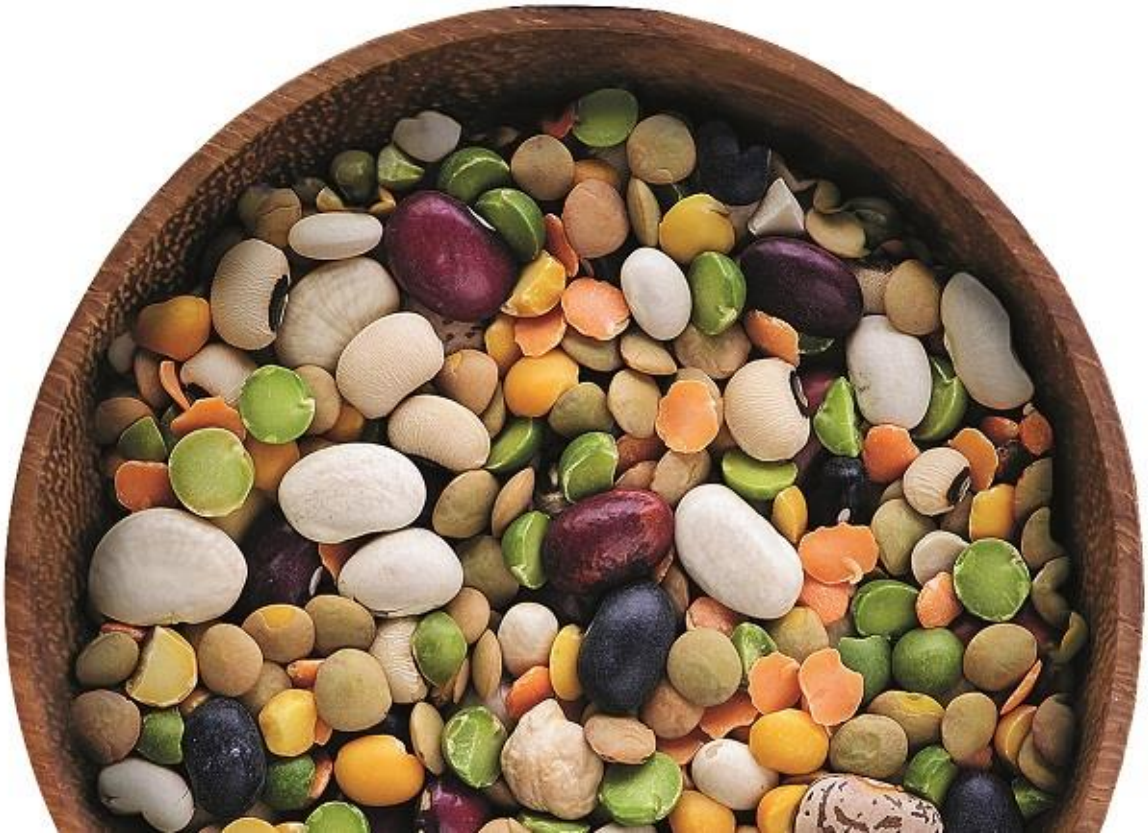
TRansition paths to sUstainable legume-based systems in Europe

# Isolation of Single Rhizobia Strains

**Date**: May, 31st 2018



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# Content



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# 1 Summary Information

## 1.1 Partner Summary

| | |
|---|---|
| **SOP Code** | EU-TRUE_SOP_002 |
| **TRUE Partner Acronym** | JHI |
| **Primary Author** | James, E.K. (*Euan.James@hutton.ac.uk*) |
| **Other Authors** | Maluk, M., Iannetta, P.P.M. |
| **Linked Reference and Hyperlink (if available)** | NA |
| **Associated files to use with the SOP [and function]** | NA |

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## 1.2 SOP Summary

### Title

**Isolation of Single Rhizobia Strains**

### Brief description

This details the isolation of single (pure) rhizobial strains from legume nodules using standard sterile techniques. Pure strains are essential for determining the symbionts involved in nodulation and Biological Nitrogen Fixation (BNF), especially if they are to be screened for their effectiveness at BNF and promoting plant growth with the ultimate aim of defining them as potential commercial inoculant strains.

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## 2 Protocol Steps

### Isolation of Single Rhizobia Strains

1. Prepare 70% ethanol, 2.5 % Na hypochlorite (NaClO; 2.5% active chlorine)[1], sterile distilled water. Sterile 1.5 mL tubes and sterile plastic pestles[2]. Yeast mannitol agar (**YMA**[3]) plates.
2. For dry nodules, soak over-night in sterile distilled water.
   For frozen nodules, allow them to thaw out.
3. Place nodules in 1.5 mL tube.
4. Surface sterilize nodules with 70 % ethanol for 1 min.
5. Sterilize nodules with 2.5 % Na hypochlorite for 3 min.
6. Wash with sterile water 3 times.
7. Squash the nodules in 1.5 mL tube using sterile plastic pestle.
8. Disperse the nodule juices across the YMA plate using sterile plastic pestle.
9. Use the same plastic pestle to spread onto next fresh YMA plate.
10. Allow the nodule juice to sink into the plate.
11. Incubate upside down (agar side upper-most), overnight at 28°C. Do not enclose/seal the plates in any way.[4]
12. Check for growth of colonies, and select individual white/pale and mucilaginous looking colonies for further growth. NB - avoid dark red colonies/growths, or those that look 'dry' or fungal. Aim to select one colony and streak this onto a fresh YMA plate. Again, incubate overnight at 28 °C.

[1] Household bleach is generally *ca.* 12.5 % chlorine, therefore dilute this x5.

[2] Pellet pestles Z359947-100EA Sigma-Aldrich; http://www.sigmaaldrich.com/catalog/product/sigma/z359947?lang=en&region=GB.

[3] **YMA -** For 1 L: 10 g of mannitol; 0.5 g of glutamate; 0.5g of $K_2HPO_4$; 0.1 g of $MgSO_4.7H_2O$; 0.05 g of NaCl; 1 mL (40 g $L^{-1}$) $CaCl_2$; 1 mL (4 g $L^{-1}$) $FeCl_3$; 1g of yeast extract; 15 g technical grade agar. Add distilled water to 800 mL and pH to 6.8. Make volume to 1L. Autoclave, and whilst still warm (~60 °C), add 10 mL.$L^{-1}$ of filter sterilised Congo Red Stock Solution (sterile; 0.25 % [w/v]). Dispense ~20 ml *per* 9 cm diameter Petri dish.

[4] Fresh nodule extracts will yield cultures that generally grow well overnight. However, isolation from the other nodules types (dry or frozen), may take up to 3-4 days to appear as colonies that are large enough to discern their phenotype.

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13. Once re-grown, take a single colony from the pure isolate on YMA plate and inoculate a sterile 5 ml **TY** broth[5].

14. Grow the TY at 28 °C (with shaking). After 24h (at log phase), take 0.9 mL of culture and combine with 0.9 mL of sterile 50% glycerol. Mix and freeze in liquid-$N_2$. Store the glycerol stock at -80 °C.

15. The stock may now be used directly for bacterial DNA extractions and/or for PCR or infection tests.

[5] **TY** - For 1 L: 5 g of tryptone; 3 g of yeast extract; 0.913 g of $CaCl_2.2H_2O$. Add distilled water to almost 1L, pH to 6.8, make volume to 1L. Autoclave. Dispense into 30 mL sterile universals (**Sterilin™ Polypropylene 30mL Universal Containers**; Catalog # : 128A; ThermoFisher) as 5 mL aliquots.

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## 3 Linked SOPs

| SOP Code | SOP Function |
|---|---|
| EU_TRUE_SOP_001 | Measuring '%Ndfa' and 'Biological Nitrogen Fixation' |
| EU_TRUE_SOP_003 | Identification of root nodule isolates by 16S-rRNA PCR |

## 4 Disclaimer

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## 6 Citation

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