

# Towards a novel tetraether lipid producer *Haloferax volcanii*

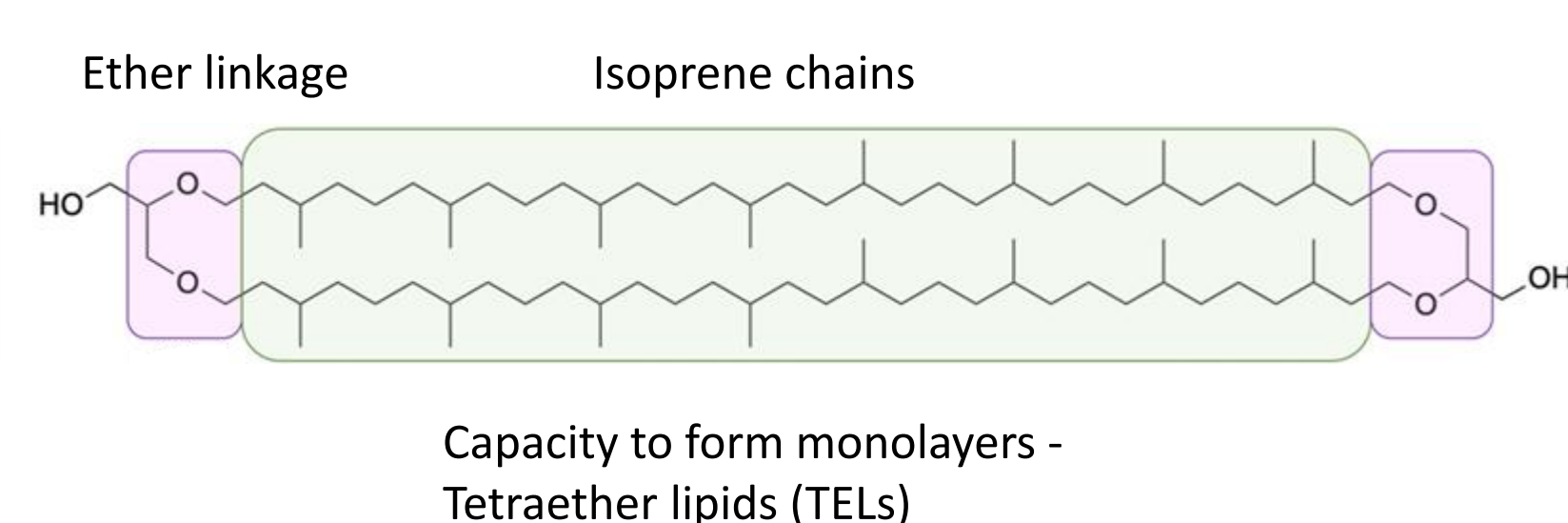
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## Background: Archaeal lipids & Applications<sup>1,2,3</sup>

- Archaeal vs conventional lipids (Bacteria and Eukarya)



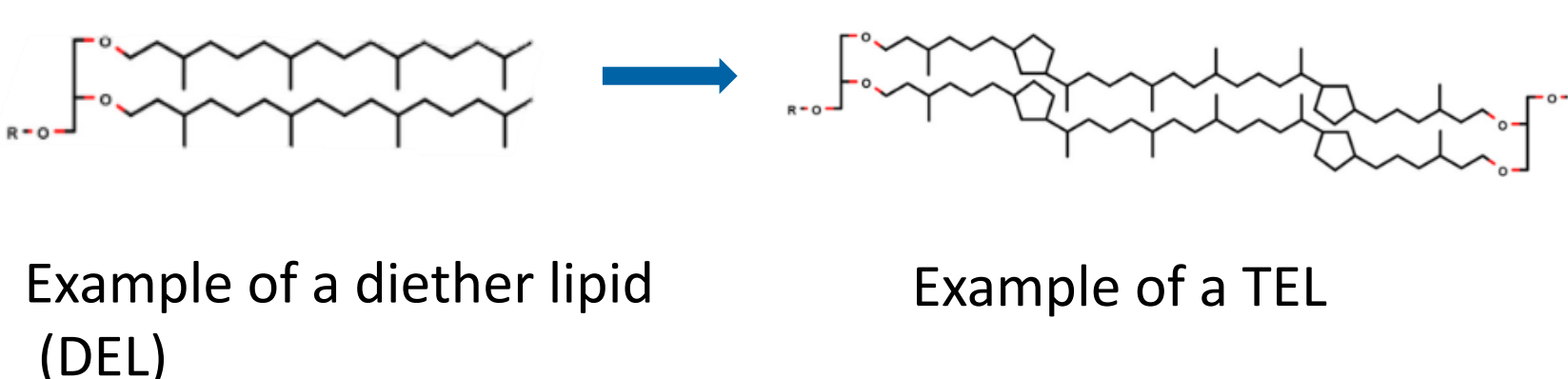
- Advantages of TELs (vs conventional lipids)

- higher stability against oxidative, hydrolytic, thermal and enzymatic stresses

- Application of TELs in pharmaceutical industry,

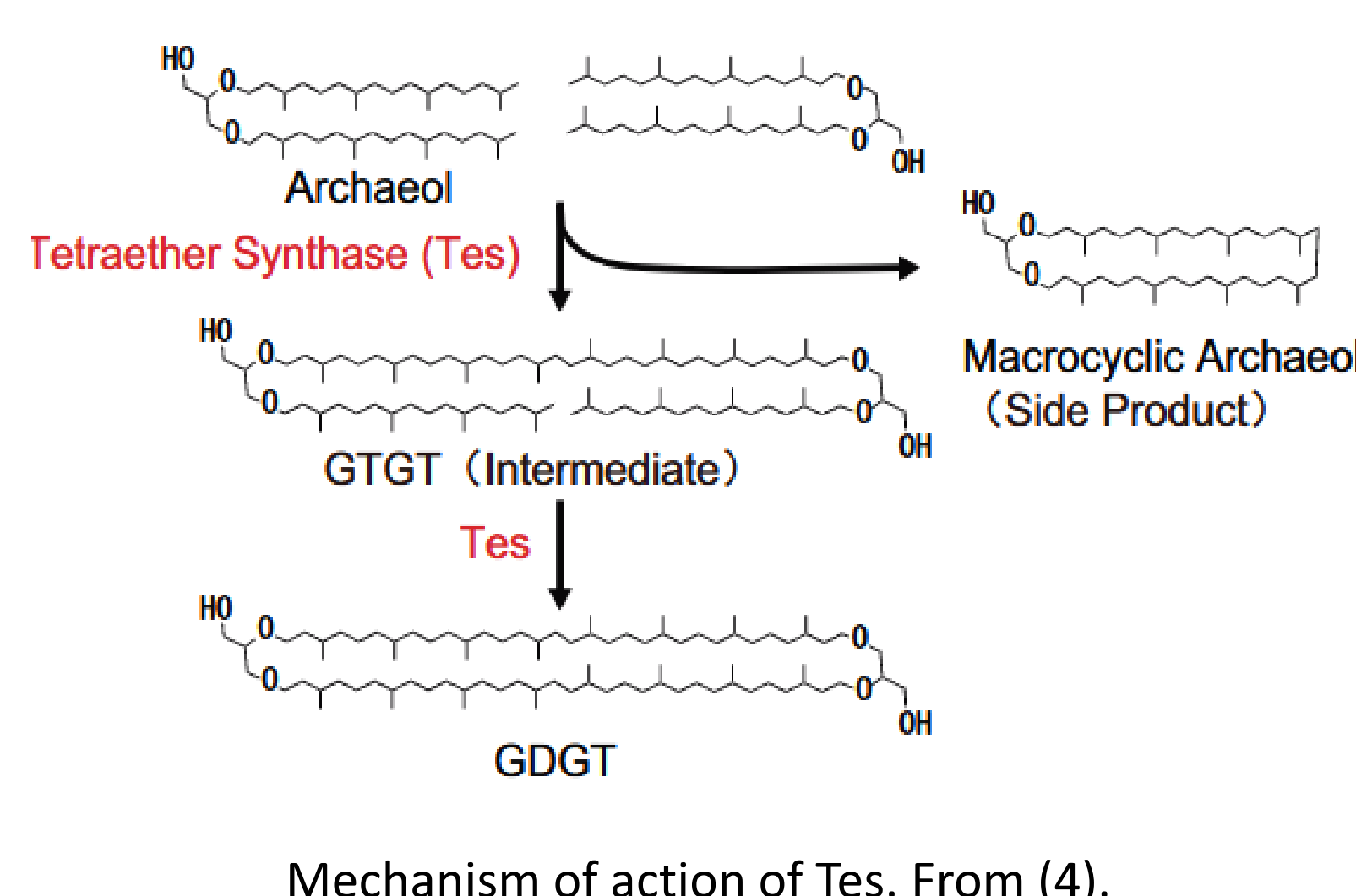
- liposomes → oral delivery of small molecules, peptides and proteins
- lipid nanoparticles → nucleic acid delivery.

- TELs are a result from selective pressure from extreme environments.



## Tetraether synthase (Tes)<sup>4,5</sup>

- Tetraether synthase (Tes) is the enzyme responsible to join **two saturated DELs** and produce 1 TEL.



- The enzyme requires 4 [Fe<sub>4</sub>S<sub>4</sub>] cluster and a Fe<sup>2+/3+</sup> as cofactors and can be able to join lipids with different types of headgroups.

## Goals

- Develop a workflow for *H. volcanii* mutant generation;
- Introduce the *tes* gene for expression in *H. volcanii*;
- Characterize mutants phenotypically.

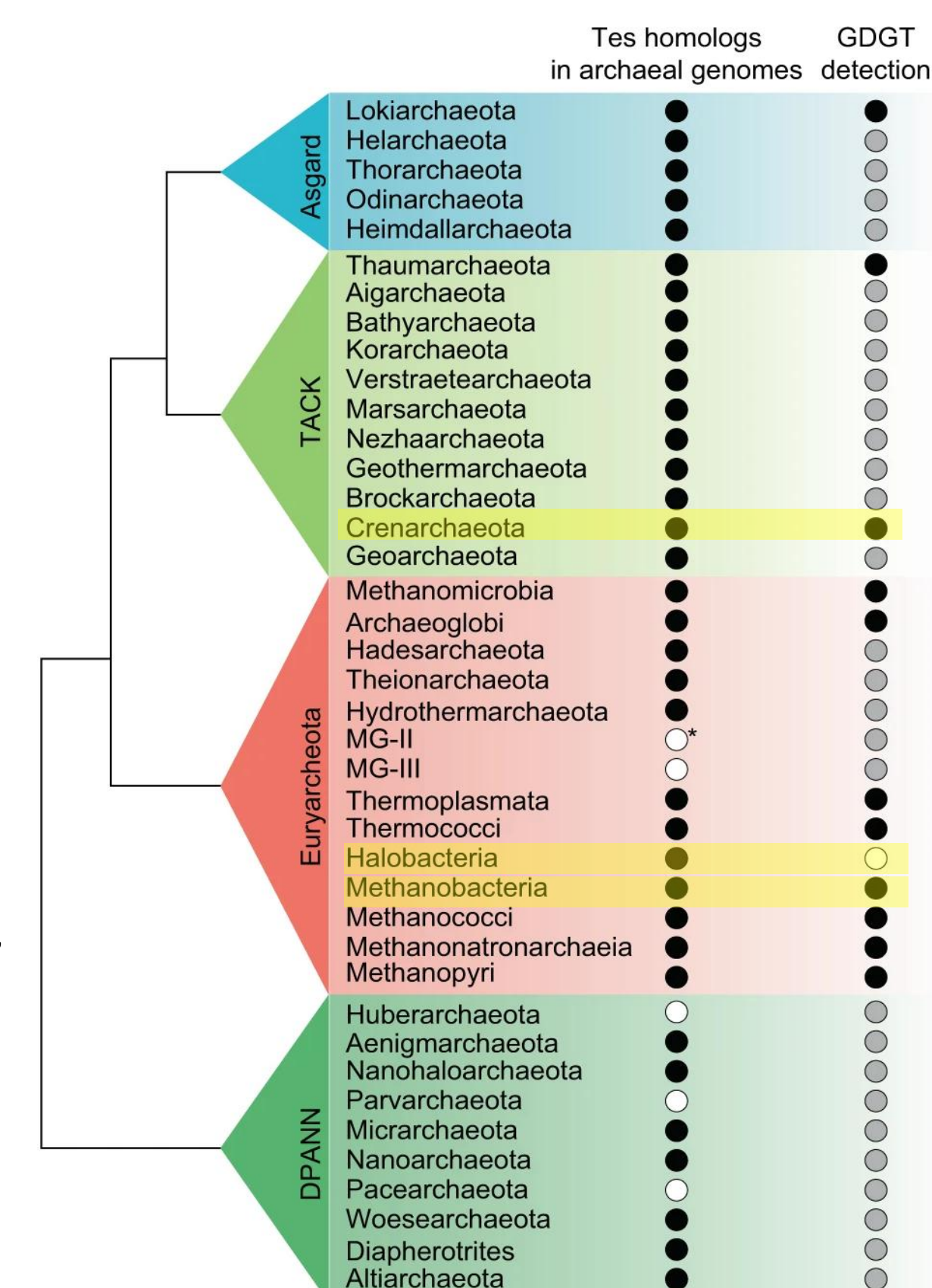
## Future perspectives

- ✓ Screen for optimal induction strength to maintain growth and promote TELs in *H. volcanii* mutants;
- ✓ Attempt protein purification and analyse resulting lipid profile;
- ✓ Explore the effects of other promoters for *tes* expression;
- ✓ Integrate *tes* into genomic DNA.

## Presence of Tes across Archaea<sup>5</sup>

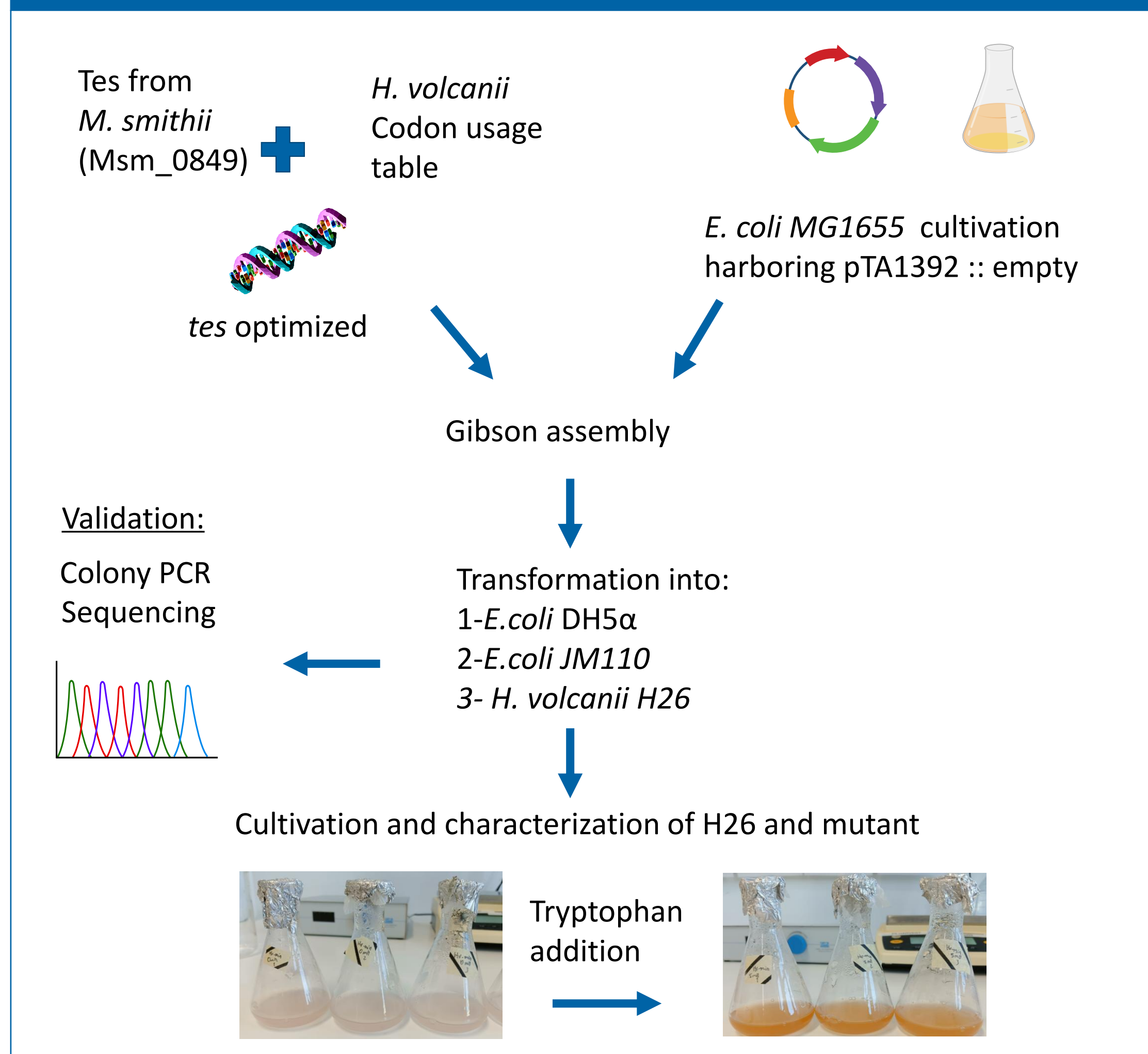
### *Haloferax volcanii*:

- same pathway for isoprenoid synthesis as other Archaea.
- produces both saturated and unsaturated DELs as a response to its high saline conditions.
- Grows faster than model TELs organisms (*Sulfolobus acidocaldarius*).
- Methanobrevibacter smithii* (from human gut) produces TELs grows faster than *S. acidocaldarius*.



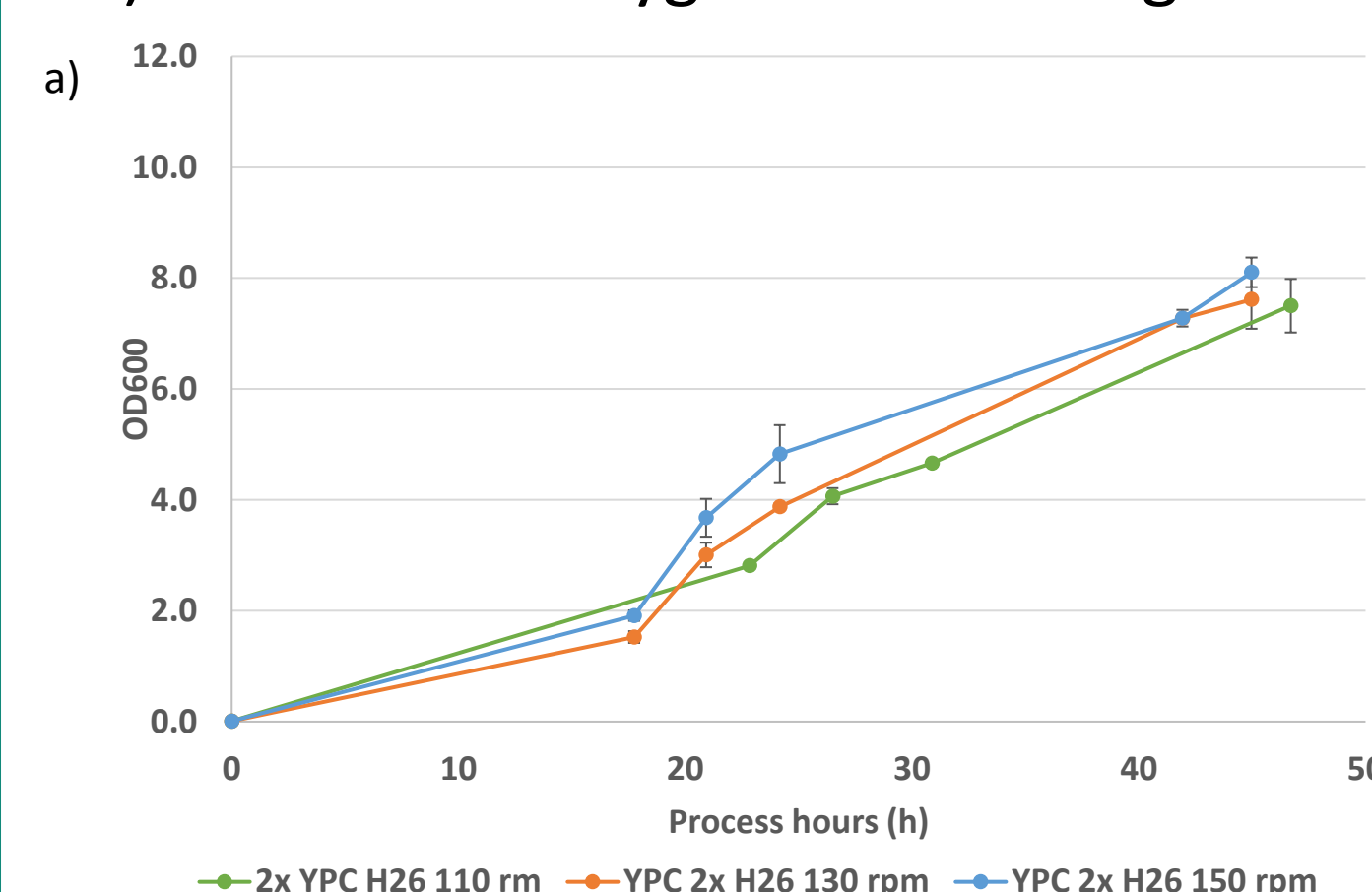
Distribution of Tes homologues in Archaea. Highlight: Organisms used in this study. Black circles: present; White circle: Absent; Gray circles: Not available. From (5).

## Materials and Methods

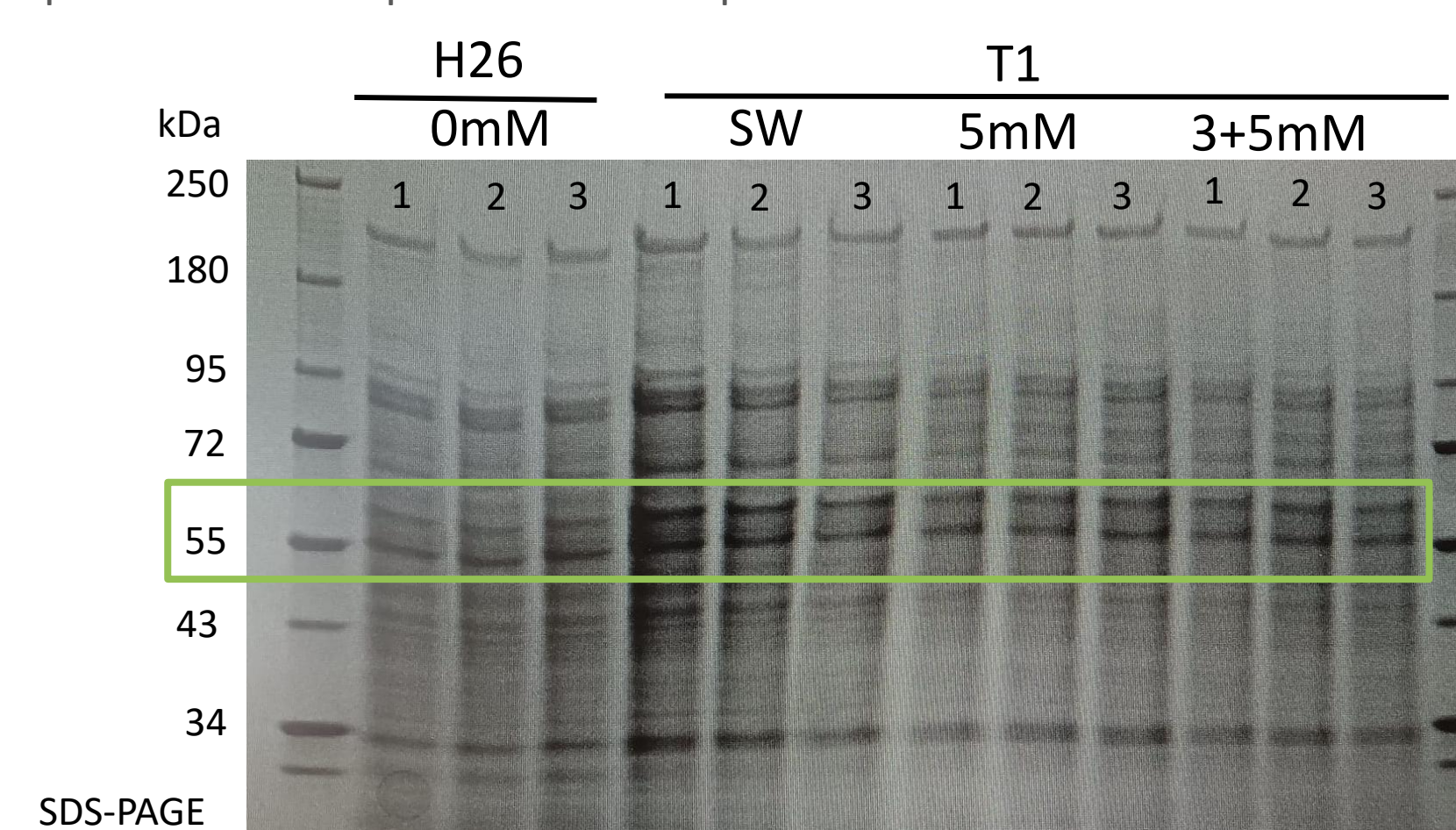
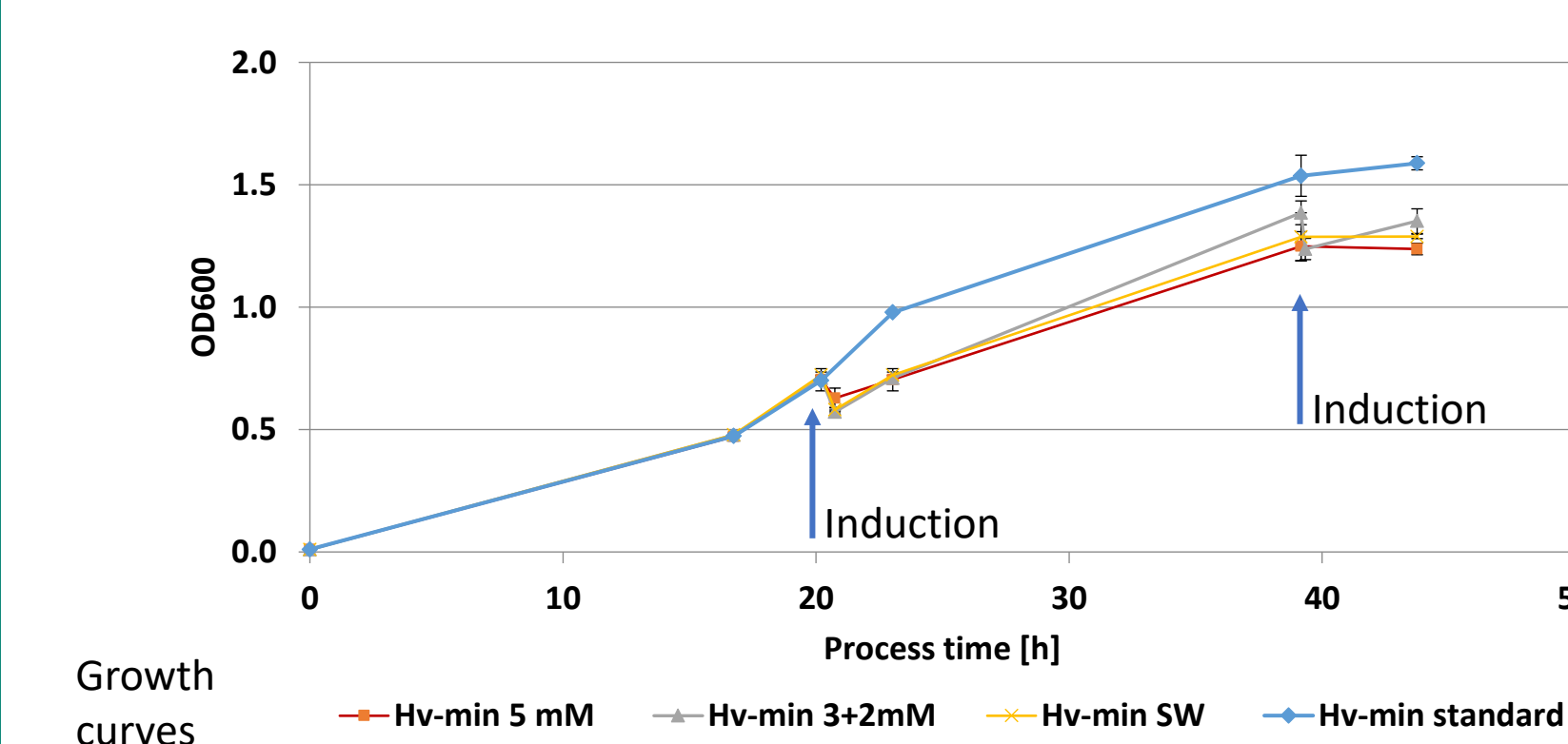


## Results

### 1) Presence of oxygen influences growth



### 1) Comparison between one and two step induction



	YPC 1x (literature)	YPC 2x (Experiments)
Basis	Salt water 18% (SW)	
Yeast Extract (g/L)	5	10
Peptone (g/L)	1	2
Casamino Acids (g/L)	1	2
1 M KOH (mL/L)	1.7	3.5
1 M CaCl (mL/L)		3

## References:

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