Properties of Alanine Dehydrogenase from *Pediococcus acidilactici* FAM18098

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Abstract

Pediococcus acidilactici occurs regularly in fermented food such as cheese. When *P. acidilactici* FAM18098 was used as an adjunct culture in cheesemaking, these cheeses showed reduced amounts of free serine and threonine and developed significantly higher amounts of alanine and 2-aminobutyrate during ripening than cheeses without the adjunct. Fermentation assays with various media showed that *P. acidilactici* FAM18098 also synthesized alanine and 2-aminobutyrate *in vitro*, and that biosynthesis of both compounds was dependent on the medium used. Since alanine is reported to add to the perceived sweetness of dairy products, *P. acidilactici* could be used as flavor-forming adjunct culture in the production of fermented products. For a better understanding of the alanine metabolism and its regulation, the genome data from *P. acidilactici* FAM18098 was searched for genes involved in alanine metabolism. Two genes encoding putative alanine dehydrogenases were identified, cloned and expressed in *E. coli* to study their activities. Indeed, one of the purified recombinant proteins catalyzed the reversible amination of pyruvate and 2-ketobutyrate to alanine and 2-aminobutyrate, respectively. However, expression analysis showed that the gene was constitutively expressed and that the expression did not correlate with alanine biosynthesis indicating that the gene plays a minor role in this pathway. An inducible alanine dehydrogenase activity was discovered when cell-free extract of *P. acidilactici* FAM18098 was separated by native electrophoresis and assayed for alanine dehydrogenase activity.

Kinetic parameters of the recombinant alanine dehydrogenase Ald2

Substrate	nonvaried Substrate	Km [a] (mM)
Alanine	NAD	0.38 ± 0.06
2-Aminobutyrate	NAD	1.42 ± 0.41
NAD	Alanine	0.16 ± 0.05
Pyruvate	NADH, ammonium	n. d. [b]
2-Ketobutyrate	NADH, ammonium	1.80 ± 0.51
Ammonium chloride	NADH, 2-Ketobutyrate	54.4 ± 6.5

[a] Values represent the means (± S.D.) of four repetitions
[b] not determined, since activity was inhibited above 2 mM pyruvate

Relative quantitation of *ald2* mRNA in *P. acidilactici* FAM18098 grown in two different media

Broth	Day 1	Day 2	Day 3
Gal-ST	19.9 ± 1.9 [a]	20.0 ± 0.04	23.1 ± 0.3
MRS	26.8 ± 0.5	21.8 ± 0.01	23.0 ± 0.03

[a] Values represent the CT levels determined by real-time RT-PCR analysis and are the mean (±S.D) of triplicates.

Formation of alanine and 2-aminobutyrate by *P. acidilactici* FAM18098

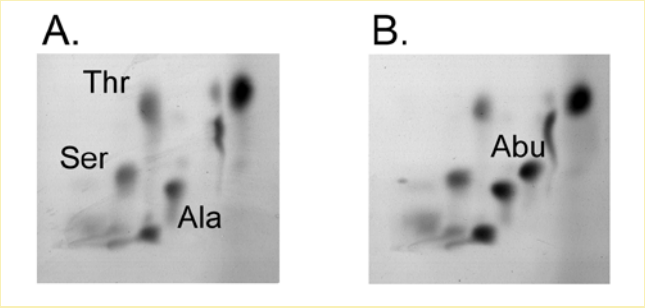

Fig. 1. The composition of amino acids in the culture supernatants of *P. acidilactici* grown in Gal-ST medium was analyzed before (A.) and after fermentation (B.) with 2D thin-layer chromatography. No formation of 2-aminobutyrate or alanine was observed when the bacterial strain was cultivated in MRS medium (data not shown).Thr: threonine, Ser: serine, Ala: alanine, Abu: 2-amino-butytrate

Colorimetric detection of alanine dehydrogenase activity in cell-free extract

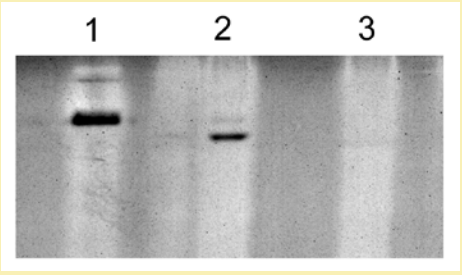

Fig. 2 Cell-free extracts of *P. acidilactici* FAM18098 grown in Gal-ST (2) and MRS medium (3) were separated under native conditions in a polyacrylamide gel. The gel was then incubated with alanine, NAD, phenazine methosulfate and nitroblue tetrazolium. Thereby, alanine dehydrogenase activity is visualized by the formation of an insoluble formazan product (dark bands). Recombinant alanine dehydrogenase from *B. subtilis* was used as control (1).

Conclusion

The alanine dehydrogenase Ald2 from *P. acidilactici* FAM18098 clearly synthesized alanine and 2-aminobutyrate *in vitro*. However, gene expression analysis indicated that the enzyme may not be involved in the synthesis of both compounds. Further studies are in progress to elucidate the pathway leading to the formation of alanine and 2-aminobutyrate in cheese and to understand its influence on cheese aroma and quality.

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