Can we fix it? Yes we can! Engineering nitrogen fixation in agriculturally relevant, non-fixing, cereal-associated bacteria

Ben Gascoyne, Albert Fàbregas-Flavià, Philip Poole

Department of BIOLOGY, UNIVERSITY OF OXFORD

Rhizosphere — *Investigating the influence of roots*

Motivation

With increasing populations and urbanisation food production must increase by 70%[1].

Nitrogen is the most important factor for productivity behind water[2].

Synthetic fertiliser use and production is unsustainable.

Bacteria can naturally fix nitrogen. Crops that naturally utilise this are highly productive without artificial nitrogen inputs.

Cereals make up to >50% of global calories[3] so are a good target to increase productivity.

Approach

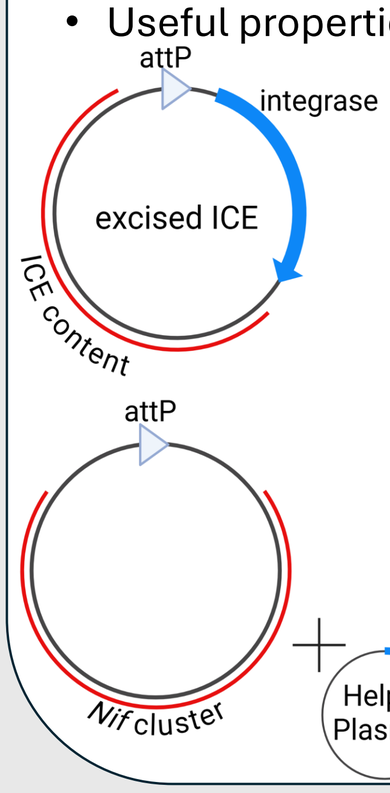
Engineer non-fixing bacteria that show:

- Strong cereal root colonisation
- Competitive in the soil
- Useful properties (e.g. biocontrol)

Good engineering targets:

Enterobacter Ludwigii AA4

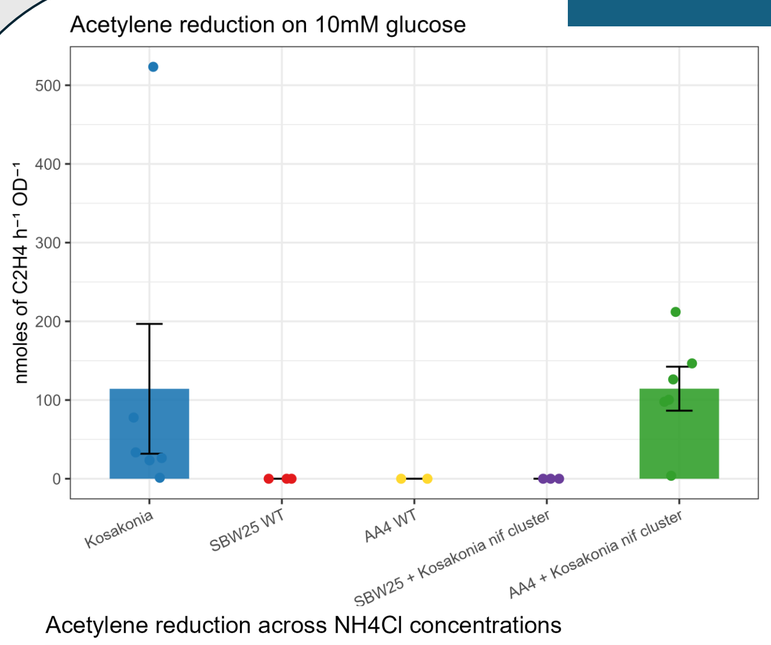
Pseudomonas fluorescens SBW25



Use a modified integrative and conjugative element (ICE) as a stable genomic integrative vector. [4]

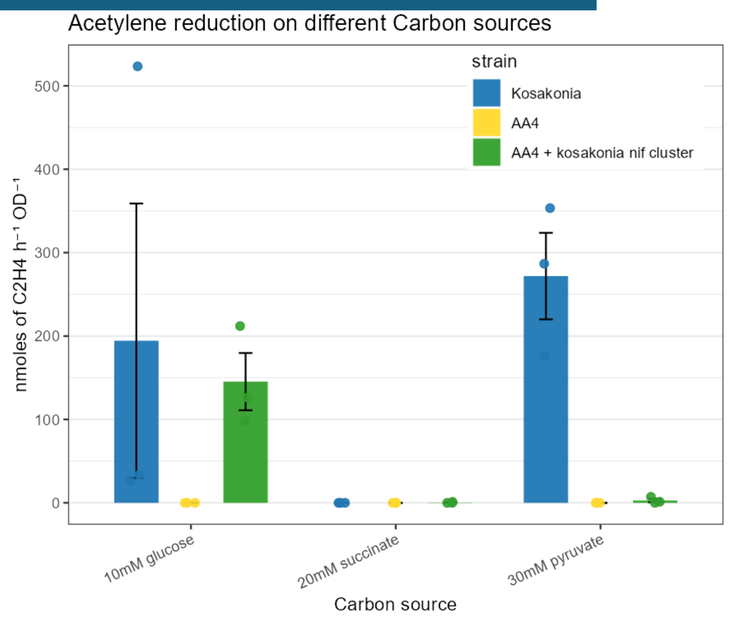
Integrate the *Kosakonia radicincitans* DMS16656 *nif* cluster.

Findings



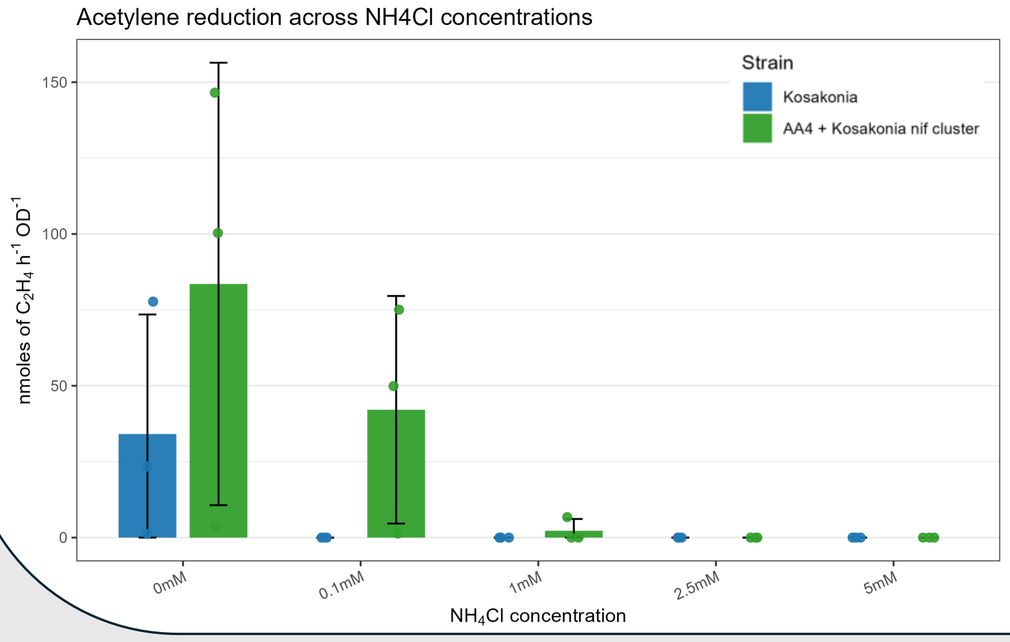
AA4 with *Kosakonia nif* cluster integrated can fix nitrogen at comparable levels to WT *Kosakonia*.

When the same *nif* cluster is integrated into SBW25, it does not result in nitrogen fixation.



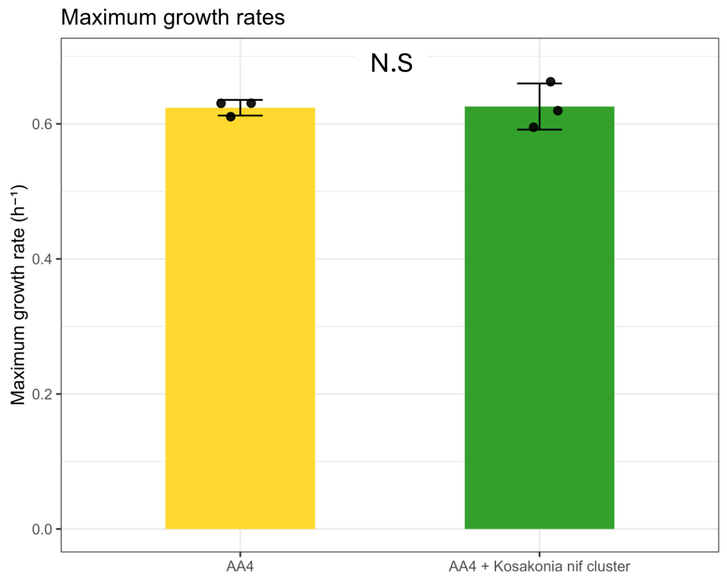
Engineered AA4 fixes nitrogen extremely poorly on 30mM pyruvate, compared to WT *Kosakonia*.

Nif cluster exhibits different carbon-dependent fixation when transferred to a new host.



Engineered AA4 can fix at higher ammonia concentrations.

Kosakonia nif cluster is less sensitive to ammonia when transferred to AA4.



No significant difference between maximum growth rates of WT AA4 and engineered AA4.

There is no fitness penalty associated with integrating a ~30kb *nif* cluster construct when grown in rich media.

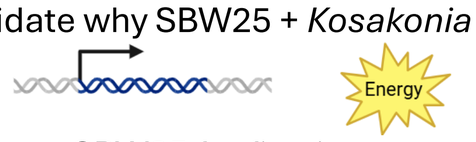
Implications

- ✓ *Nif* clusters can be integrated into heterologous hosts to engineer fixation in non-fixing bacteria. → Proof of concept in agriculturally relevant bacteria
- ✓ *Nif* clusters show strain-specific differences → Novel system to understand regulation of *nif* genes.
- ✓ Integration is without fitness penalty → *Nif* genes unlikely to be lost or disrupted during manufacturing and in field applications.
- ✓ Decreased ammonia sensitivity in engineered AA4 vs WT *Kosakonia* → Transferring *nif* cluster disrupts natural repression

Engineered AA4 is a good biofertiliser candidate ✓

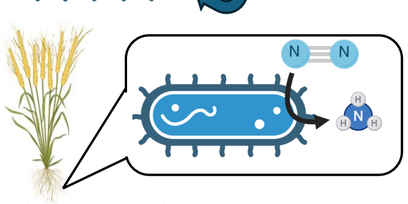
Next Steps

- Elucidate why SBW25 + *Kosakonia nif* cluster does not fix

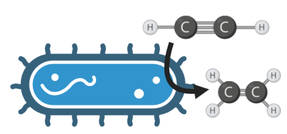


- Engineer SBW25 for fixation

- Engineer regulatory control
  - Cereal-specific promoters



- Test engineered AA4 for ammonia secretion
- In planta experiments:
  - Acetylene reduction assay
  - Plant growth promotion



[1]UN Food and Agriculture Organization, 2009. How to feed the world in 2050. [2] Setten, L., Soto, G., Mozzicafreddo, M., Fox, A.R., Lisi, C., et al., 2013. PLoS ONE, 8(5), p.e63666. [3] Human Progress, n.d. Grain cornucopia. Available at: https://humanprogress.org/trends/grain-cornucopia/ [4] Miyazaki, R., van der Meer, J.R., 2013. Applied and Environmental Microbiology, 79(14), pp.4440–4447.