# Investigation of three members of the dihydrodipicolinate synthase enzyme family from the (hyper)thermoacidophilic crenarchaeon *Sulfolobus Solfataricus* P2

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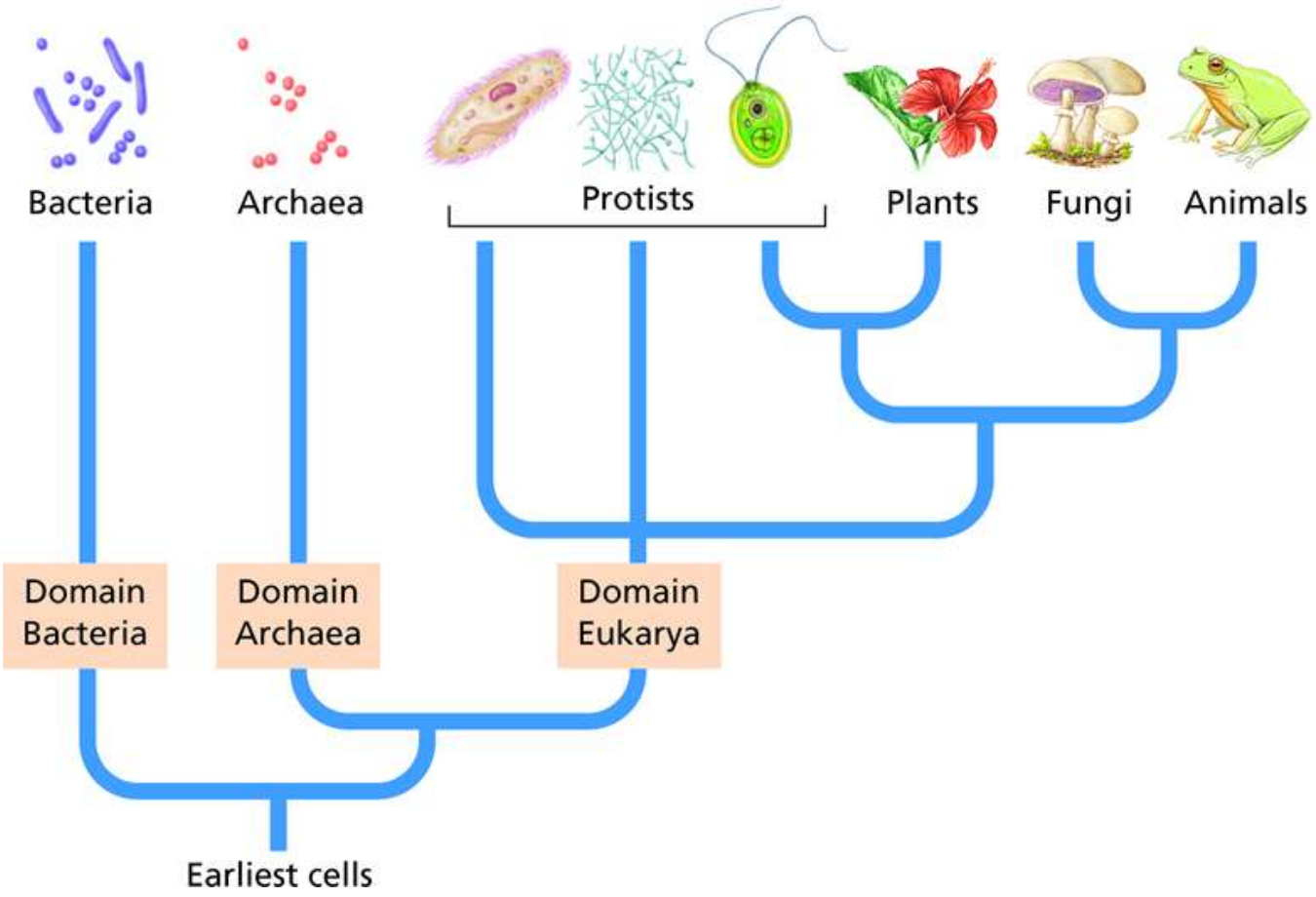
# Introduction



# *Introduction*

- Model organism: *Sulfolobus Solfataricus*
  - *Thermoacidophillic crenachaeon ( 80°C, pH 2-4 )*
  - *Isolated from geothermal springs by Brock 1972*

➡ Source for novel, thermostable enzymes

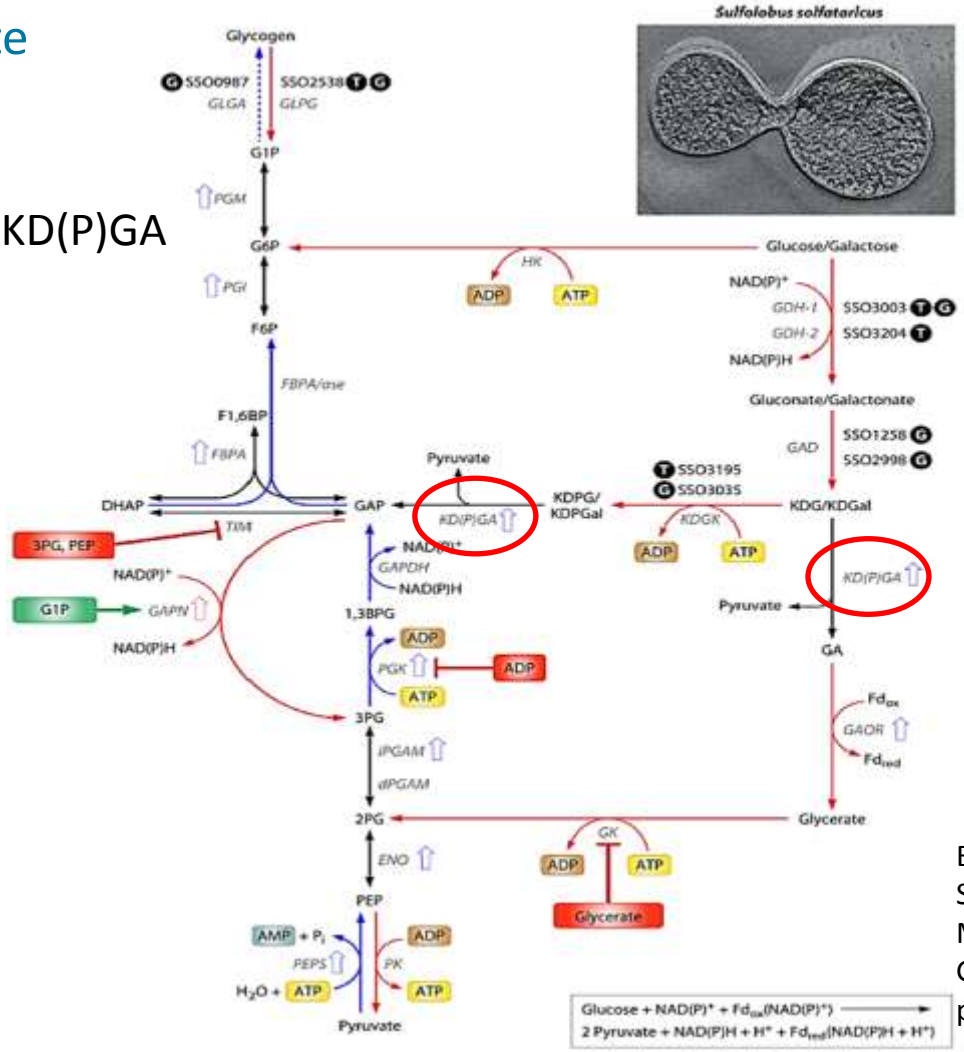
Hot spring in Yellostone National Park, USA

# Aim of the study

## Central carbohydrate metabolism

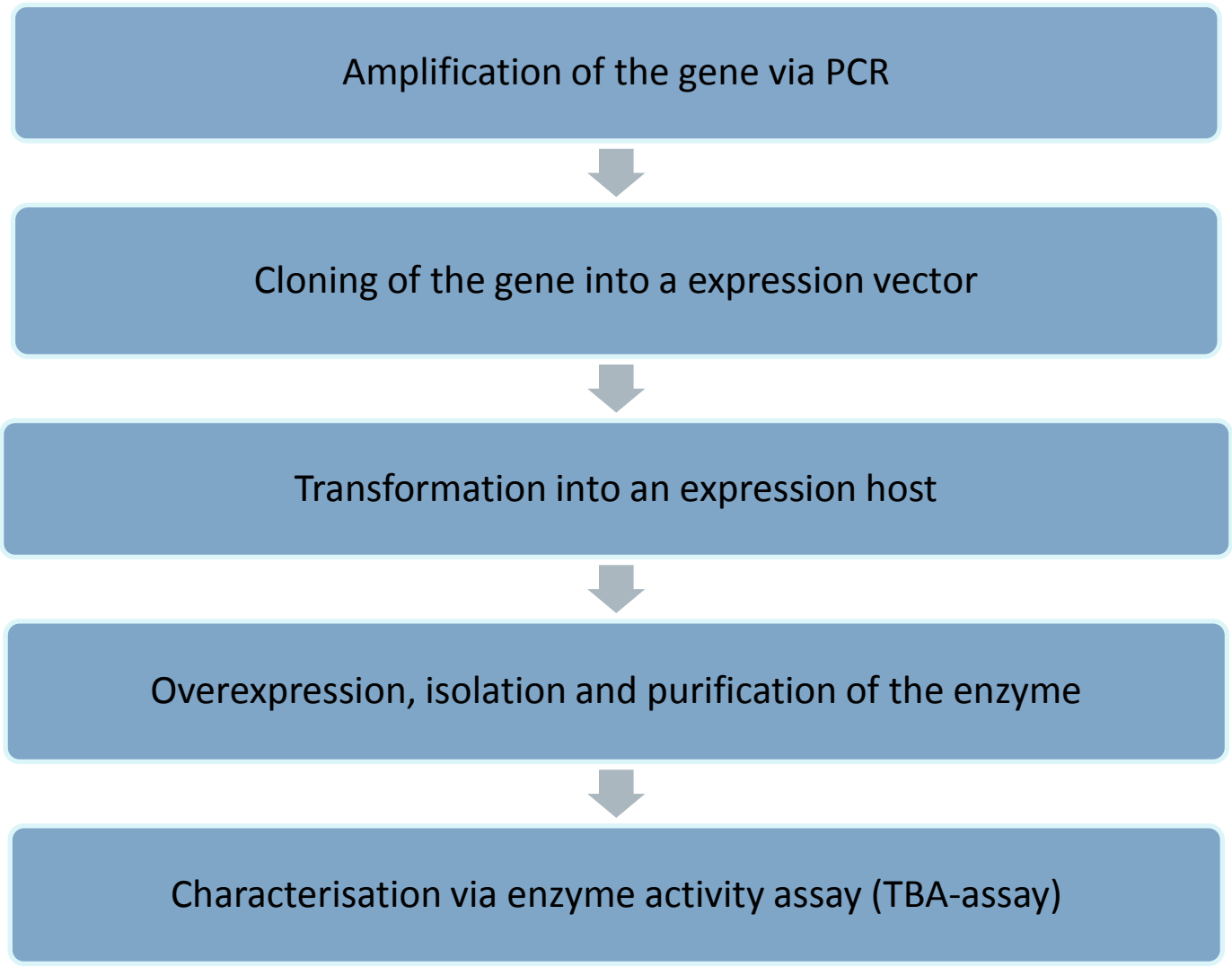
Putative isoenzymes of KD(P)GA

- *DapA-1*
- *DapA-2*
- *DapA-3*



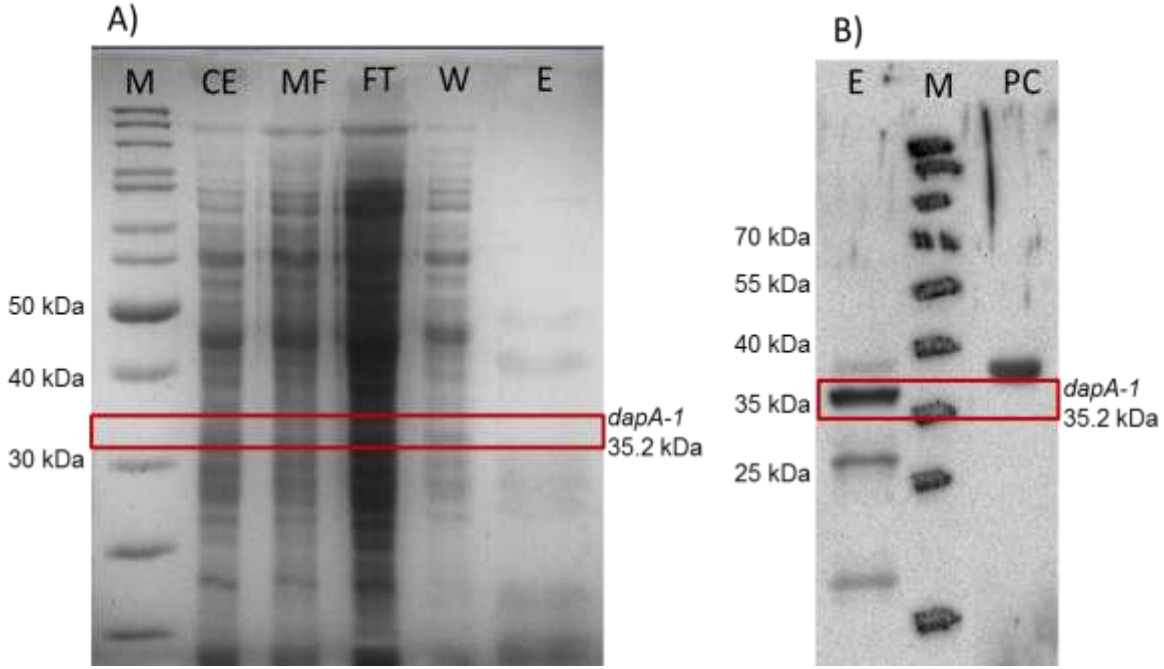

Electron micrograph courtesy of Sonja-Verena Albers, Max Planck Institute, Marburg, Germany, reproduced with permission

# *DapA-1*


Amplification of the gene via PCR
Cloning of the gene into a expression vector
Transformation into an expression host
Overexpression, isolation and purification of the enzyme
Characterisation via enzyme activity assay (TBA-assay)

# *Results*

- Succesful expression of *DapA-1* in *Sulfolbus acidocaldarius*



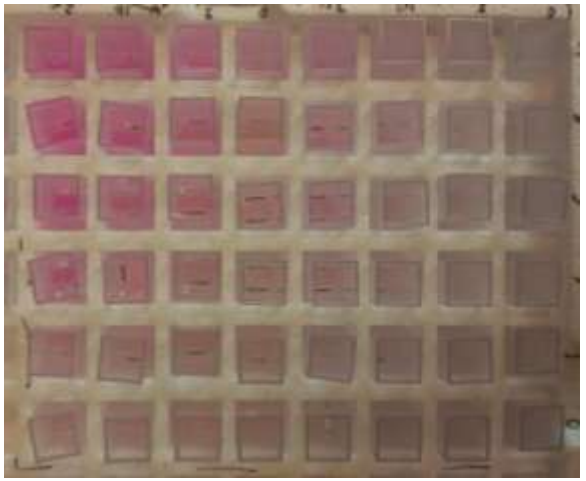

Protein fractions were separated by SDS-PAGE with a 12.5% separating gel and a 4% stacking gel A) and transferred to a PVDF-Membrane B)

# Results

- TBA-Assay
  - Best activity shown with GAP followed by GA and GlyA

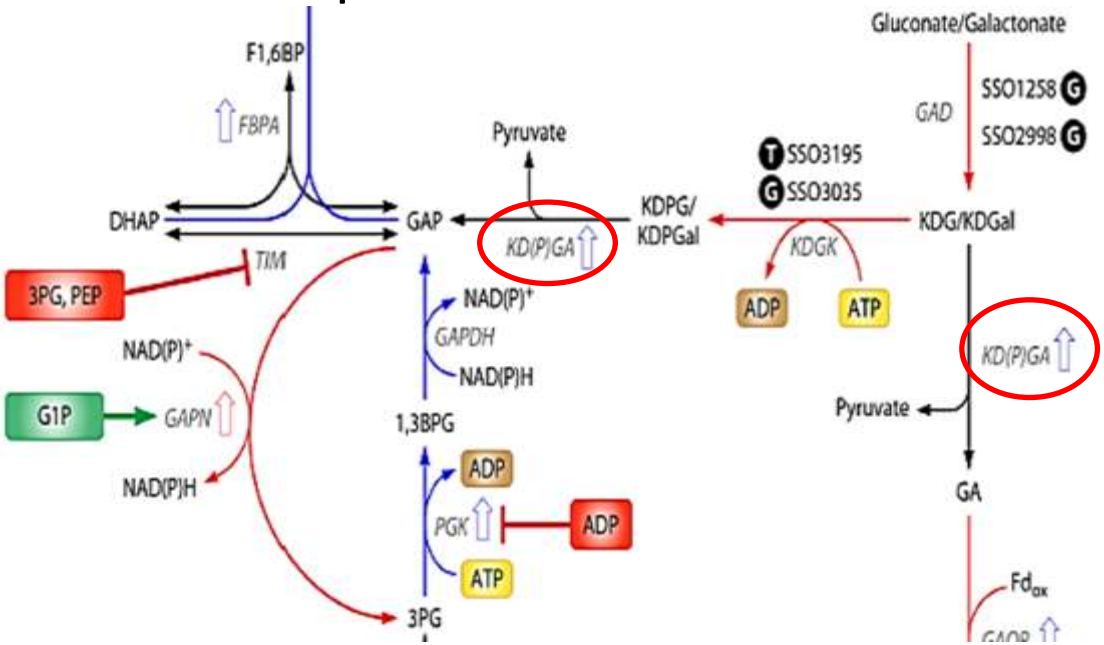
| **Substrates** | Pyruvate | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | GA | GAP | GlyA | SSA | FA | Thr | Ery | Gly | DOP |
| **Soluble Protein** | ✓ | ✓ | ✓ | x | x | x | x | x | x |

GA = glyceraldehyde, GAP = glyceraldehyde-3-phosphate,
GlyA = glycolaldehyde,
SSA = succinic semialdehyde, FA = formaldehyde, Thr = D-Threose,
Ery = Erythrose, Gly = glyoxylate, DOP = 2,5-dioxopentanoate



# Conclusion

- *DapA-1* seems to be an isoenzyme of the KD(P)G- aldolase
- Show same substrate preferences




- Further investigation concerning the stereochemical control needed

# Experience & Advice

- Read Papers
- Make proper notes and mark all your samples neatly (can save you quite some work and time….)
- Western blotting is hard work
- A great research group eases everything
- Do not take it too serious

## Special thanks to:

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