Expression Profiling of *Pediococcus acidilactici* FAM18098 with a focus on threonine and serine catabolism

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Introduction

Proteolysis and amino acid catabolism are the most important biochemical processes taking place during cheese ripening. When *Pediococcus acidilactici* FAM18098 was used as adjunct culture in cheese production, it degraded arginine, serine and threonine and synthesized ornithine, alanine and 2-aminobutyrate. The latter we did not observe under laboratory conditions using MRS broth, a medium commonly used for lactic acid bacteria. By examining a variety of new compositions we were able to find a medium in which this strain shows an amino acid metabolism similar to the one observed in cheese (Fig.1). In order to understand the transcriptional regulation of genes involved in amino acid catabolism we analyzed the transcriptome of *P. acidilactici* FAM18098.

Methods

P. acidilatici FAM18098 was grown at 30°C in a basal medium (BM) containing 2 g/L glucose (Glc-BM) and galactose (Gal-BM), respectively. After 24, 48 and 65 hours the bacteria were harvested for RNA isolation. Reverse transcribed RNA was sequenced on an Ion Torrent PGM.

Amino acid composition in the culture supernatants

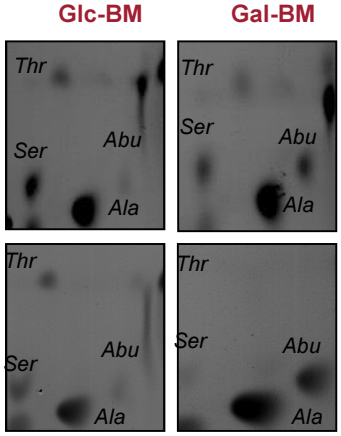

Fig.1: The 2D thin-layer chromatography of the culture supernatants of *P. acidilatici* FAM18098 grown in Glc-BM and Gal-BM for 24 and 65 h at 30°C shows the degradation of threonine (Thr) and serine (Ser) and the formation of alanine (Ala) and 2-aminobutyrate (Abu). This is in good agreement with the amino acid metabolism observed in cheese.

Read statistics

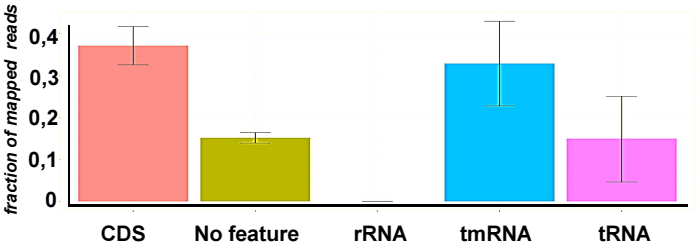

Fig.2: Read mapping. CDS: coding sequence, rRNA: ribosomal RNA; tmRNA: transfer-messenger RNA; tRNA: transfer RNA (Glc-BM, N=3 and Gal-BM, N=3) .

Most significantly regulated genes Glc-BM versus Gal-BM

FAM18098_01636	PTS system
FAM18098_01637	Galactitol permease IIC component
FAM18098_01064	UDP-glucose 4-epimerase
FAM18098_01065	Galactokinase
FAM18098_01635	PTS system galactitol-specific transporter subunit IIA
FAM18098_01070	Lactose permease
FAM18098_01634	Lactose phosphotransferase system repressor
FAM18098_00052	PTS system lactose-specific EIICB component
FAM18098_01632	Galactose-6-phosphate isomerase subunit LacB
FAM18098_01063	Galactose-1-phosphate uridylyltransferase
FAM18098_01631	Tagatose 1
FAM18098_00051	Lactose-specific phosphotransferase enzyme IIA
FAM18098_01633	Galactose-6-phosphate isomerase subunit LacA

Tab.1: Comparing Glc-BM (24/48/65 h) with Gal-BM (24/48/65 h) the most significantly regulated genes (upregulated in Gal-BM (p-value <0.05)) are involved in sugar metabolism.

GO term enrichment focusing on nitrogen metabolism

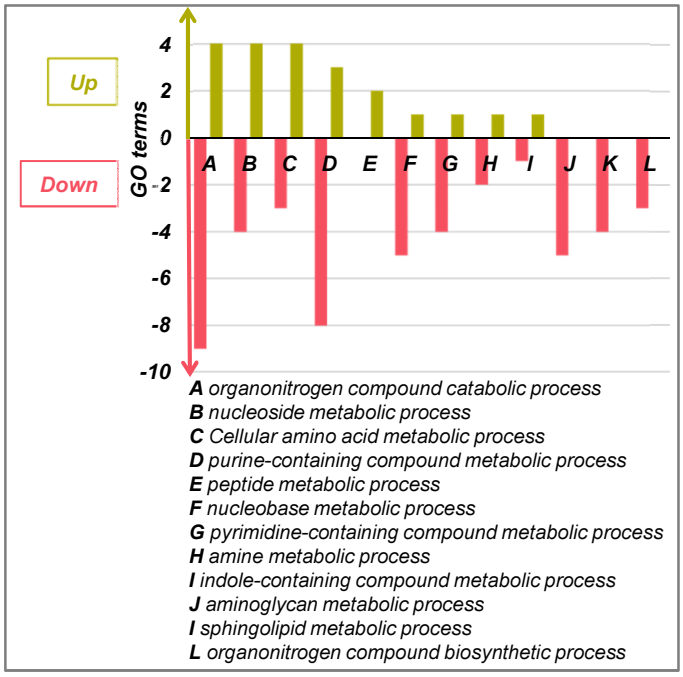

Fig.3: The Gene Ontology (GO) term enrichment of the regulated genes involved in the nitrogen metabolism of the samples Glc-BM (24/48/65 h) and Gal-BM (24/48/65 h) illustrates the biological processes which are regulated (green=up, red=down) by media composition.

Conclusions

We established a protocol to analyze and compare whole transcriptomes of *P. acidilactici* with next generation sequencing technology to study the amino acid metabolism (Fig. 1). We found the following findings:

- Efficient depletion process (Fig.2)
- Reliable statistic results (Tab.1)
- First identification of significantly regulated biological processes by GO term enrichment analysis (Fig.3)

With this methodology we intend to gain deeper insights into the regulation of metabolic pathways of lactic acid bacteria.

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