Investigation of four sugar kinases and one phosphoglucose isomerase from the (hyper)thermoacidophilic creanarchaeon *Sulfolobus solfataricus* P2

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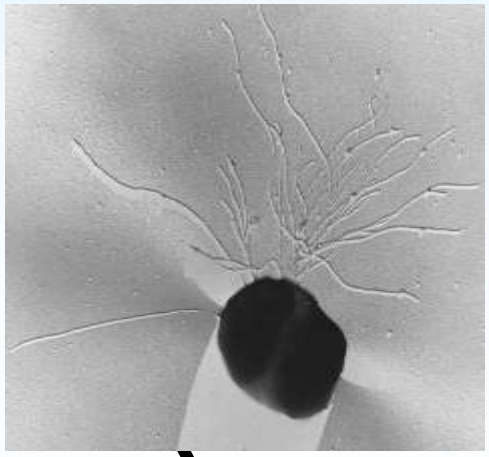
* Introduction I

Bacteria

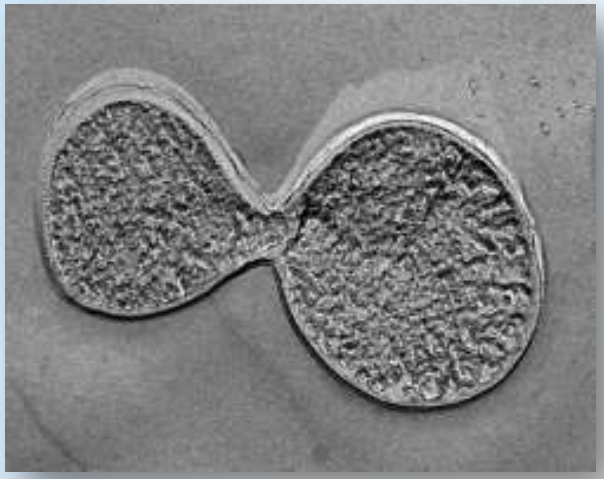
Archaea



Eukaryotes

Woese & Fox (1977)

Introduction I



http://www.sulfosys.com/sulfolobus-solfataricus.html

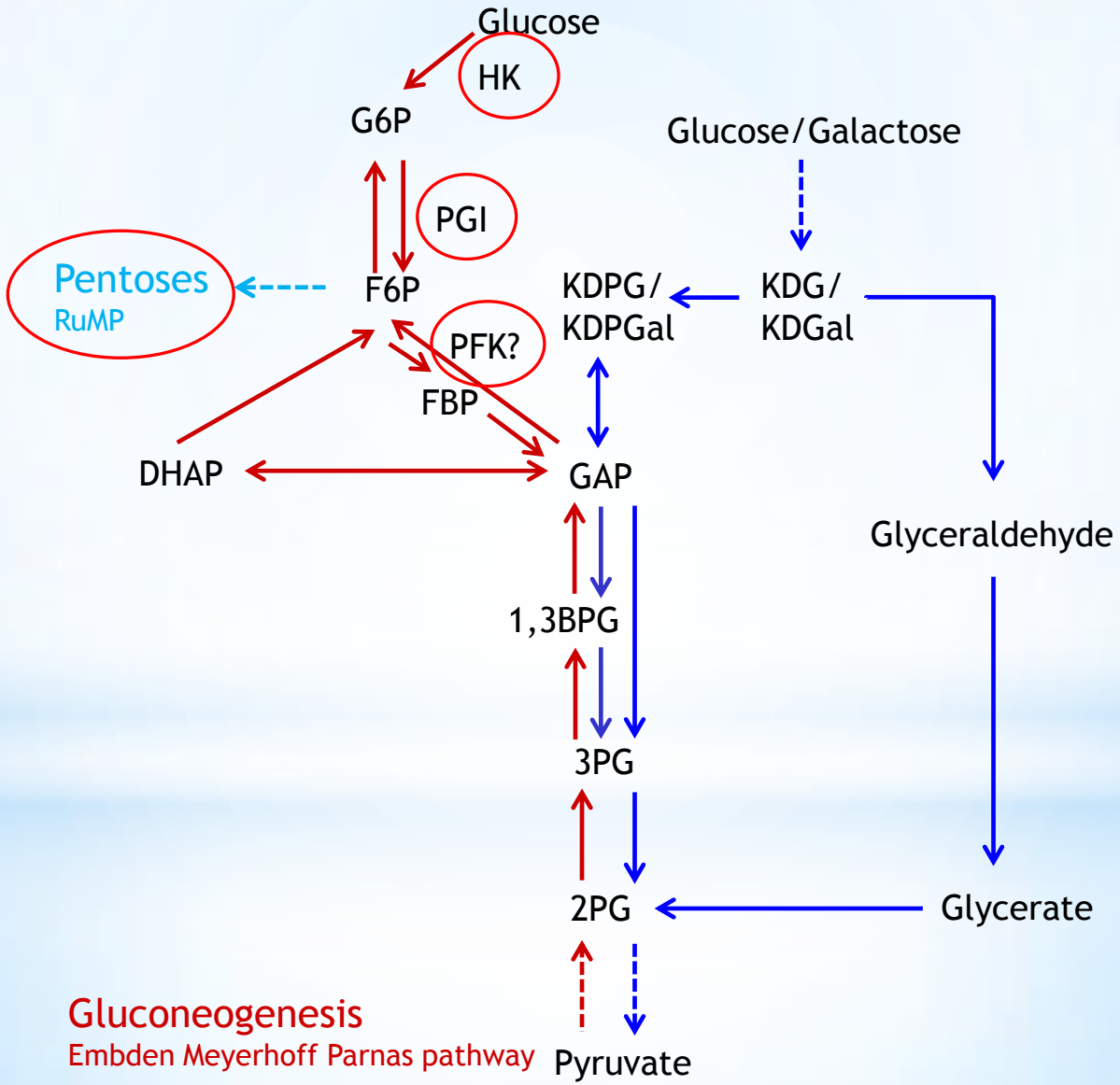
* *Sulfolobus solfataricus*
* *Domain: Archaea*
* *(hyper)thermoacidophil (T=80°C, pH=2-3)*
* *carbon and energy source: carbohydrates, amino acids*

Yellowstone National Park, USA

Pisciarelli, Italy

Aim of the work

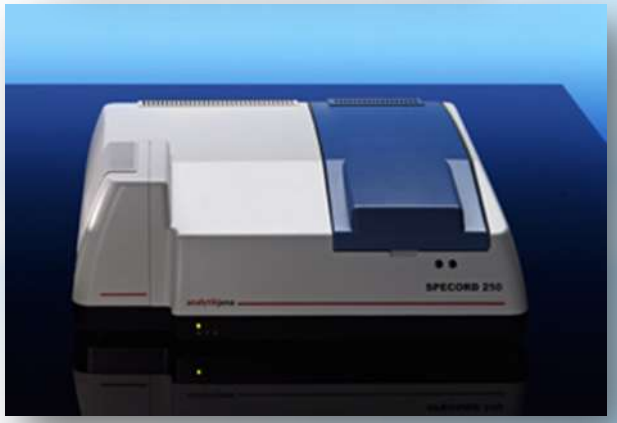
* Central carbohydrate metabolism of *S. solfataricus*



Methods

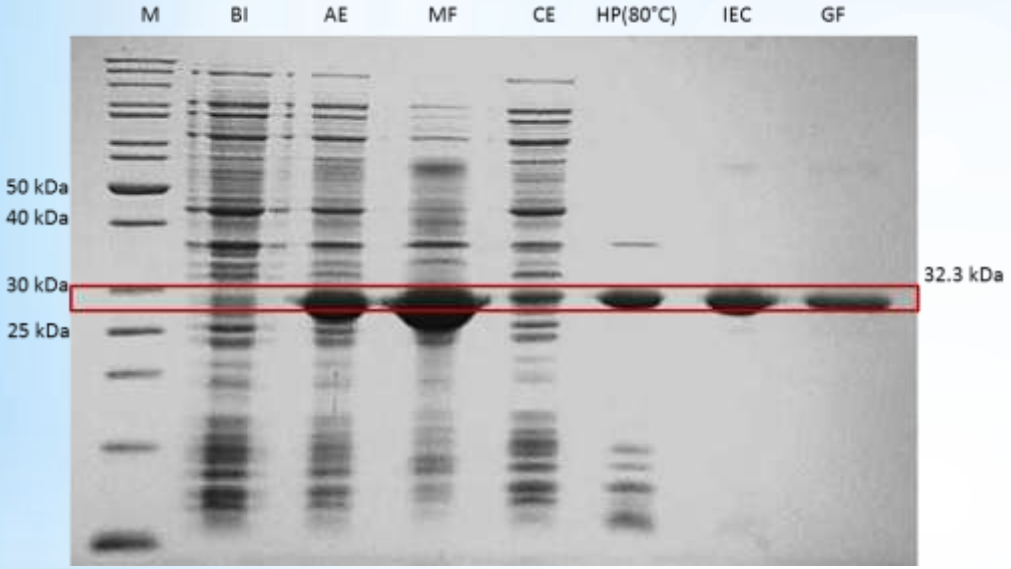
putative hexokinase: SSO3218

* Heterologues overexpression of the putative hexokinase in *E.coli*
* Purification to apparent homogeneity
  - heat precipitation, ion exchange chromatography and gel filtration
* Charaterization via enzyme activity assay at 340 nm (60°C) and determination of kinetic parameters
  - v_{max} (maximal reaction velocity), k_m (Michaelis-Menten constant),k_{cat} (turnover number) k_{cat}/k_m (catalytic efficiency)



Results I

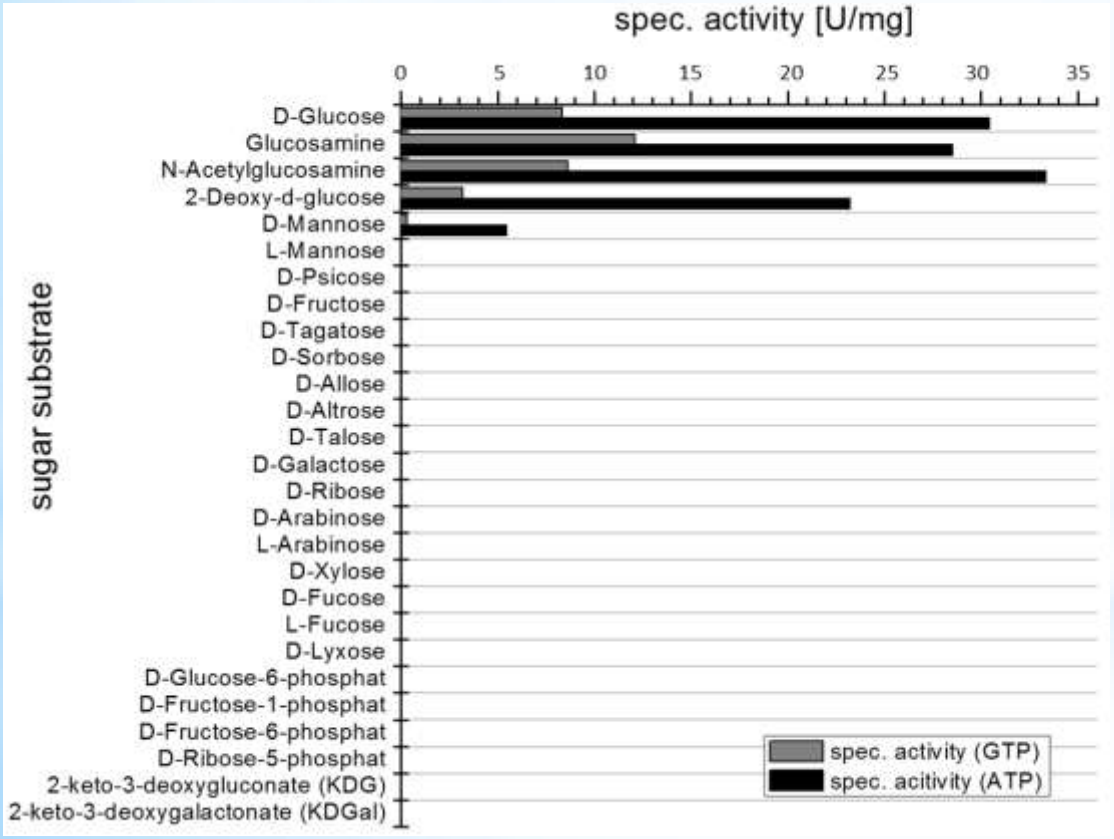
putative hexokinase: SSO3218

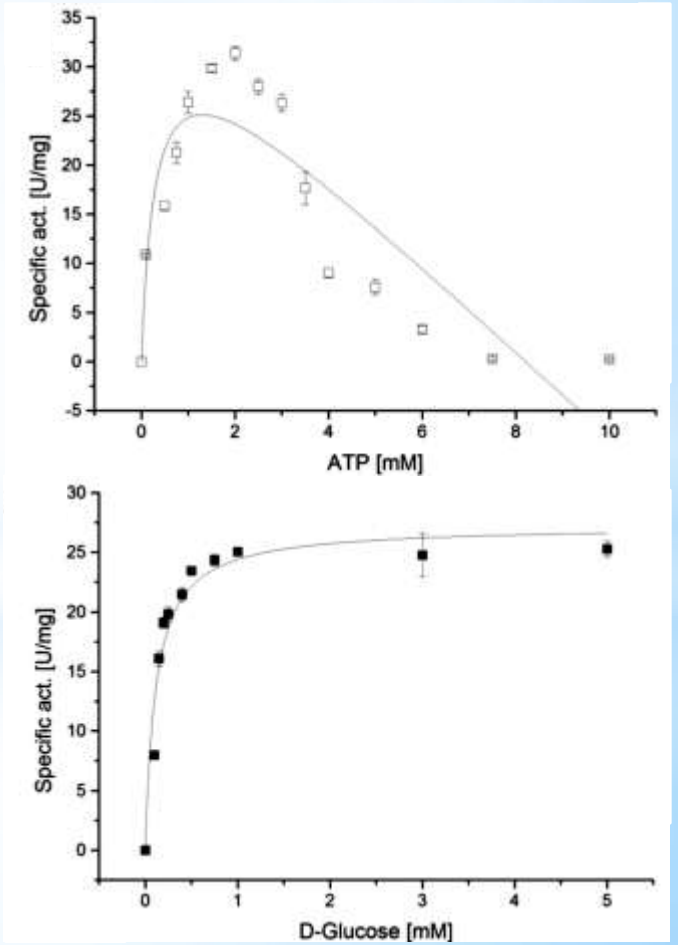


* Successfully soluble expressed in *E. coli*
* Size 32.3 kDa
* Purified to apparent homogeneity

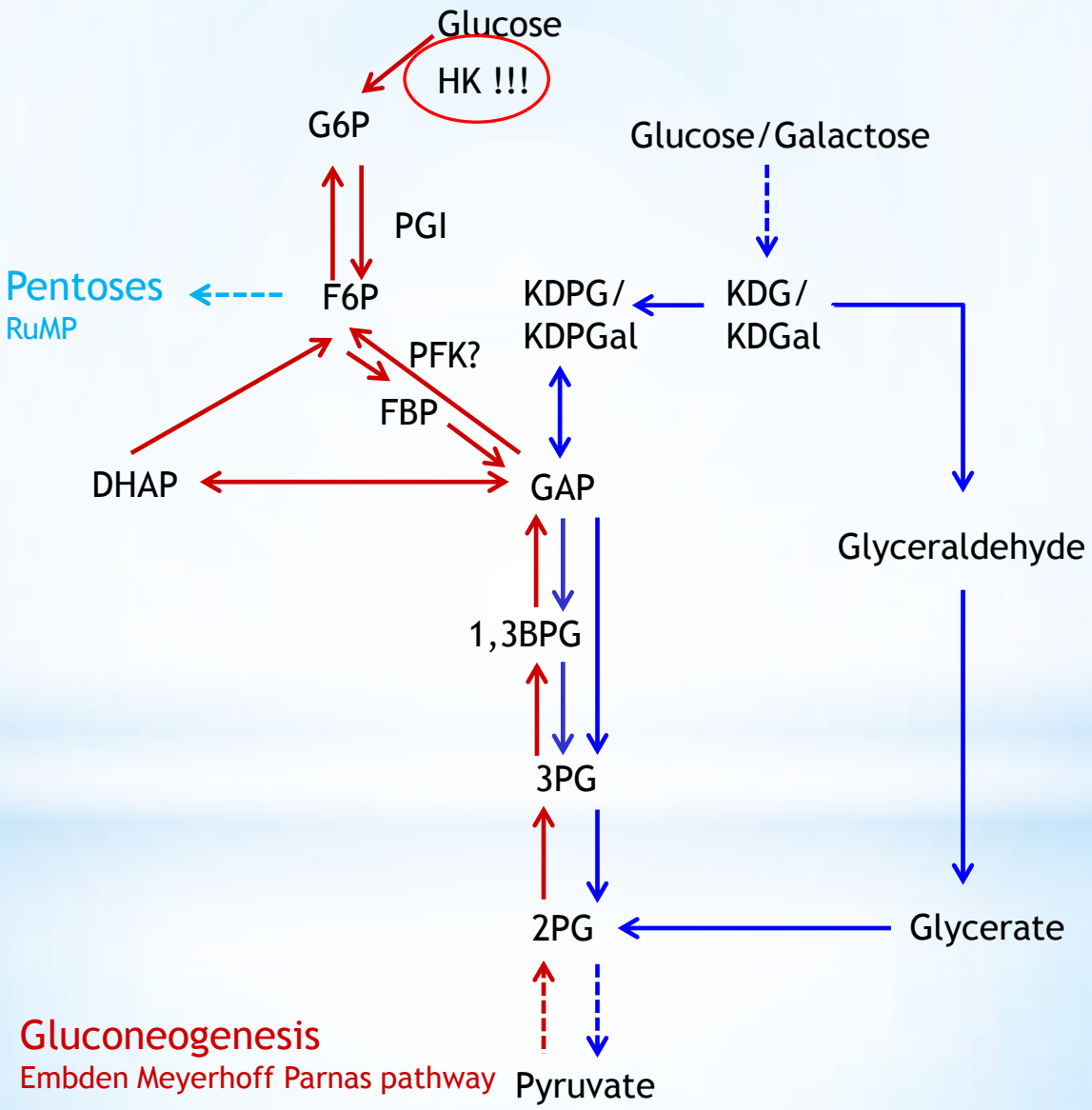
Results II

* SSO3218: Hexokinase activity
* Sugar substrates: D-Glucose, D-Glucosamine, N-Actetylglucosamine, 2-Deoxy-d-glucose, D-Mannose
* Phosphoryl donors: ATP, GTP





* Conclusion I



Conclusion II

- first report of an ATP dependent hexokinase in *S.solfataricus*

- Preferred sugar substrate is D-Glucose
  -catalytic efficiency (k_{cat}/k_m): 127.27 [$mM^{-1}s^{-1}$]
  -Glucosamine and N-Actelyglucosamine reduced compared to D-Glucose.

- Hexokinases from *Sulfolobus* species presumably build a separate hexokinase family with unique substrate specificity

* Experience

* Independent work and organization
* It does not always work at the first try
* Self confidence by positive results

* Advices

* Read paper!
* Create a good schedule!
* Backups, backups and backups!!!
* Have fun
* Do not pipette by mouth!

* Thanks for your attention

Special thanks to:

* Prof. Dr. Bettina Siebers and the whole group
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