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Study of *Penicillium* Strains Enzymatic Pattern Involved in the Elaboration of *Sumaia*

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Abstract: The main objective of this study was to value the enzymatic pattern and its relationship with the possible antimicrobial activity of some strains of the genus *Penicillium* used when elaborating *Sumaia* (typical sausage from Catalonia). From the results obtained, it may assess that these strains own a marked phosphatase, hydrolase and glucosidase activity directly related to their antimicrobial capacity. This fact allows to considerate that their addition in meat products elaboration process helps likewise a better control of undesirable microbiota.

Key words: *Sumaia*, sausage, *Penicillium* sp., enzymatic activity, antimicrobial activity

INTRODUCTION

During the elaboration of some meat products, it is common to use some mould strains over the product surface in order to help its maturity and conservation. In addition, these strains lend certain organoleptic characteristics to the final product. These products, due to their composition, are capable of being substratum for undesirable microorganisms development, that may spoil them, becoming unfit for human consumption. Along this study period, the possibility that the added microorganisms over the product surface may be able of controlling undesirable microbiota is analysed.

The selected product has been the *Sumaia,* a typical sausage from Catalonia, elaborated with pork meat, salt, sugar and pepper. Likewise, during the elaboration process, vitamin C is added as antioxidant, nitrified salt and all is stuffed into eatable natural gut.

These products are added with some mould strains of the genus *Penicillium* over their surface. These strains colonize the product surface and let them acquire their typical whity dusty texture.

The main objective of this study was to value the enzymatic pattern of the different recovered mould strains from these products and to assess their possible capacity of inhibiting certain spoiling and/or pathogenic bacteria growth. In this kind of product, no previous references about the enzymatic activity of these strains were found.

MATERIALS AND METHODS

First of all, three *sumaia* were studied and from their surface mould strains were recovered under sterile conditions. In the same way, three strains used in these products where recovered as well. These three strains were supplied by the company that produces the studied *Sumaia.*

All these cultures were incubated for a week at 28°C in Sabouraud broth with antibiotic. After this time, the grown mould colonies were microscopically identified and subsequently isolated in tubes containing Sabouraud Dextrose Agar for their conservation and further studies.

In like manner, with the aim of reaching the enzymatic pattern of the different mould strains recovered, a modification of API-ZYM® (Biomérieux)[1] commercial method was used. After resuspending the strains in Ringer ¼ solution, a gallery for each strain was inoculated and it was kept in incubation for 48 h at 28°C. After this period of time, colorimetric reactions were observed and their enzymatic pattern was established. The enzymatic activity studied concerned the following substrata: 2-naphtyl-phosphate, 2-naphtyl-butyrate, 2-naphtyl-caprylate, 2-naphtyl-myristate, L-leucyl-2-naphtylamide, L-valyl-2-naphtylamide, L-cystyl-2-naphtylamide, N-benzoyl-DL-arginine-2-naphtylamide, N-glutaryl-phenylananine-2-naphtylamide, 2-naphtyl-phosphate, naphtol-AS-BI-phosphate, 6-Br-2-naphtyl-αD-galactopyranoside, 2-naphtyl-βD-galactopyranoside, Naphtol-AS-BI- βD-glucuronide, 2-naphtyl-αD-glucopyranoside, 6-Br-2-naphtyl-βD-glucopyranoside, 1-naphtyl-N-acetyl-βD-glucosaminide, 6-Br-2-naphtyl-αD-mannopyranoside and 2-naphtyl-αL-fucopyranoside.

Finally, the possible inhibition capacity of the studied fungal strains was evaluated according to Campbell's method[2] against certain spoiling/pathogenic microorganisms, selecting with this aim Gram positive bacteria, Gram negative bacteria and a yeast.

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RESULTS AND DISCUSSION

All cultures developed in Sabouraud broth were identified as genus *Penicillium* strains, belonging to the asymmetric biverticillate[3,4]. These cultures were also isolated in Czapek Agar plates and tubes and Malt Extract Agar 2% and after a week at 28°C, both the supplied strains and the recovered strains from the products, showed a white obverse and orange reverse.

From the nineteen enzymes studied in the galleries, only those with colorimetric reaction observed for any of the strains are mentioned (Table 1). This reaction is semiquantitative and gives an approach to the hydrolyzed substratum nanomoles amount.

The finding of N-acetyl-β-glucosaminidase is directly related to the capacity of altering the bacterial wall.

Table 2 showed the results obtained when performing the Campbell inhibition test. *Penicillium* strain 2 was the more active from the all the strains assayed, due to the fact that inhibits growth of all the bacteria and yeast chosen for the test.

Table 1: Results from the API-ZYM® galleries

	Supplied strain 1	Supplied strain 2	Supplied strain 3	Strain isolated from *Sumaia*
Alkaline phosphatase	1	0	1	2
Esterase	1	0	2	2
Acid phosphatase	2	0	2	2
Naphtol-AS-BI-Phosphohydrolase	2	5	4	5
B-glucosidase	4	1	1	3
N-acetyl-β-glucosaminidase	3	1	2	2

The numbers indicate the hydrolyzed substratum nanomoles amount: 0 means negative reaction, 1 means 5 nanomoles, 2 means 10 nanomoles, 3 means 20 nanomoles, 4 means 30 nanomoles, and 5 means 40 or more nanomoles

Table 2: Results of inhibition test according to Campbell's method

	Supplied strain 1	Supplied strain 2	Supplied strain 3	Strain isolated from *Sumaia*
Bacillus subtilis	+	+	+	+
Proteus mirabilis	-	+	+	+
Salmonella typhimurium	+	+	+	+
Staphylococcus aureus	+	+	-	+
Escherichia coli	+	+	-	-
Pseudomonas aeruginosa	-	+	-	+
Klebsiella pneumoniae	+	+	+	+
Saccharomyces cerevisiae	+	+	-	+

- : Means that no inhibition is observed and +: Means present inhibition

CONCLUSIONS

With the results obtained, we may assess that the studied strains belong to the genus *Penicillium* and that the isolated strain in all cases coincide with the supplied ones due to their microscopic and macroscopic characteristics.

The enzymatic patterns obtained from all the analysed cultures are highly similar, showing a marked phosphatase, hydrolase and glucosidase activity.

With regard to the inhibition produced by the fungal strains against the different microorganisms tested, a similar activity was obtained against Gram positive, Gram negative and yeasts. This fact, can be correlated with the glucosidase activity able to hydrolyze NAM-NAG bonds that belong to the peptidoglycan molecules, component of some microorganisms cell wall[5,6].

Therefore, as a conclusion we may assess that the possible inhibitory capacity of these fungal cultures added to meat products in order to inhibit spoiling and/or pathogenic microorganisms growth was verified. This antimicrobial duty can then be added to the traditional duty lent to the fungal specie added when *sumaia* is elaborated: to get certain organoleptic characteristics and improving its conservation.

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