Carbon metabolism in Streptococcus thermophilus: co-utilization in mixtures and role of sugar nature and concentration in gene regulation

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Carbon metabolism in *Streptococcus thermophilus*: co-utilization in mixtures and role of sugar nature and concentration in gene regulation

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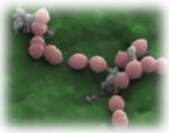


Context

Streptococcus thermophilus is a bacterium widely used in the production of yogurts and cheeses, where it efficiently ferments lactose, the saccharide naturally present in milk. However, when used in sweetened dairy products or plant-based products, *S. thermophilus* may encounter other saccharides (i.e. alone or in mixtures). To date, *S. thermophilus* growth and metabolic capacities in such contexts as well as carbon metabolism regulation mechanisms remain poorly characterized.

Questions

- Is there genetic and/or phenotypic diversity of sugar use in *S. thermophilus*?
- What are the sugars consumed?
- How is sugar metabolism regulated?

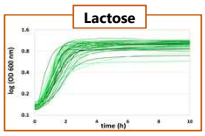
in presence of single or mix sugars

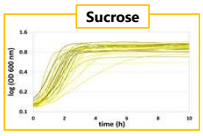
Methods

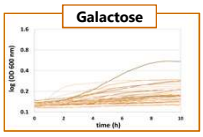
- Screen and comparison of genomes of 39 *S. thermophilus* strains for potential carbon metabolism (CM) genes
- CM gene promoter activities (*lux* transcriptional fusions)
- CM gene mutant construction (gene deletion)
- Growth and acidification (M17 medium)
- Glycolytic flux estimation (lactate prod. estimation)
- Dosage of sugars (HPLC)

lactose, sucrose, glucose, galactose, fructose or sugar mixes in various concentration

Bacterial growth, sugar consumption and glycolytic flux

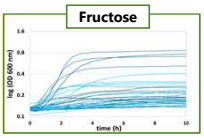


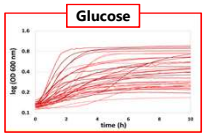




→ → growth of all strains – high final biomass

→ no/low growth for most strains



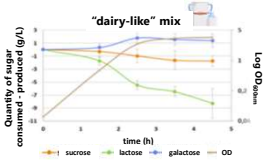


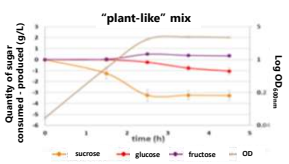
→ growth of the 4 strains having an intact *fruA* gene

→ high variability of growth

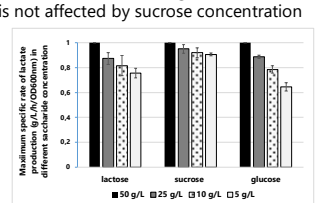
- ***S. thermophilus* LMD-9 first consumes**
  - → lactose over sucrose
  - → sucrose over glucose

when sugars are mixed





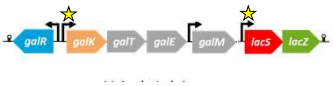
- **Lactate production**
  - → is higher in presence of lactose or glucose, compared to sucrose
  - → decreases as lactose or glucose concentration decrease
  - → is not affected by sucrose concentration



In silico analysis

- **The 39 strains possess genes coding for the potential use of 5-6 carbohydrates**

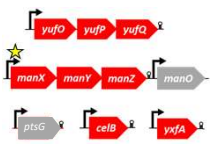
Lactose - Galactose



Fructose

→ ***fruA* : pseudogene for 36 strains over 39**

Glucose - Mannose



Transporter
Hydrolase
Kinase
Regulator

Pseudo-gene for few strains
Pseudo-gene for all strains
Potential *cre* box

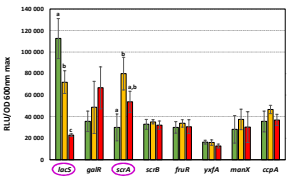
Sucrose

→ ***scrR* : pseudogene for 3/39 strains**

→ **low genetic diversity for carbohydrate metabolism genes (sequence and gene organization conserved)**

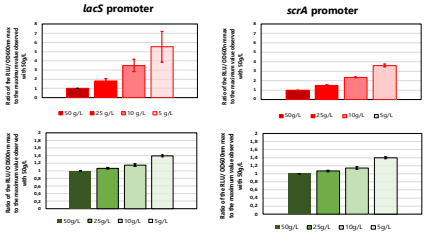
Regulation of carbon metabolism genes

- **All promoters of CM genes tested are active in presence of lactose, glucose or sucrose and the activities of only *lacS* and *scrA* ones are modulated by the nature of the sugar**



Promoter activity for 8 carbohydrate metabolism genes in *S. thermophilus* LMD-9 grown in presence of high concentration of lactose, sucrose, or glucose (50 g/L).

- *lacS*: lactose permease; *galR*: galactose regulator; *scrA*: sucrose/glucose transporter; *scrB*: sucrose hydrolase; *fruR*: fructose regulator; *yxfA*: potential glucose permease; *manX*: potential glucose PTS transporter; *ccpA*: CCR general regulator.
- Experiments were performed in triplicate. Different letters indicate significant differences in promoter activity for a given gene (ANOVA and Tukey-Kramer post-hoc tests, $p<0.05$).



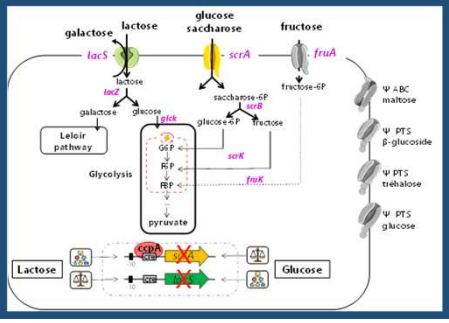
Promoter activity for *lacS* and *scrA* genes in *S. thermophilus* LMD-9 grown in presence of different concentrations of glucose or lactose (50, 25, 10, or 5 g/L).

Each bar represents the ratio of the maximum RLU/$OD600_{nm}$ value for a given set of experimental conditions to the maximum value in the condition with 50 g/L of glucose.

- **Glucose and lactose repress promoter activity for transporter-encoding genes *lacS* and *scrA* in a concentration-dependent manner, whereas sucrose not**
- **Glucose maintains its repressive effects in mixed-saccharide media on *lacS* and *scrA* promoter activities**

- ***lacS* promoter activity is no more repressed in a Δ*ccpA* mutant, whatever the lactose or glucose concentration (10, 25 or 50 g/L)**
- **the promoter profil activity for *scrA* is similar in a Δ*scrR* mutant than in the wild-type strain, in glucose, lactose or sucrose (whatever their concentration)**

- **The sucrose transporter ScrA is necessary for growth in LMD-9 strain in presence of glucose or sucrose as a single carbon source (no growth in a Δ*scrA* mutant)**
  - → **ScrA is responsible for the transport of glucose and sucrose in LMD-9 strain**
  - → **An explanation for the effect of glucose on *scrA* promoter activity modulation?**



Conclusions

- ***S. thermophilus* is able to co-consume several sugars when mixed**
- **Carbohydrate metabolism regulation principally involves lactose and sucrose transporter gene expression via the general regulator CcpA**
- **Regulatory profiles for *lacS* and *scrA* genes according to sugar concentration and glycolytic flux are similar in LMD-9**
- **Catabolic repression in *S. thermophilus* LMD-9 does not strictly represses the consumption of less preferred sugars; instead, saccharides are jointly consumed**

References:

- Gasser et al. Co-utilization of saccharides in mixtures: moving toward a new understanding of carbon metabolism in *Streptococcus thermophilus*, Int J Food Microbiol, doi 10.1016/j.fm.2022.104080
- Gasser et al, New insight on carbohydrate metabolism in *S. thermophilus*: role of sugar nature and concentration in operon regulation via carbon catabolite control, under review.