Accelerating growth of *Pseudomonas putida* through media optimization to improve rhamnolipid production

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Rhamnolipids

- Amphiphilic molecules
- Possible **applications**: plant protection, bioremediation [1], [2]
- Naturally produced by e.g. *Pseudomonas aeruginosa* [3]
- Recombinant production in ***Pseudomonas putida* KT2440 SK4**
- Current media developed for low cell densites [5]

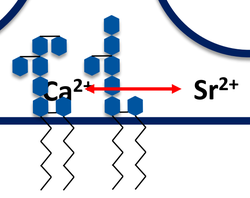
Aim

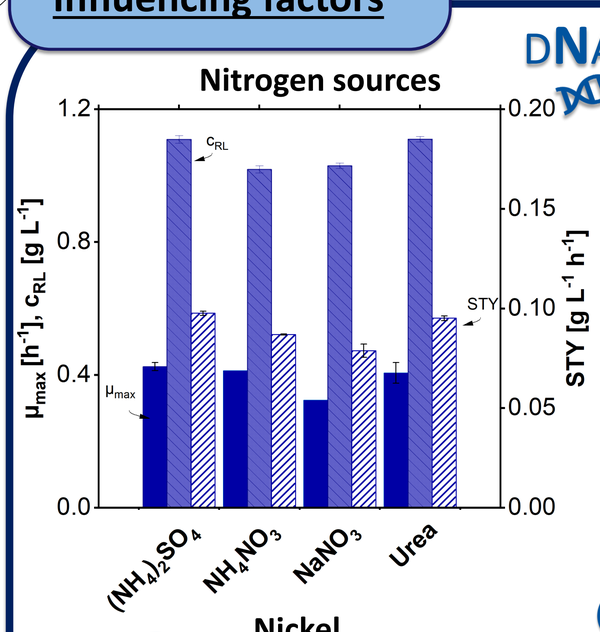
- Investigation of **influencing factors** on rhamnolipid production
  - Nitrogen source
  - Trace elements
- Development of an optimized medium for **high-cell-density** cultivations

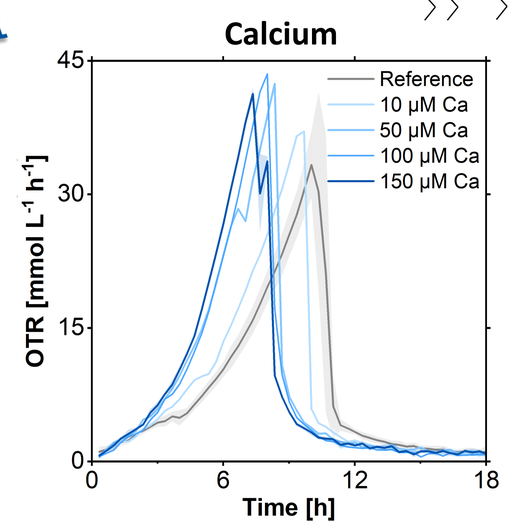
No improve

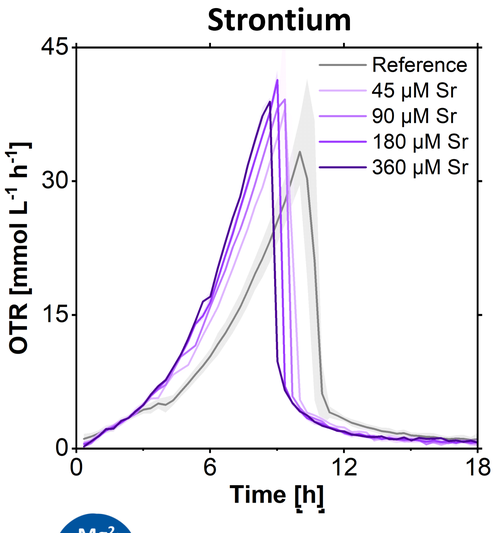
- Tungsten
- Aluminium
- Cobalt
- Copper
- Molybdenum
- Selenium
- Boron

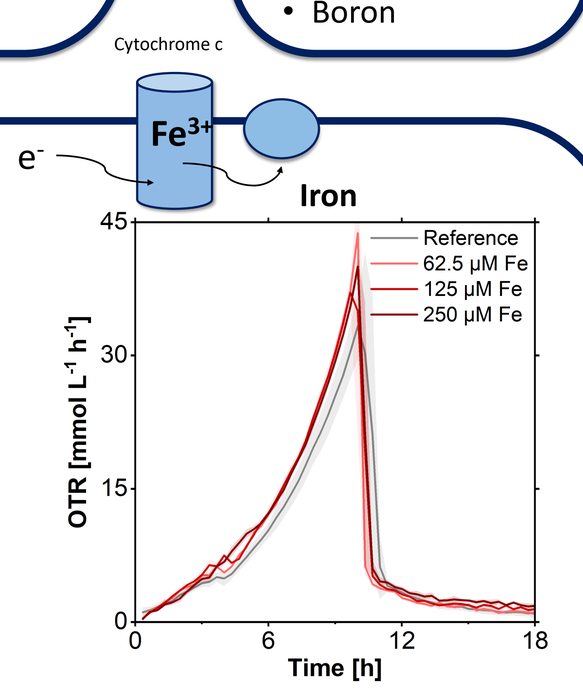
Influencing factors

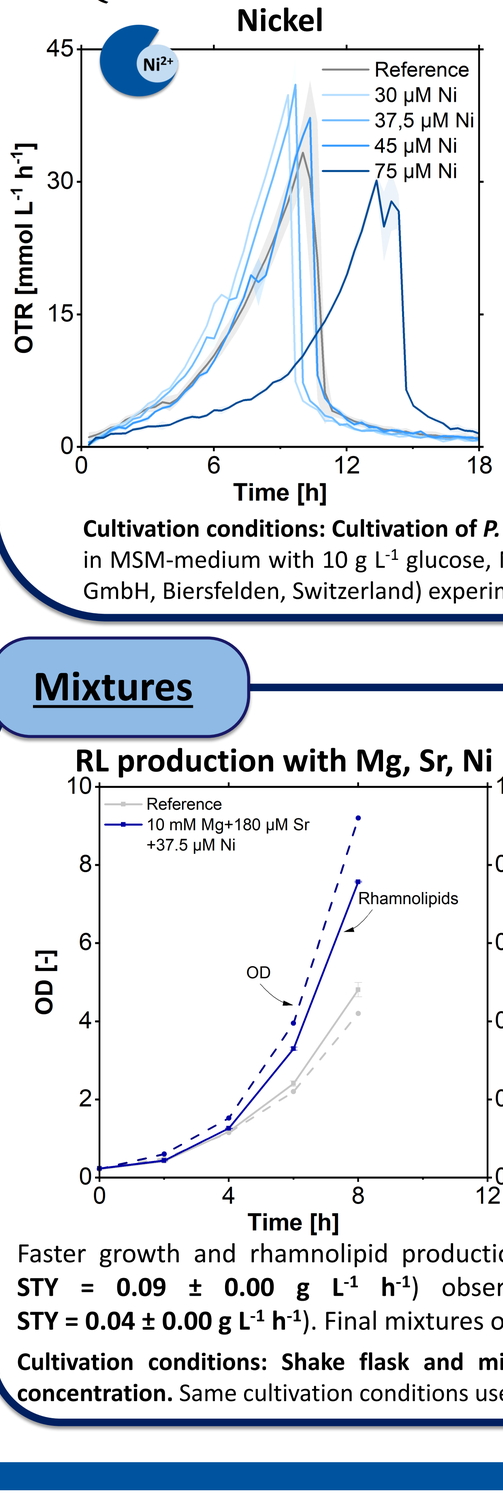


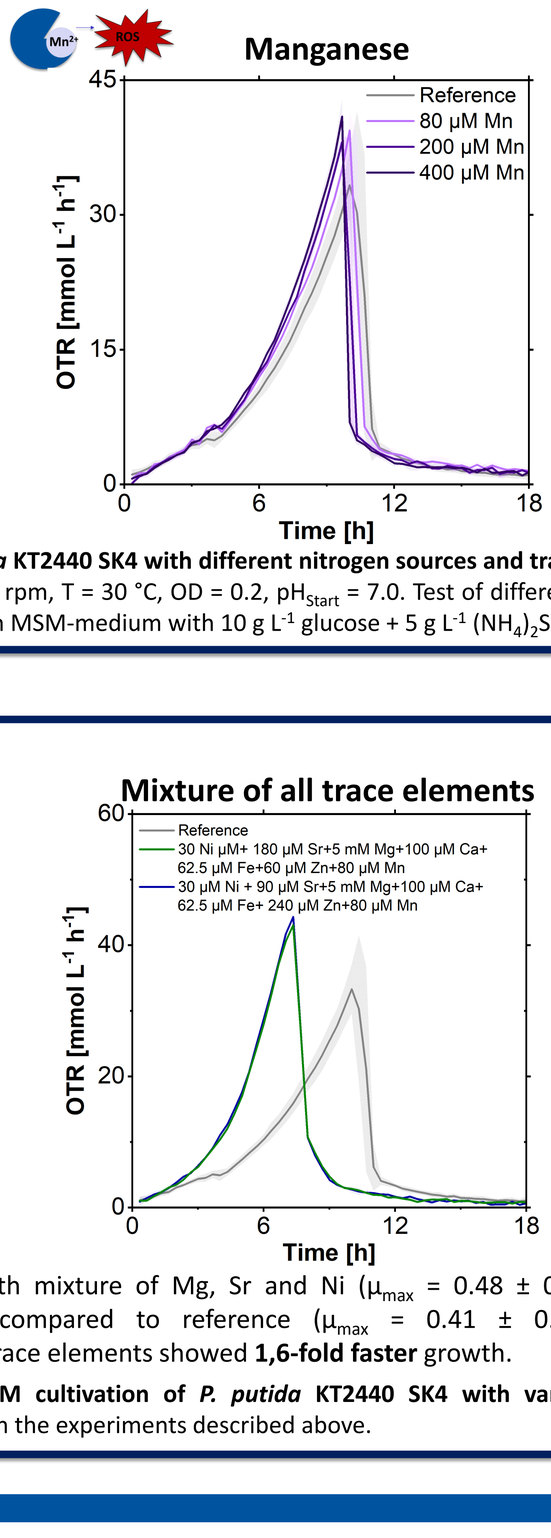


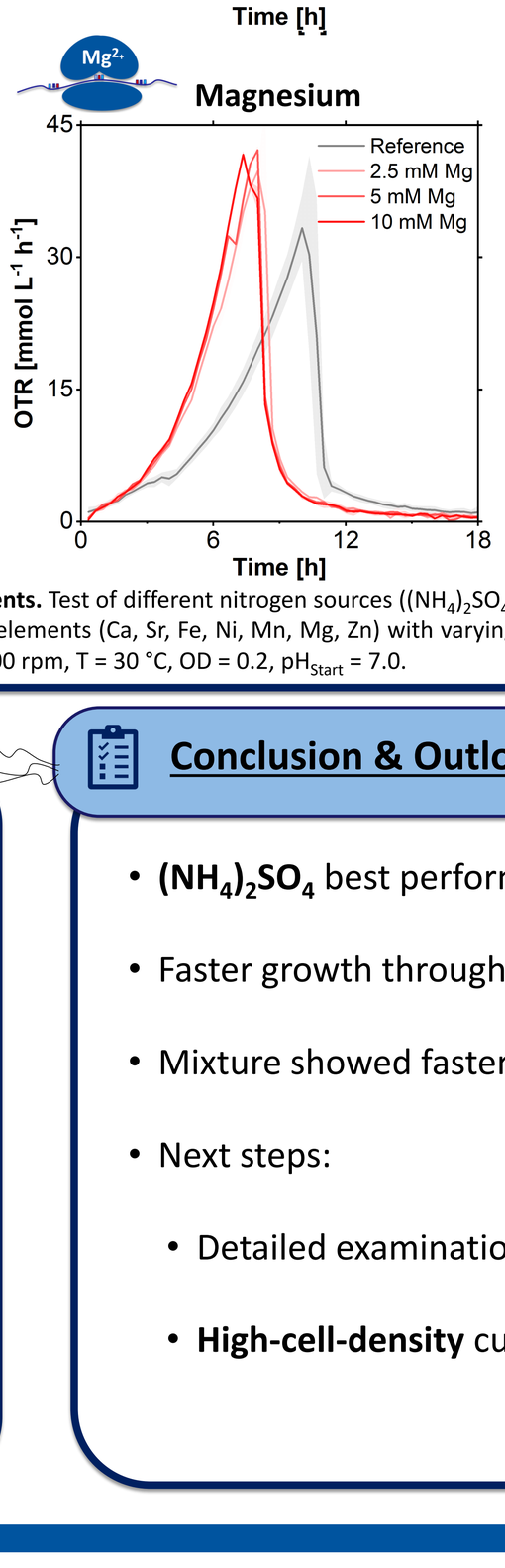


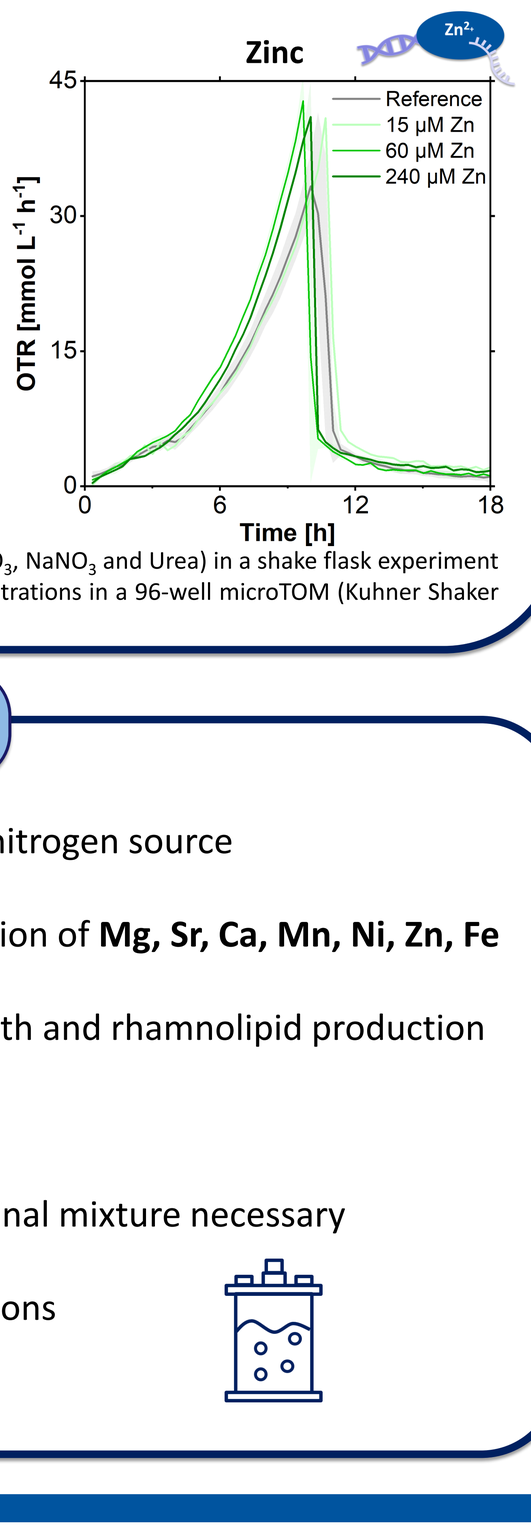












Cultivation conditions: Cultivation of *P. putida* KT2440 SK4 with different nitrogen sources and trace elements. Test of different nitrogen sources ($(NH_4)_2SO_4$, $NH4NO_3$, $NaNO_3$ and Urea) in a shake flask experiment in MSM-medium with 10 g L^{-1} glucose, N=250 rpm, T = 30 °C, OD = 0.2, pH_{Start} = 7.0. Test of different trace elements (Ca, Sr, Fe, Ni, Mn, Mg, Zn) with varying concentrations in a 96-well microTOM (Kuhner Shaker GmbH, Biersfelden, Switzerland) experiment in MSM-medium with 10 g L^{-1} glucose + 5 g L^{-1} $(NH_4)_2SO_4$, N = 300 rpm, T = 30 °C, OD = 0.2, pH_{Start} = 7.0.

Mixtures

Faster growth and rhamnolipid production with mixture of Mg, Sr and Ni (μ_{max} = 0.48 ± 0.00 h^{-1}, **STY = 0.09 ± 0.00 g L^{-1} h^{-1}**) observed compared to reference (μ_{max} = 0.41 ± 0.01 h^{-1}, **STY = 0.04 ± 0.00 g L^{-1} h^{-1}**). Final mixtures of all trace elements showed **1,6-fold faster** growth.

Cultivation conditions: Shake flask and microTOM cultivation of *P. putida* KT2440 SK4 with varying TE concentration. Same cultivation conditions used as in the experiments described above.

Conclusion & Outlook

- **$(NH_4)_2SO_4$** best performing nitrogen source
- Faster growth through addition of **Mg, Sr, Ca, Mn, Ni, Zn, Fe**
- Mixture showed faster growth and rhamnolipid production
- Next steps:
  - Detailed examination of final mixture necessary
  - **High-cell-density** cultivations

References

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[4] Hartmans et al. (1989), Appl. Eniviron. Microbiol., 55: 2850-2855

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Kuhner shaker