A59

EXOPEPTIDASE ACTIVITY IN MICROORGANISMS ISOLATED FROM DRY-CURED SAUSAGES

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BACKGROUND

Dry-cured sausages experience a sensible proteolysis resulting in the accumulation of certain noticeable amounts of free amino acids especially during the drying period. The generation of these compounds is attributed to exopeptidase activity from both endogenous and bacterial origin (Verplaetse et al., 1989). According to Demeyer (1992), exopeptidases liberating amino acids are more important in the later drying period. Lactic acid bacteria and micrococci are known to have exopeptidase activity in other fermented foods (Bhowmik and Marth, 1989; Visser, 1993) and they have to be taken into account since they constitute the main components of the microflora of dry-cured sausages.

OBJECTIVE

To determine exopeptidase activity in *Micrococcaceae* and Lactic Acid Bacteria strains isolated at two stages (fermentation and end of curing) in the processing of dry-cured sausages.

METHODS

The microorganisms used for enzymatic assays were firstly assigned to their corresponding genus according to the identification schemes of previous numerical taxonomic studies (Baker, 1984; Hugas et al., 1993). *Micrococcaceae* and lactic acid bacteria were grown in Brain Heart Infusion (Difco) and MRS broth (Merck), respectively, at 30ºC for 24 h. Cells were harvested by centrifugation (6000 g, 15 min.), washed with 100mM phosphate buffer (pH=6,5) and resuspended in the same buffer. The washed whole cell suspensions were used as enzymatic extract.
Aminopeptidase activity was measured fluorimetrically against different aminoacyl-methylcoumarin substrates (L-Phenylalanine-AMC, L-leucine-AMC and L-Tyrosine-AMC) using a multiscan fluorimeter (Fluoroskan). The three substrates were previously choosen from a total of 13 using type strains provided by CECT (Spanish Colection of Type Cultures). The reaction mixture contained 50 µl of enzymatic extract and 25 µl of 100mM phosphate buffer (pH=6,5) with 0,1 mM of each substrate and was incubated at 37ºC for 1 h. The intensity of the activity was expresed as µmol of liberated AMC in 15 min.

RESULTS AND DISCUSSION

Micrococcaceae strains isolated during the fermentation stage (94% *Staphylococcus*) exhibited reduced exopeptidase activities (see table 1). In fact, 50% of the total number of strains did not show any activity. Hovewer, micrococcaceae isolated at the end of the curing process (95% *Staphylococcus*) showed a significative increase in all activities (see table 1). So, only 10 % of the strains did not hydrolyze any substrate. The activity of the rest of strains was 2-3 times that of the strains isolated in the previous stage. Leucyl-hydrolyzing activity was the most significative.
On the other hand, the exopeptidase activity detected in lactic acid bacteria (80 and 100% *Lactobacillus* at fermentation and curing stages, respectively) was higher than that show by the group of *Micrococcaceae*. The hydrolysis of Leu-AMC was very intense even in the fermentation stage (see table 2) for most of the strains although some of them also hydrolyzed significative amounts of Phe-AMC and, in a less scale, Tyr-AMC. These last substrates were more intensively hydrolyzed by the strains isolated at the end of the process (see table 2).

CONCLUSIONS

There is a substantial and significative increase of exopeptidase activity of the strains isolated along the process.
The highest specificity is against Leu-AMC.
Exopeptidase activities are especially high in lactic acid bacteria.

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Table 1.- Exopeptidase activity against aminoacyl-AMC derivatives of *Micrococcaceae* strains isolated at two curing stages.

FERMENTATION

Strains code[a]	Number of strains	Phe-AMC	Leu-AMC	Tyr-AMC
M1	1	nd	*	*
M2	2	*	*	*
M3	2	nd	nd	*
M4	4	*	**	*
M5	9	nd	nd	nd

CURING

M6	1	*	***	*
M7	1	*	***	**
M8	1	nd	*	*
M9	1	*	**	*
M10	1	***	*	*
M11	3	*	****	*
M12	3	*	*	*
M13	3	**	*	*
M14	3	**	***	**
M-15	2	nd	nd	nd

[a] Strains with similar activity profiles
* Intensity of activity (each * = 3.5 µmol/15 min)
nd non detected

Table 2.- Exopeptidase activity against aminoacyl-AMC derivatives of lactic acid bacteria isolated at two curing stages.

FERMENTATION					CURING				
Strains code[a]	Number of strains	Phe-AMC	Leu-AMC	Tyr-AMC	Strains code	Number of strains	Phe-AMC	Leu-AMC	Tyr-AMC
L1	1	****	******	**	L13	1	****	**********	***
L2	1	****	**********	**	L14	1	******	**********	****
L3	1	****	*	*	L15	1	******	**********	***
L4	1	****	***	**	L16	1	****	**********	**
L5	1	*	****	*	L17	1	**	*********	*
L6	1	*	*	*	L18	1	**	**********	**
L7	2	***	**********	*	L19	1	**	*********	*
L8	2	**	*	*	L20	2	***	**********	**
L9	2	***	**********	**	L21	2	******	*******	****
L10	2	**	**********	**	L22	2	***	**********	**
L11	3	*	**********	*	L23	3	****	**********	**
L12	3	**	**********	*	L24	4	*****	**********	***
					L25	9	****	**********	**

[a] Strains with similar activity profiles
* Intensity of activity (each * = 3.5 µmol/15 min)
nd non detected