DUAL GLYCOLYTIC PATHWAYS OPERATING IN *LACTOBACILLUS REUTERI* ATTCC 55730

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Introduction

The bipartition of the lactobacilli into homofermentatives and heterofermentatives, based on the type of central metabolic pathway, should be revised. It is expected that a major part of the lactobacilli possess the potential to have the dual central metabolic pathways running simultaneously (Årsköld et al 2008, Claesson et al 2006, Pieterse et al 2005).

Redox problem

The maximum specific growth rate and biomass yield obtained on glucose was low and growth was linear (Fig. 3). This was accompanied by low ATP levels (Fig. 4), although the ATP production flux is as high as in sucrose-metabolizing cells due to two-fold increase of enzyme activities in both glycolytic pathways (Table 2). Adding fructose as an external electron acceptor improved growth on glucose, indicating that the observed behaviour is due to a redox imbalance causing energy starvation.

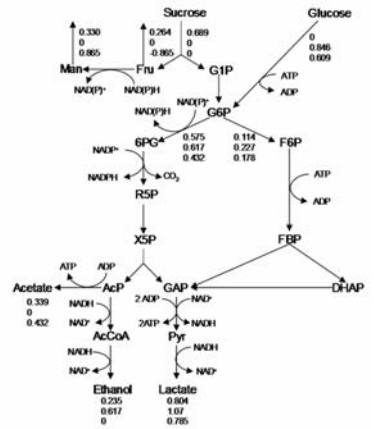

Fig 1. MFA of sucrose and glucose catabolism in *L. reuteri* ATCC 55730.

The fluxes (mol·mol biomass^{-1}·h^{-1}) represent that of cultures on 50 g·l^{-1} sucrose, 50 g·l^{-1} glucose, and 25 g·l^{-1} glucose plus 25 g·l^{-1} fructose, respectively.

Fru, fructose; Man, mannitol; G1P, glucose-1-phosphate; G6P, glucose-6-phosphate; 6PG, 6-phosphogluconate; R5P, ribulose-5-phosphate; X5P, xylulose-5-phosphate; AcP, acetyl phosphate; AcCoA, acetyl-CoA; F6P, fructose-6-phosphate; FBP, fructose-1,6-bisphosphate; DHAP, dihydroxyacetone phosphate; GAP, glyceraldehyde-3-phosphate; Pyr, pyruvate. All carbon balances were complete.

Table 1. Specific activities of fructose-1,6-bisphosphate aldolase (ALD), phosphofructokinase (PFK), and phosphoglucoisomerase (PGI) in sucrose and glucose grown cultures of *L. reuteri* ATCC 55730**.** Values are means of triplicates.

Enzyme	Activity (μmol^{-1}·min^{-1} mg protein^{-1})	
	Sucrose	Glucose
ALD	0.11 ± 0.004	0.21 ± 0.01
PFK	0.18 ± 0.16	0.27 ± 0.16
PGI	0.23 ± 0.07	0.59 ± 0.19

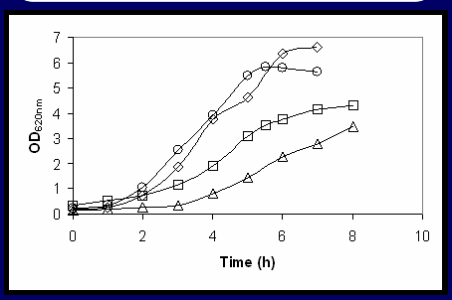

Fig 3. Growth of *L. reuteri* ATCC 55730 on 50 g·L^{-1} glucose (□), 50 g·L^{-1} sucrose (◊), 25 g·L^{-1} glucose and 25 g·L^{-1} fructose (o), and 20 g·L^{-1} glucose (Δ).

Results

Proof of dual glycolytic pathways

L. reuteri ATCC 55730 has both pathways operable under normal growth conditions, as was proven via 4 independent approaches (Årsköld et al 2008):

(i) metabolic flux analysis (MFA) (Fig. 1)
(ii) bioinformatics
(iii) activities of key enzymes of both pathways (Table 1)
(iv) *in vitro* ^{31}P-NMR analysis (Fig. 2)

In all cases studied so far with *L. reuteri* ATCC 55730, the main flux is through the phosphoketolase pathway (PKP), while the Embden Meyerhof pathway (EMP) is used as a shunt.

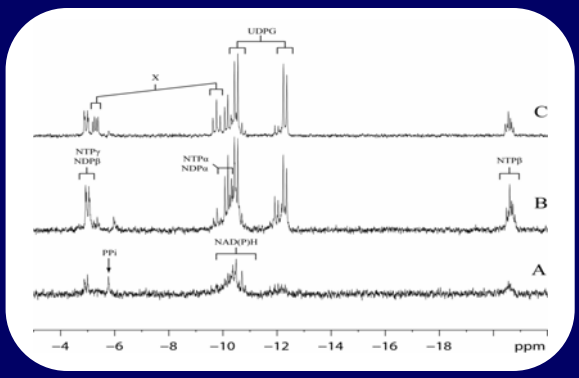

Fig 4. ^{31}P NMR spectra of glucose (A), sucrose (B) and glucose plus fructose (C) metabolizing *L. reuteri* ATCC 55730. Labels: PP_i = pyrophosphate; α, β, γ NTP or NDP = phosphates of nucleoside tri and diphosphates; NAD(P)H = nicotinamide adenine dinucleotide (phosphate)/reduced and oxidized; UDPG = UDP-glucose peaks; and X = unknown peaks.

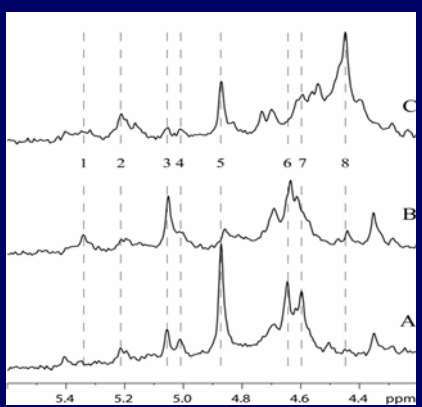

Fig 2. ^{31}P NMR spectra of the expanded phosphomonoester region of glucose (A), sucrose (B) and glucose plus fructose (C) metabolizing *L. reuteri* ATCC 55730. Peak 1, 6-phosphogluconate; peak 2, glucose-6-P; peak 3, xylulose-5-P; peak 4, ribulose-5-P; Peak 5, 3-phosphoglycerate; peak 6, ribose-5-P; peak 7, fructose-6-P; peak 8, unidentified.

Table 2. Maximum specific growth rate, molar growth yield and Y_{ATP} of *L. reuteri* ATCC 55730 growing on sucrose, glucose and glucose plus fructose.

Substrate	μ_{MAX} h^{-1}	Y_{SX} g biomass· mol substrate^{-1}	Y_{ATP} g biomass· mol ATP^{-1}
Sucrose	0.82 ± 0.16	22.0 ± 2.0	10.5 ± 1.6
Glucose	0.45 ± 0.01	15.3 ± 2.3	5.2 ± 0.1
Glucose + Fructose	0.66	33.2 ± 1.7	7.7 ± 0.2

Conclusions

- Dual operating glycolytic pathways enhance metabolic flexibility and might be an adaptation to exposure to continuously changing environments, such as the GIT
- The dominant PKP indicates a common use of external electron acceptors, which ultimately points to new probiotic properties.

References

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