H_2-driven production of substituted piperidines in *Cupriavidus necator*

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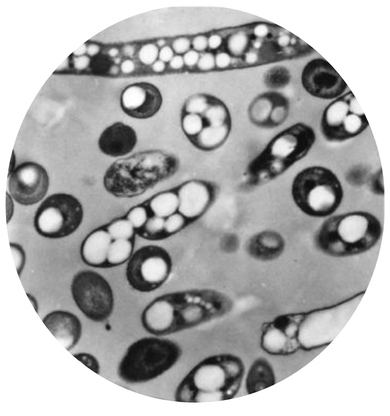
Background

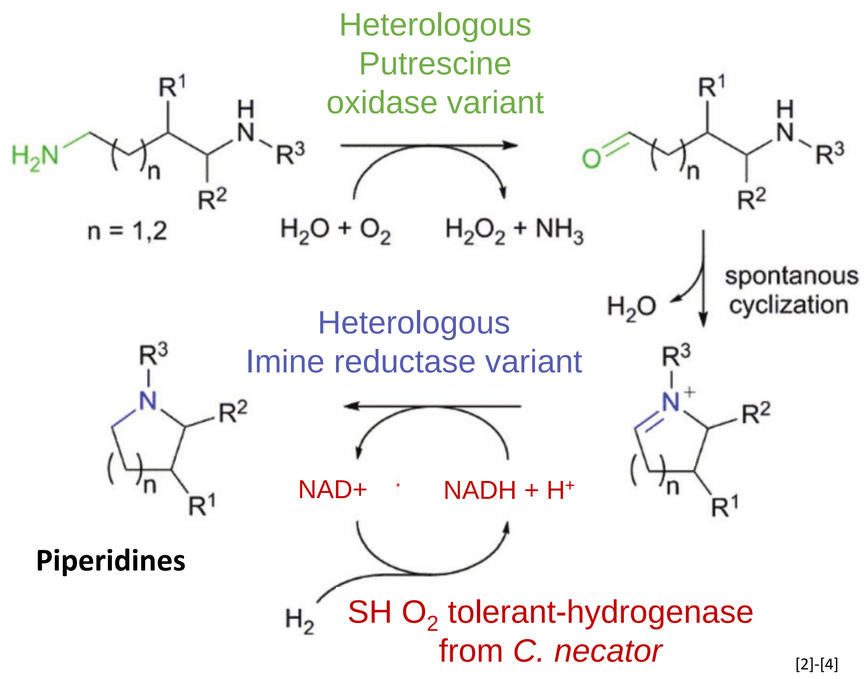


Cupriavidus necator.

- ✓ Facultative hydrogen-oxidizing bacterium.
  - ✓ CO_2 carbon source.
  - ✓ H_2 as an electron donor.
  - ✓ O_2 as an electron acceptor.

[1]

Enzymatic cascade in whole-cell system for piperidine production



Piperidines

[2]-[4]

- ✓ ConCO$_2$rde develops **biorefineries** using *C. necator* to transform CO_2 into **fine chemicals.**
- ✓ **Piperidines** are important compounds to produce a wide array of pharmaceuticals.
- ✓ Native imine reductase (IRED) from *Myxococcus stipitatus* converts imines to piperidines using NADPH as cofactor.
- ✓ An engineered NADH-variant of IRED can be coupled to the SH hydrogenase from *C. necator.*.
- ✓ NADH-dependent IRED production in *C. necator* was studied.

C. necator strains for IRED production

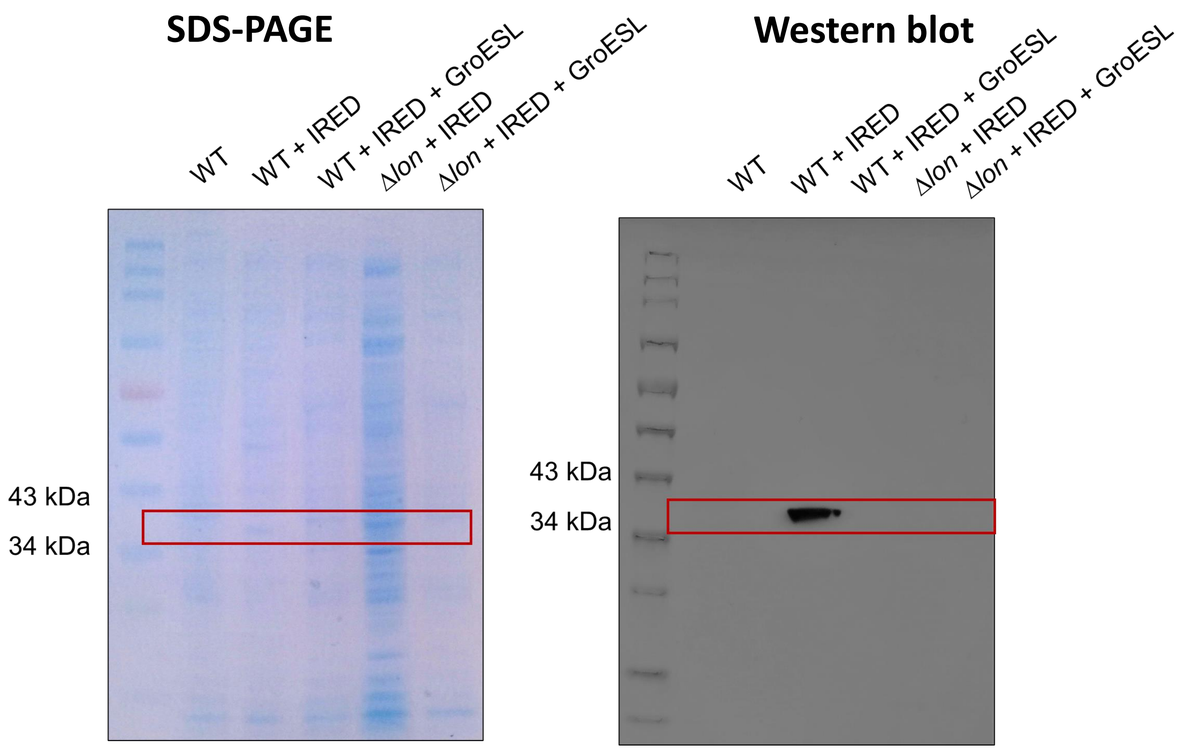
Use of different strains to optimize IRED production

- Microorganism: *C. necator*
- Genetic background: Wild Type **H16**; Deletion of Lon protease **Δ *lon***
- pKR plasmid, Rhamnose induction: NADH-dependent Imine reductase **(IRED)**
- pEG19 plasmid, Arabinose induction: Heat-shock chaperonin **GroESL**

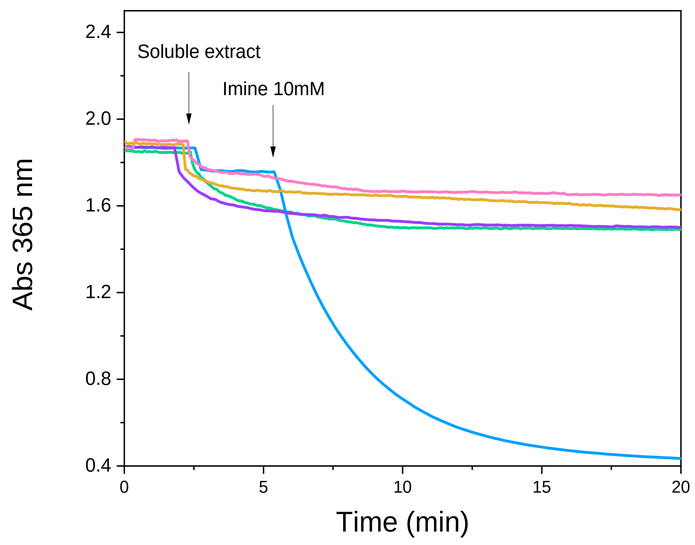
[5]

IRED was heterologously produced in *C. necator*

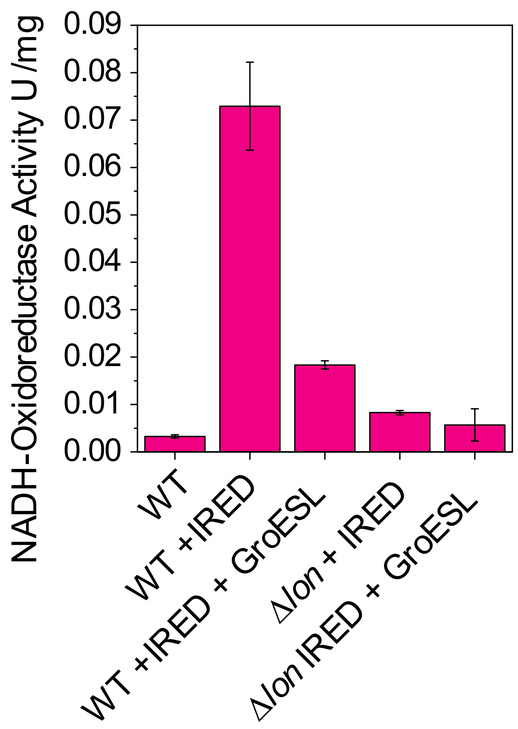
IRED production was analyzed in the different *C. necator* strains.
Expected size of IRED is 35kDa, indicated in red.
IRED was detected for the WT + IRED strain.



IRED showed oxidoreductase activity in soluble extract



- Raw trace of IRED activity measured using soluble extract.
- NADH consumption was followed spectrophotometrically at 365 nm.



Oxidoreductase activity was observed in the WT + IRED and WT + IRED + GroESL strain.

WT + IRED showed the highest activity, 0.072 U/mg.

Outlook

- IRED activity was observed in *C. necator.*
- Influence of GroESL chaperonin and Lon protease in IRED production needs to be further evaluated.
- Coupling IRED with putrescine oxidase will allow us to implement a whole-cell biotransformation system.

References: [1] Panich J., Fong B., Singer S., (2021) *Trends in Biotech*. 39, 4, 412-424. [2] Al-Shameri, Borlinghaus N., Weinmann L., Scheller P., Nestl B., Lauterbach L., (2019), *Green Chem*, 21, 1396–1400. [3] Borlinghaus N., Nestl B., (2018), *ChemCatChem* 10,183–187. [4] Borlinghau N., Weinmann L., Krimpzer F., Scheller P., (2019) *ChemCatChem*, 11, 5738–5742. [5] Marc, J., Grousseau, E., Lombard, E., Sinskey, A., Gorret, N. and Guillouet, S., (2017). *Metabolic Engineering*. 42, 74-84.

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