Screening of microbial strains producing amines and isolated from meat products.

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BACKGROUND

Biogenic amines like tryptamine, phenylethylamine (PEA), histamine, tyramine, putrescine, cadaverine have been found in fermented meat products (Vandekerckhove, 1977). Such amines may involved food poisoning, especially in conjonction with potentiating factor such as amine oxidase, inhibiting drugs, alcohols, other food amines and gastrointestinal disease (Askar and Treptow, 1986).

The synthesis of biogenic amines are usually generated by amino-acid decarboxylase from micro-organisms present in fermented meat products. The decarboxylase positive strains may constitute part of the natural population of the food or may be introduced as starter culture in sausages (Ten Brink et *al.*, 1990). Actually, high amount of tyramine (1000ppm) in sausage was associated to *Lactobacillus casei* subsp. *tolerance* (Maijala and Eerola, 1993). Moreover, Eitenmiller et *al.* (1978) attributed tyramine content in sausage to *Enterococcus faecalis*. But, amine production by species such as *Lactobacillus sake*, *Lactobacillus curvatus*, *Staphylococcus xylosus* or *Staphylococcus carnosus* and other species of the natural flora of sausages has never been tested in liquid media.

OBJECTIVES :

The objective of the present work was to investigate if strains isolated from meat products (natural microflora, starter culture) produced amines in liquid media. In a second part, the impact of the pH and the conditions of stirring on the amine production has been studied.

MATERIAL AND METHODS

Strains and media

Microbial strains studied were : ❖ **12** bacterial strains used as starter culture in sausages : *L. sake* (4), *L. curvatus* (2), *Lactobacillus plantarum* (1), *Pediococcus acidilactici* (1), *S. carnosus* (1), *S. xylosus* (2) *Micrococcus varians* (1);

❖ **10** contaminant strains found in meat products : *Carnobacterium divergens* (3), *Carnobacterium piscicola* (2), *Staphylococcus warneri* (1), *Staphylococcus saprophyticus* (4)

❖ **54** strains isolated from natural flora of artisanal and industrial french sausages on the following media : lactic acid bacteria (16) on MRS (Difco), *Micrococcaceae* (26) on chapman agar (Difco), *Pseudomonas* (0) on CFC (Oxoid), Yeast and Mould (2) on PDA, (Difco) and *Enterococcus* (10) on KF agar (Merck).

After overnight incubation, the cultures were centrifuged 15 min. at 5000 g; the cells were washed with saline solution (NaCl : 8.5‰) and resuspended at a density of 10^7 - 10^8 cells per ml of saline solution. An aliquot of the suspension (0.2 ml) was inoculated into 2 ml of Maijala's medium (1993) supplemented with 7 precursor amino acids (0.4%) : lysine, phenylalanine, ornithine, arginine, tryptophane, tyrosine, histidine. The pH of the medium was adjusted at 5.4 and 7, and incubated in static and shaking (150 RPM) conditions. After 5 days incubation at 30°C, the medium was centrifuged, its pH measured and the supernatant was kept at -20°C until biogenic amine analysis.

HPLC method.

Medium supernatants were dansylated and analysed on a C18 ODS2 spherisorb column as described by Eerola et *al.* (1993). The gradient run was carried out with eluent A : ammonium acetate (0.1M) and eluent B : acetonitrile. Amines were eluted by increasing linearly the concentration of eluent B from 57% to 90% in 12.35 min. at 40°C and with a flow rate of 1ml/min. The total run time with equilibration was 16 min. Dansylated amines were detected by UV absorption at 254 nm. Standard of amines were chromatographied in the same conditions. Peak areas were quantified in reference with calibration curves of amines by using an informatic integration.

RESULTS AND DISCUSSION.

Among the lactic acid bacteria tested, none of the strains of *L. sake*, mainly used as starter culture in sausages, produced amines (Table 1). On the other hand, the species *L. plantarum* and *L. curvatus* produced simultaneously phenylethylamine and high amount of tyramine (Table 1). For *L. curvatus*, these findings are consistent with those of Straub et *al.* (1994) who isolated from sausages, a strain of *L. curvatus* producing tyramine but at less amount (200µg/ml). Furthermore, the production of amines by our strains of *L. curvatus* was higher at pH = 5.4 than pH = 7 (Figure 1). The increase of amine production in acid conditions has been widely noticed. For example, Maijala (1994) correlated tyramine production of a strain of *lactobacillus* with pH decrease in the medium. Considering the strains of the genus *Carnobacterium*, tyramine was the main amine produced by *C. divergens* (1711µg/ml) and *C. piscicola* (759µg/ml). These results were confirmed by Masson et *al.* (1996) who found more than 2000µg/ml of tyramine produced by all the strains of *C. divergens* (19), *C. piscicola* (14) and *C. gallinarum* (1) tested. However, higher tyramine production is obtained at pH = 5.4 than pH = 7 (Figure 1). But the pH had more marked effect on the tyramine production of *C. divergens* than *C. piscicola*.

Among the *Micrococcaceae*, only one strain of *Staphylococcus carnosus* 833 produced phenylethylamine (500µg/ml) and in a less extent tryptamine (57µg/ml), after 12 days incubatin time (Figure 2). For both amines, the production was better in shaking than static conditions and highly enhanced at pH = 7 (Figure 2). The effect of shaking conditions could be correlated to a better growth of

S. carnosus in aerobic conditions. Around 400µg/ml of putrescine was released by one strain of *Micrococcus varians* (Table 1) also used as starter culture in sausage. Furthermore, putrescine production was noticed for 3 strains of *Staphylococcus saprophyticus* (Table 1).

The natural microflora isolated from sausages (yeast, *Micrococcaceae*, lactic acid bacteria) mainly produced tyramine and phenylethylamine (Table 1). Actually, 2 strains of yeast isolated from PDA produced 3267µg/ml of tyramine and 849µg/ml of phenylethylamine. 5 strains of *Micrococcaceae* produced around 2432µg/ml of tyramine. Furthermore 3 of these strains released simulteanously more than 100µg/ml of phenylethylamine. Thus it appeared a correlation between the production of tyramine and phenylethylamine. Actually, the strains producing tyramine released also phenylethylamine. The same profile was also obtained for lactic acid bacteria and *Enterococcus* (Table 1).

CONCLUSION.

From the results of the study it can be concluded that *L. curvatus* and *Carnobacterium* could produced amines in dry sausages, owing that pH = 5.4 in sausages favoured their production. On the other hand the combination of starter culture like *L. sake* and *S. carnosus* or *xylosus* could be realised without any risks. Beside these strains which could be used as starter culture, the natural flora isolated from french sausages could also participate to amine production. Only few strains (4 *Enterococcus*) produced putrescine (77µg/ml) which was neverthless detected in high amount in sausages. The origin of such amount of putrescine was not elucidated. On the opposite, phenylethylamine and tyramine were mainly produced by yeasts, *Enterococcus*, *Micrococcaceae* and lactic acid bacteria.

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	Strains tested	Tryptamine		PEA		Putrescine		Cadaverine		Histamine		Tyramine		Spermidine		Spermine	
Starter culture		+[a]	Mean[b]	+[a]	Mean[b]	+[a]	Mean[b]	+[a]	Mean[b]	+[a]	Mean[b]	+[a]	Mean[b]	+[a]	Mean[b]	+[a]	Mean[b]
L. sake	4	0		0		0		0		0		0		0		0	
L. curvatus	2	1	357	1	58	1	384	0		0		2	1152	0		0	
L.plantarum	1	0		1	592	0		0		0		1	829	0		0	
P. acidilactici	1	0		0		0		0		0		0		0		0	
S. carnosus	1	0		0		0		0		0		0		0		0	
S. xylosus	2	0		0		0		0		0		0		0		0	
M. varians	1	0		0		1	408	0		0		0		0		0	
Contaminant strains																	
C. divergens	3	0		2	220	0		0		0		3	1711	0		0	
C. piscicola	2	0		1	85	0		0		0		2	759	0		0	
S. warneri	1	0		0		0		0		0		0		0		0	
S. saprophyticus	4	0		0		3	254	0		0		0		0		0	
Natural flora																	
Enterococcus	10	1	73	4	259	4	79	0		0		3	3512	1	921	0	
Lactic acid bacteria	16	1	60	6	303	0		0		0		6	3986	1	905	0	
Micrococcaceae	26	0		4	299	0		0		0		5	2432	0		0	
Yeast	2	0		2	849	0		0		0		2	3267	0		0	

Table 1 : Biogenic amines produced after 48h incubation, at 30°C, in static conditions at pH = 5.4.

+[a] : positive strains producing more than 50µg/ml of amine; Mean : mean amount of amine (µg/ml) produced by the positive strains.

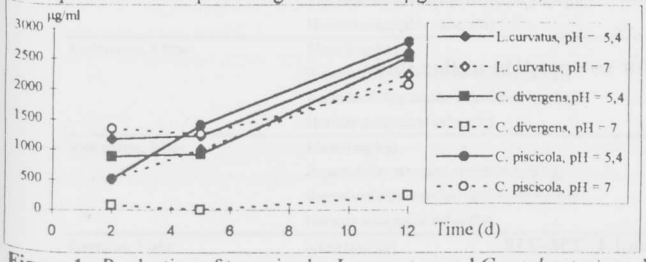

Figure 1 : Production of tyramine by *L. curvatus* and *Carnobacterium*.

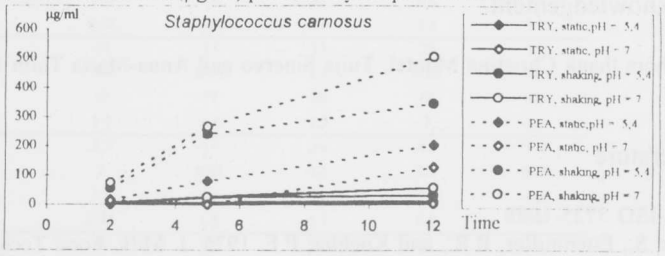

Figure 2 : Production of Tryptamine (TRY) and Phenylethelamine (PEA).