PRODUCTION OF AROMATIC COMPOUNDS FROM LEUCINE BY LACTIC ACID BACTERIA AND *STAPHYLOCOCCI*

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KEYWORDS : aminoacid catabolism, flavour, meat product.

BACKGROUND :

The characteristic flavour of dry fermented sausage is due to a complex combination of different non-volatile and volatile compounds. Volatile compounds have been identified as alkanes, alkenes, aldehydes, methylaldehydes, ethylesters, alcohols, ketones and terpenes (Berdagué *et al.*,1993). Among these aromatic compounds, 3-methyl butanal and 3-methyl butanoic acid have a strong effect on the sensorial qualities of the sausages (Berdagué *et al.*,1993 ; Stankhe *et al.*,1995). Berdagué *et al.* (1993) and Montel *et al.* (1996) have shown that sausage inoculated with *Lactobacillus sake* and *Staphylococcus carnosus* contained higher quantities of 2 or 3-methyl butanal than those inoculated with *Staphylococcus warneri* or *Staphylococcus saprophyticus*. In the same way, Stankhe *et al.* (1995) attributed an important role of 3-methyl butanal in aroma of dry sausage inoculated with *Staphylococcus xylosus*. These molecules are derived from the leucine catabolism and starter cultures may be involved in this catabolism.

OBJECTIVES :

According to these bibliographic data, the purpose of the present study was to determine the enzymatic pathways involved in the aromatic molecules production from leucine by lactic acid bacteria and *Staphylococci*.

MATERIAL AND METHODS :

Bacterial strains : *Carnobacterium piscicola* 545, *Carnobacterium divergens* 210, *Lactobacillus sake* 23K, *Lactobacillus curvatus* 411, *Lactobacillus plantarum* Lpl, *Pediococcus pentosaceus* 716, *Staphylococcus carnosus* 833, *Staphylococcus xylosus* 16, *Staphylococcus saprophyticus* 852, *Staphylcoccus warneri* 863.

Culture conditions : Strains of *C. piscicola* 545, *C. divergens* 210, *L. sake* 23K, *L .curvatus* 411, *L. plantarum* Lpl, *P. pentosaceus* 716 were grown at 30°C in the Niven medium (+0.2% ribose). All the stains of *Staphylococci* were grown at 30°C in Peptone Yeast Extract (+0.5% glucose). Cells grown to exponential growth phase were centrifugated 10000 g for 10 min at 4°C. After washing twice with saline solution, cells were resuspended in a buffer KH_2PO_4/Na_2HPO_4 (0.067M, pH 7).

Incubation media : Microbial cell suspension density estimated by the A_{600} measure was adjusted between 10 and 15 in the reaction mixture.

« Reaction 1 » : aminotransferase activity

Cells were incubated for 22 hours at 30°C in Tris HCl 0.05M pH 8.6 buffer with leucine (2mM), α-ketoglutaric acid (10mM), pyridoxal phosphate (2mM).

« Reaction 2 » : decarboxylase activity

Cells were suspended in KH_2PO_4/Na_2HPO_4 0.067 M pH 5.4 at 30°C containing 4mM of α-ketoisocaproic acid and 1.4mM of thiamine pyrophosphate. The reactions were stopped after 22 hours by adding $HClO_4$ (0.6 M). The reaction mixture was harvested 7 min at 4000 g, the supernatant was frozen.

Analytical method : Medium supernatants were analysed by HPLC on a ion-exchange column (Aminex HPX 87H) at 55°C. The metabolites were eluted by an isocratic gradient of H_2SO_4 0.075N with a flow rate of 1ml/min as described by Fernandez-Garcia and Mac Gregor (1994). Molecules were detected by measuring absorbance at 210nm and by refractometry. For the quantification of metabolites, standards were chromatographied in the same conditions.

RESULTS AND DISCUSSION :

As shown in Table 1, in the conditions of reaction 1, all the strains were able to catabolise leucine by producing α-ketoisocaproic acid ; the levels were to 7µg/ml for *C. piscicola* 545 to 147 µg/ml for *L. plantarum* Lpl. *P. pentosaceus* 716, *L.plantarum* Lpl, *L. curvatus* 411, *S. warneri* 863 accumulated α-ketoisocaproic acid. *S. carnosus* 833, *S. saprophyticus* 852, *S. xylosus* 16, *L. sake* 205 and *C. piscicola* 545 produced hydroxy α-ketoisocaproic acid. High productions of 3-methyl butanal (65µg/ml), 3-methyl butanol (28 µg/ml) and 3-methyl butanoic acid (53 µg/ml) were observed for *C. piscicola* 545.

At pH 5.4, in the conditions of reaction 2, the strains of *S. warneri* 863, *L. sake* 23K, *L. curvatus* 411, *C. divergens* 210, *L. plantarum* Lpl produced hydroxy α-ketoisocaproic acid, 3-methyl butanoic acid without detection of 3-methyl butanal. Besides 3-methyl butanoic acid, *S. saprophyticus* 852, *S. carnosus* 833 and *C. piscicola* 545 produced also 3-methyl butanal. The highest production of aldehyde was observed for *C. piscicola* 545 (643 µg/ml) and *S. xylosus* 16 (332 µg/ml).

For all the strains studied, the production of α-ketoisocaproic acid could be attributed to an aminotransferase activity as shown in Figure 1. The production of 3-methyl butanal by *C. piscicola* 545 and *S. carnosus* 833, *S. xylosus* 16, *S. saprophyticus* 852 was due to the decarboxylation of α-ketoisocaproic acid. The lowest activity of this enzyme in the reaction 1 may be attributed to higher pH value than that of reaction 2. For *S. carnosus* 833, *S. saprophyticus* 852, *C. piscicola* 545, the presence of 3-methyl butanoic acid may correspond to the dehydrogenation of the aldehyde or to the activity of the multienzymatic α-ketoacid dehydrogenase complex. For the strains of *S. warneri* 863, *L. sake* 23K, *L. curvatus* 411 and *C. divergens* 210,

the production of 3-methylbutanoic acid without detection of 3-methyl butanal may correspond to a multienzymatic α-ketoacid dehydrogenase complex which directly convert α-ketoisocaproic acid into carboxylic acid.

SOUCHES	REACTION 1					REACTION 2			
	capro	OH capro	al	oic	ol	OH capro	al	oic	ol
S. carnosus 833	52	69	11	70	<5	137	8	161	54
S. xylosus 16	81	32	20	<5	<5	5	332	<1	<5
S. saprophyticus 852	30	24	<5	25	<5	75	15	135	<5
S. warneri 863	11	<1	<5	<1	<5	116	<5	132	<5
L sake 23K	61	5	<5	<1	<5	14	<5	4	<5
L. curvatus 411	12	<1	<5	<1	<5	<1	<5	33	<5
C. divergens 210	55	75	<5	<1	<5	60	<5	31	<5
C. piscicola 545	**7**	**15**	**65**	**53**	**28**	**6**	**643**	**25**	**<5**
L.plantarum Lpl	147	<1	<5	<1	<5	108	<5	14	<5
P. pentosaceus 716	37	<1	<5	<1	<5	<1	<5	3	<5

Table 1: Metabolites produced from leucine (reaction 1) and α-ketoisocaproic acid (reaction 2) after 22 hours incubation.
Results in µg of metabolites produced /ml of reaction mixture.
Capro : α-ketoisocaproic acid ; OH capro : hydroxy α-ketoisocaproic acid ; al : 3-methyl butanal ; ol : 3-methyl butanol ; oic : 3-methyl butanoic acid.

CONCLUSION :

From the results of the study, it can be concluded that all strains which can be present in fermented meat products were able to catabolise leucine. The limiting factor for leucine catabolism seems to be the pH because aminotransferase had higher activity at pH 8.6 than pH 5.4. At pH 5.4, all strains produced 3-methyl butanoic acid from α-ketoisocaproic acid but only *S. carnosus*, *S. xylosus*, *S. saprophyticus* and especially *C. piscicola* produced 3-methyl butanal. For example, the association of *L. sake* and *S. carnosus* commonly used as starter culture could participate to aromatic compounds production. *C. piscicola* 545 has never been used as starter culture but it would be interesting to test it. Nevertheless, further works on regulation of synthesis and activities of enzymes (aminotransferase, decarboxylase and dehydrogenase) will be necessary to establish the catabolic pathways of leucine, in order to control the production of aromatic compounds by starter cultures in fermented meat products.

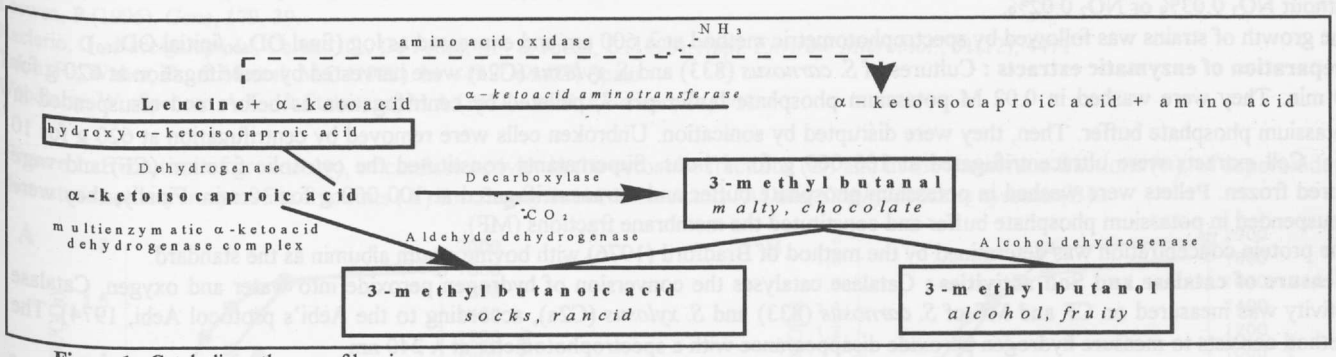

Figure 1 : Catabolic pathways of leucine

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