Methane Oxidation by the Oleaginous Yeast, *Rhodotorula glutinis* – Fact or Fiction?

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Significance

- With an atmospheric life span of 7-12 years, the warming potential of methane (CH_4) is 25 times greater than for carbon dioxide (CO_2) over a 25 year period.
- More than 63% of atmospheric CH4 (346 Tg Y^{-1}) is derived from anthropogenic activities with landfills, oil and gas, and enteric fermentation being the main pollution sources.
- Methane oxidising organisms can fix CH_4, providing a biological carbon sink by converting CH_4 to biomass carbon.
- This biomass can then be used for the production of value-adding renewable products (e.g. bioplastics, pigments, and lipids) providing added positive inputs into the techno-economics of CH_4 remediation.

Background

- Four species of CH_4-fixing yeasts were identifeid by Wolf *et al.*, (1980):
  - *Sporobolomyces roseus* and *S. gracilis*
  - *Rhodotorula rubra* and *R. glutinis*
- The ability of R. glutinis to utilise CH4 as a sole carbon source was reported by Wolf *et al.* (1979, 1980).
- Reported CH_4-oxidation rates were: 0.18 µmol CH4.mg dry weight.
- *Rhodotorula glutinis* is a unicellular, oleaginous, saprophytic yeast (Fig. 1) with a high lipid (>60%) and high carotenoid contents.
- As C18:1 was reported as the dominant fatty acid, *R. glutinis* fatty acids have potential for bioplastic production and the carotenoids (β-carotene, torulene and torularhodin) have high values on the antioxidant market (feed additives in aquaculture, natural food colourants, pharmaceuticals).
- Therefore, this study investigated the CH_4-remediation potential of *R. glutinis* to evaluate benefits in a CH_4-remediation context.

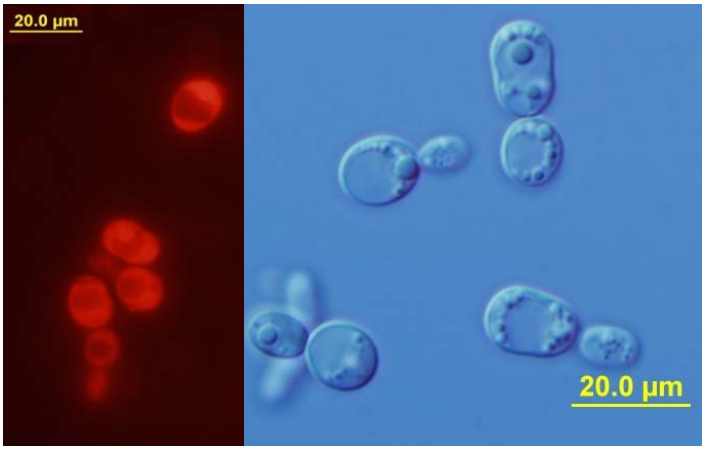

Fig 1. (Left) Fluorescence micrograph of *R. glutinis* stained with Nile-blue (Right) Differential interference micrograph of budding yeast cells (1,000x)

Methods

- Cultivation: *Rhodotorula glutinis* cultures were grown in fed-batch reactors at 30 °C without shaking in basal medium (BM).
- Methane treatments: *R. glutinis* was purged with 20% CH_4 in air in batch cultures and 5% CH_4 in air in fermenter batch mode (Fig. 2). BM supplemented with CH_4 without *R. glutinis* served as control for non-biological CH_4 dissipation.
- Carbon substrate utilisation: *R. glutinis* was cultured in BM supplemented with acetate, glycerol, glycerol-diesel,or glycerol peptone.
- Growth was measured spectrophotometrically at 540 nm and via direct cell counts using a Neubauer haemocytometer.

Fig 2. Cultivation of *R.glutinis* in fermenter batch-mode, incubated with 5% CH_4 in air.

Results

- Repetition of the exact same cultivation conditions used by Wolf *et al*. (1980) showed that *R. glutinis* did not fix CH_4;
- Instead observed CH_4 declines were due to dissipation of CH_4 in the medium (non-organism control, Fig. 3).
- Best growth was achieved when cultivated in basal medium supplemented with glycerol-peptone (1%+0.001% w/w), while no growth was observed with CH_4 as the sole carbon source (Fig. 4).
- 22-day β-carotene productivities of *R. glutinis* on glycerol-peptone supplemented BM were 0.05 $mg.g^{-1}$.dry weight.L^{-1}.

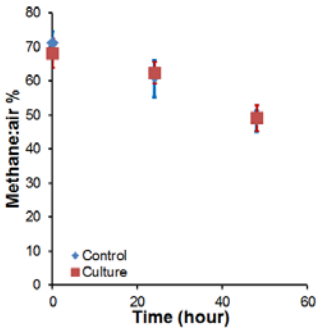

Fig 3. CH_4 dissolution in BM with (culture) and without *R. glutinis* (control)

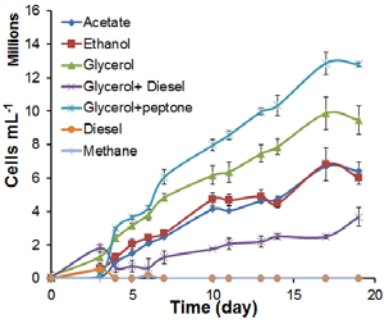

Fig 4. Growth of *R. glutinis* on different carbon sources

Conclusions

- Our results show that *R. glutinis* is not a methane-oxidising yeast and refutes earlier reports by Wolf et al. (1979, 1980).
- *Rhodotorula glutinis* is not suitable for methane remediation.
- β-carotene productivities in glycerol-peptone-supplemented basal medium are not competitive with other natural sources.

References

Wolf, H. J., and R. S. Hanson. "Isolation and characterization of methane-utilizing yeasts." *Journal of General Microbiology* 114.1 (1979): 187-194.
Wolf, H. J., M. A. R. C. I. A. Christiansen, and R. S. Hanson. "Ultrastructure of methanotrophic yeasts." Journal of bacteriology 141.3 (1980): 1340-1349.