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A Study on Metabolic and Antimicrobial Activities of *Pediococcus pentosaceus* Isolated from Fermented Sausage

[1]Özlem Turgay Erdoğrul, [2]Ömer Çetin and [2]Özer Ergün
[1]Kahramanmaraş Sütçü Imam University, Agricultural Faculty,
Department of Food Sciences, Kahramanmaraş, Turkey
[2]Istanbul University, Veterinary Faculty,
Department of Food Hygiene and Technology, Avcılar- Istanbul, Turkey

Abstract: A total of 34 *Pediococci* have been isolated from 7 different sausage samples produced by various retailers in Kahramanmaraş, a city in the south-east of Turkey, and the results obtained after identification showed that 4 (11.7 %) isolates were *Pediococcus pentosaceus*. The amounts of lactic acid and H_2O_2 produced by the strains were in the ranges of 0.11-0.32 % and 0,41-0,81 µg/ml. The results also made it clear that the strains produced various amounts of acids and H_2O_2 and had inhibitory effects against *Staphylococcus aureus* ATCC 25923.

Key words: Metabolic and antimicrobial activity, *Pediococcus pentosaceus*, fermented sausage

Introduction

In Turkey, fermented sausage has a considerable share of 42% in all fermented meat production (Anonymous, 1991). In developed countries, fermented meat products are produced in integrated plants and starter cultures are used in the process. With the introduction of these cultures, meat production has not only been standardized but also been improved in quality. That explains the reason why starter cultures have widely been used in meat production during past 25 years. Especially in the last decade of this 25-year period these cultures have almost become an indispensable part of fermented meat production (Erdoorul, 1998). The starter cultures used in meat production are namely; *Lactobacillus plantarum, L. sake, L. curvatus, Pediococcus acidilactici* and appropriate combinations of *P. pentosaceus* (Askar *et al.*, 1999; Gokalp *et al.*, 1994). Apart from lactic cultures, *Micrococcus* or *Staphylococcus* types of bacteria are also used (Askar *et al.*, 1999; Smith and Polumbo, 1983).

The antagonistic effects of lactic acid bacteria are attributed to some of their biochemical features. Most of these bacteria can turn carbohydrates into organic acids as lactic acid or acetic acid. The majority of food-borne contaminants, either pathogenic or nonpathogenic, are sensitive to these acids and accordingly to low pH values (Lewus *et al.*, 1991; Mortvedt *et al.*, 1991). H_2O_2, which is produced by lactic acid bacteria in microaerophilic conditions, may have inhibitory effects on a number of microorganisms (Juven *et al.*, 1991; Mortvedt *et al.*, 1991). It has been reported in the literature that diacetyl (Harlender and Spelhaug, 1989; Lewus *et al.*, 1991) and CO_2 may play a part in the acquisition of antagonistic effects by lactic acid bacteria. Bacteriocin or bacteriocin-like compounds produced by some lactic acid bacteria have recently been another point of interest with regard to the antagonistic activity mechanisms of such bacteria (Harlender and Spelhaug, 1989; Juven *et al.*, 1991; Lücke, 1986). It has so far been shown in various studies that some *Pediococcus* bacteria produce a kind of bacteriocin named as pediocin. This bacteriocin is basically a protein, and it inhibits the reproduction of food-borne pathogenic and sporing bacteria and of bacteria that are close in structure to its own species (Berry *et al.*, 1990; Bhunia *et al.*, 1988; Motlagh *et al.*, 1991).

The isolation of *P. pentosaceus* strains from various brands of sausage on sale in the Turkish markets and detection of some metabolic products formed by these strains and observation of the antimicrobial activities of such products is what this study aims to deal with.

Materials and Methods

Materials: Seven samples of different brands of fermented sausages manufactured in Kahramanmaraş, a city in the south-east of Turkey, have been used in this study is performed Sütçü Imam University, Agricultural Faculty, Department of Food Science, Kahramanmaraş, Turkey.

In order to isolate *Pediococcus* sp. bacteria, 10 g of each sample has thoroughly been mixed with 90 ml of sterile physiologic water/serum physiologic. The dilution of each sample has been planted in Elliker Agar (Difco) culture media by evenly spreading it over the whole surface of the plates and these plates have been incubated for a period of 72 hours at 37°C. The white, transparent or grey colonies which reproduced in the media were then transferred into Elliker Broth medium and thereby activated. Afterwards, activated cultures were microscopically studied, and bacteria observed to be in pairs and tetrads were replanted in 10% litmus milk and kept in 1.5 ml Eppendrof tubes at −20°C (Askar *et al.*, 1999; Toksoy and Beyatlı, 1999).

Identification of Bacteria: In separating *Pediococcus* bacteria from other groups, the following inspections and tests were carried out on isolates:

* Their tetrad shapes, immobility and capability of sporing,
* Gram and catalytic reactions,
* White-transparent-grey colours and structures of the colonies in the solid culture media,
* Homofermentative qualities of the isolates and their ability to form ammonia from arginine.

However, in distinguishing *Pediococcus pentosaceus* strains from other *Pediococci*, the physiological and biochemical qualities of *P. pentosaceus* were utilized. General identification methods were used in all the identification tests and evaluations was carried out on the bacteria that are dealt in this study (Barrow and Feltham, 1993).

Determination of the acid production of strains: The amount of the acids produced by the strains has been determined in a medium containing 10% skimmed milk in which 2% of active culture has been inoculated and the preparation was incubated at 37°C for a period of 24 hours. At the end of this period, acid production rates of strains were determined by titrating the cultures with 0.1N NaOH.

Determination of the hydrogen peroxide (H_2O_2) production of strains: Two percent of each active culture has been inoculated in Elliker Broth culture medium and was incubated at 37°C for a period of 24 hours. The amount of hydrogen peroxide (H_2O_2) in the cultures was then determined by a spectrophotometer and calculated as µg/ml in 400 nm (Walter and Herman, 1949).

Determination of the inhibitory effects of strains on the test bacteria: The strains used in the study were *Bacillus brevis* FMC 3, *Bacillus megaterium* DSM 32, *Bacillus subtilis* IMG 22, *Micrococcus luteus* LA 2971, *Mycobacterium smegmatus* RUT, *Staphylococcus aureus* ATCC 25923, *Yersinia enterocolitica*, O:3 P 41797 and *Pediococcus pentosaceus.* The general inhibitory effects of these strains on the indicator bacteria were determined by means of the agar diffusion method (Klaenhammer, 1988). Two percent of active *Pediococcus* strains was inoculated in a medium of 10 ml skimmed milk (10%) and incubated at 37°C for a period of 24 hours. Following incubation, the medium was centrifuged at 5,000 rpm for a period of 15 minutes and the supernatant obtained in this way was filtered through a 0.45 μm membrane filter.

The test bacteria were activated in Nutrient Broth and *P. pentosaceus* strains in Elliker Broth. In order to test the inhibitory effects of *Pediococcus* strains, 100 μl of this culture was poured into holes opened in the agar medium containing the indicator bacteria, and the preparation was incubated at 37°C for a period of 24 hours (Reinhamier *et al.,* 1990).

Determination of the effects of bacteriocin and bacteriocin-like substances: The production of bacteriocin by the isolated *Pediococcus* strains has been noticed to occur in an anaerobic medium. *Pediococcus pentosaceus* culture of 2% was inoculated in an Elliker Broth medium containing 0.02% sugar, and the preparation was incubated at 37°C for a period of 24 hours. Following incubation, the pH of the culture was adjusted to a degree of 6.5. The culture was then centrifuged at 16,000 rpm for 15 minutes and was washed in 5nM sodium phosphate of 20% at a pH value of six. In next step, it was processed in 0.1M sterile NaCl at pH 2, and was mixed at 4°C for 2 hours. This was followed by the centrifugation of the culture at 29,000 rpm for 20 minutes. The cells were finally processed in 5mM sodium phosphate at pH 5. The inhibitory effect of the supernatant obtained on the test bacteria was determined by means of the general inhibition method as explained earlier (Reinhamier *et al.*, 1990).

Results

A total of 34 species of *Pediococcus* have been isolated in this study. Four (11.7%) of 34 species were identified by biochemical analysis to be *Pediococcus pentosaceus,* the strains of which were observed to produce acid, hydrogen peroxide and hydrogen sulphur. The amount of acid produced by these strains was found to be between 0.11 and 0.32, and that of hydrogen peroxide between 0.41 and 0.81 (μg/ml). The findings are shown in Table 1.

Table 1: The amount of lactic acid and H_2O_2 produced by *Pediococcus pentosaceus* strains.

Number of strains	% Acidity	H_2O_2 (µg/ml)
Pediococcus pentosaceus 1	0.11	0.53
Pediococcus pentosaceus 2	0.14	0.41
Pediococcus pentosaceus 3	0.32	0.81
Pediococcus pentosaceus 4	0.29	0.80

Table 2: The inhibitory effect of *Pediococcus pentosaceus* strains (diameter/mm)

	Number of strain			
	1	2	3	4
B. brevis	-	-	-	-
B. megaterium	-	-	-	-
B. subtilis	-	-	-	-
Mic. luteus	-	-	-	-
Myco. smegmatus	-	-	-	-
S. aureu	-	-	10	-
Y. enterocolitica	-	-	-	-

The general inhibitory effect of *P. pentosaceus* strains on the test bacteria has only been tested on *Staphylococcus aureus* ATCC 25923 (1 cm), and the findings are given in Table 2.

Discussion

One of the most significant functions of starter cultures during the fermentation process is that they have an antimicrobic effect on some food-borne, pathogenic and contaminant bacteria that are found in the micro flora of meats. The antimicrobial activities of starter bacteria are caused by metabolites. The metabolites that are produced by starter cultures, and that have an antimicrobial effect are mainly lactic acid, H_2O_2, H_2S, bacteriocin and bacteriocin-like substances (Lewus *et al.*, 1991).

Hydrogen peroxide, which is produced by some starter cultures in fermented meats, is an undesirable by-product due to the colour, taste and smell it leaves on the meat. However, many researchers have so far shown that hydrogen peroxide has an antimicrobic effect on a number of pathogenic and nonpathogenic microorganisms (Fernandes *et al.*, 1987; Geisen *et al.*, 1992).

In this study, which has, in particular, attempted to find the effects of *P. pentosaceus* strains on sausage produced in Turkey, the H_2O_2 production of *P. pentosaceus* strains have been discovered (0.41-0.81 μg/ml), and bacteriocin and bacteriocin-like production of these strains have been examined. From the results obtained, one strain was found to be effective on *Staphylococcus aureus,* which was one of the test bacteria.

The inhibitory effects of the bacteriocins and bacteriocin-like substances in the *Pediococcus pentosaceus* strains were determined as follows: *Bacillus brevis* FMC 3, *Bacillus megaterium* DSM 32, *Bacillus subtilis* IMG 22, *Micrococcus luteus* LA 2971, *Mycobacterium smegmatus* RUT, *Staphylococcus aureus* ATCC 25923, *Yersinia enterocolitica*, O:3 P 41797.

It has been reported in the literature that in a study dealing with a similar subject to ours, only two of the 16 different *Pediococci* were found to be (Bac^+) and the rest (Bac^-). The argument put forward in that study was that it is possible to manufacture microbiologically the safer fermented meat products using lactic acid bacteria which produce bacteriocin (Klaenhammer, 1988).

Close analysis of the results attained in present research has shown that *P. pentosaceus* strains normally produce a moderate amount of lactic acid, and that their production of hydrogen peroxide is low. Furthermore, the research has also ascertained that the antagonistic effects of the pathogenic and nonpathogenic contaminants of these strains on the bacteria vary in degree.

The authors hope that their findings will be of some assistance to those who may wish to carry out further studies on *P. pentosaceus* strains, which are presently being tried out as a starter culture on fermented meat products.

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