PEPTIDASIC ACTIVITIES OF STARTER CULTURES

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SUMMARY

The proteolytic and peptidasic activities of the species of *Lactobacillus*, *Pediococcus*, *Staphylococcus* used as starter cultures were determined in resting cells and in supernatant cultures.
None species tested hydrolysed hemoglobin, casein, bovine albumine. Only *Lactobacillus plantarum* showed an activity on azocasein.
Each species was characterized by arylamidase pattern. *Pediococcus pentosaceus, L. plantarum* and *Lactobacillus sake* were active on a large number of substrates, whereas *Carnobacterium piscicola* and *Staphylococcus carnosus, Staphylococcus xylosus, Staphylococcus saprophyticus* only hydrolysed a few.
In particular *L. sake* showed a high activity with hydrophobic amino acid (leucine, alanine or phenylalanine) and diaaminomonocarboxylic (lysine or arginine). Some activities were released in the supernatant by lysis of bacteria.
The peptidase activities were more important in lactic acid bacteria than in *Staphylococcus* strains .

INDRODUCTION

Some amino acids contribute to flavour development of sausages, either directly or as precursors (MAILLET and HENRY, 1960 ; BERDAGUE *et al.* , 1992). During the ripening of sausages the levels of free amino acids increase and more specially those of leucine and alanine (STANCULESCU *et al.*, 1971 ; SAJBER *et al.*, 1971 ; DIERICK *et al.*, 1974). This increase result from microbial and endogenous peptidases activities.
The role of bacterial proteases and peptidases is not demonstrated. A few reports pointed out that the proteolytic activities of *Micrococcus* species (SAJBER *et al.*, 1971) and lactic acid bacteria (REUTER and LANGNER, 1968 ; LAME, 1976 ;G ARRIGA *et al.*, 1986) could lead to an increase of amino acids in sausages. DEMASI *et al.* (1990) reported that sausages fermented with *P. pentosaceus* had a higher content in amino acids than non fermented sausages.
To attempt to determine at what extend some bacterial species (lactic acid bacteria and *Staphylococcus*), commonly used as starter cultures in sausage could affect the amino acids production, their proteolytic activities were examined.

MATERIAL and METHODS

Culture of cells

The species studied were : *Lactobacillus sake* (L110, Texell), *Lactobacillus curvatus* (678, INRA); *Lactobacillus plantarum* (719, INRA), *Carnobacterium piscicola* (525, INRA), *Pediococcus pentosaceus* (717,INRA), *Staphylococcus carnosus* (Texell), *Staphylococcus warneri* (863, INRA), *Staphylococcus xylosus* (Texell); *Staphylococcus saprophyticus* (M2, INRA).
These bacteria were grown at 30°C on the following medium : Meat extract (10g/l) ; yeast extract (2g/l) ; NaCl (5g/l) ; glucose (5g/l) ; pH 7. At the begining of the stationary phase (after 14 to 18 h of cultures) the cultures were centrifugated at 8000g. The activities were measured in the supernatant and in the corresponding resting cells prepared by suspending the pellet in 0,01 M Tris buffer pH 7.

Aminopeptidase activities.

They were measured by assaying the quantity of β naphtylamine produced from the substrats amino-acids - naphtylamide derivatives by the fluorometric method described by WAGNER *et al.* (1979). The reaction mixture contained : 0.3 ml d'amino acyl naphtylamide (Aa Na) prepared 10^{-2} M in ethanol, 2.4 ml of Tris 0,1M buffer , 0.3 ml of cell suspension (density 3-4 MacFarland standard) or 0.3 ml of supernatant.

Proteolytic activities

The hydrolysis of azocasein, azoalbumin were noted according to the method of SARATH *et al.* (1989).

RESULTS

The species tested, except *L. plantarum*, did not hydrolyse azocasein or azoalbumin .

As shown in figure 1 the lactic acid bacteria and *Staphylococcus* significantly differed in their activities against amino acid naphtylamide. The aminopeptidase profiles were highly characteristic of each genus.
The *Lactobacillus* and *Pediococcus* group showed high exopeptidases activities by being active against at least 8 out of the 20 substrats tested. The highest aminopeptidase activities were found for *L. plantarum* followed by *P. pentosaceus* and *L. sake* . Some activities (Leu. Na, Pheala. Na.) were measured in the supernatant culture. These extracellular activities were correlated with the lysis of bacteria as it was shown for *L. sake* by finding β galactosidase activity. There are in fact typically intracellular. It is of interest that leucine, alanine derivatives were hydrolysed by these species because this amino acid increase during the ripening even if leucine could be metabolised in methyl butanal (BERDAGUE *et al.*, 1992).
In contrast all the *Staphylococcus* and *Carnobacterium* species had appreciably a small number of arylamidase activities compared to lactic acid bacteria. *Staphylococcus* species only weakly hydrolyse proline, tryptophane β naphylamide. No activity was excreted in the supernatant.
From the present work it could be concluded that these starter cultures play no role in the hydrolysis of proteins . Nevertheless the intracellular activities of lactic acid bacteria could contribute to the increase of amino acids. So further study will be focusing on the peptidases of *Lactobacillus*.

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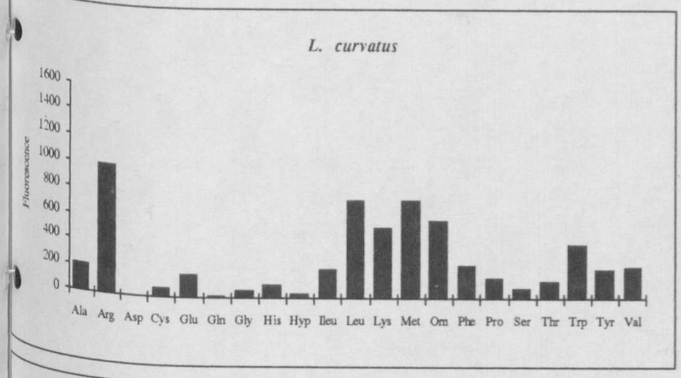
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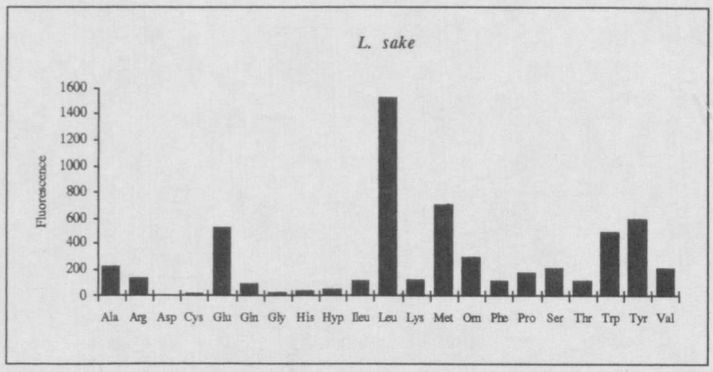
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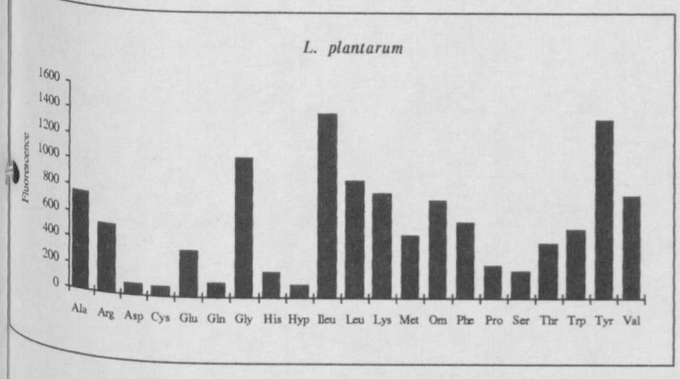
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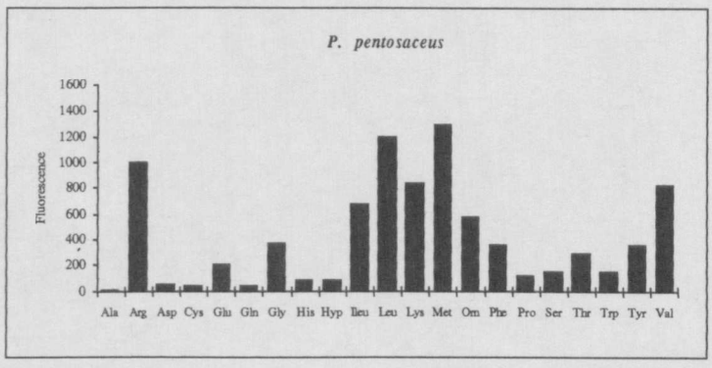
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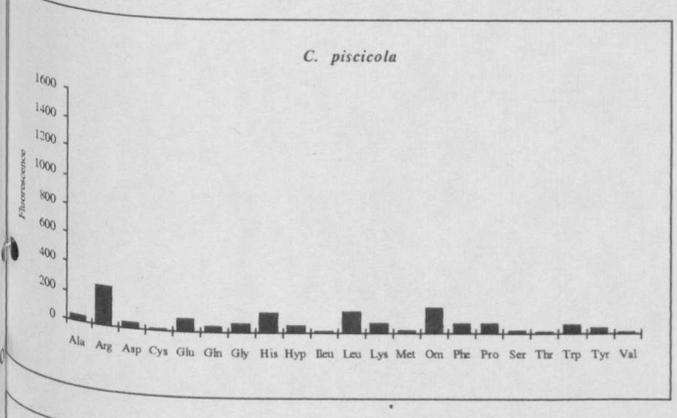
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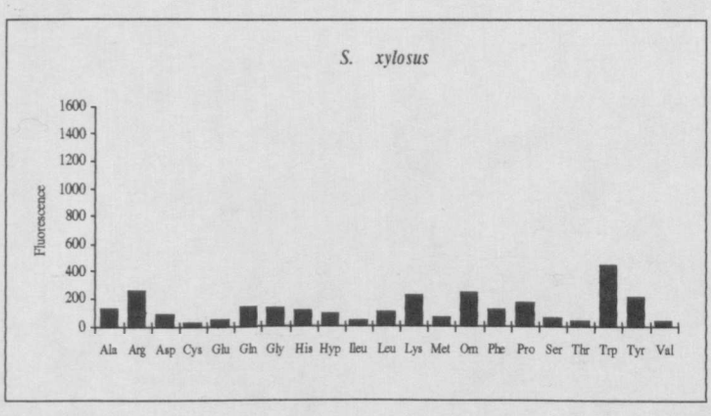
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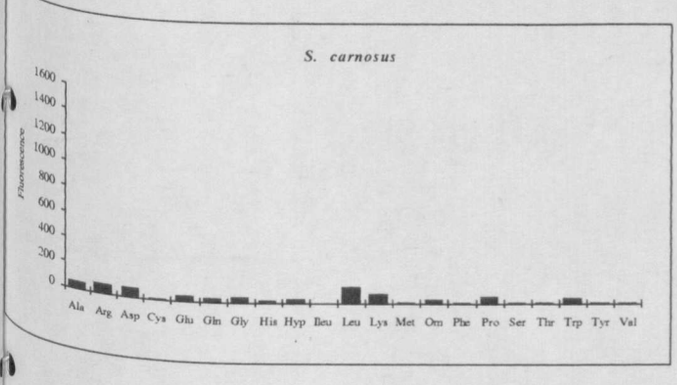
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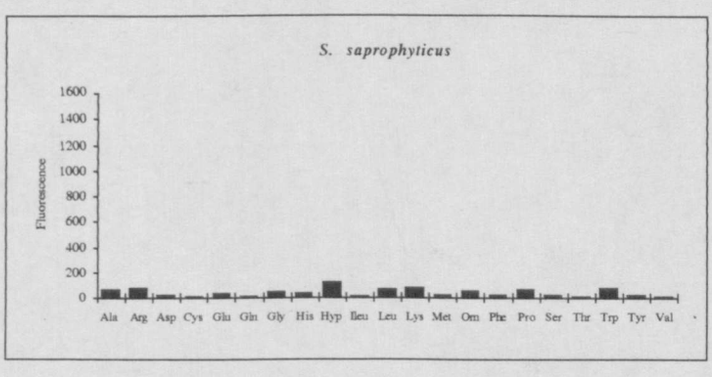
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