

Novel Starter Cultures for Fermented Meat Products (Sausages)

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Abstract

The purpose of this study was to select the suitable bacterial strains for use as a novel starter culture for producing fermented meat products. *Micrococcus roseus* (MTCC-1532), *Lactobacillus plantarum* (MTCC-1407 and L-89) and *Pediococcus acidilactici* (NCIM-2292 and NCIM-2293) were examined for their ability to grow in the presence of sodium chloride, sodium nitrite, sodium nitrate and at different temperatures. Their ability to ferment different carbohydrate was also assessed. These hurdles were used alone and in combination. *M. roseus* (MTCC-1532), *L. plantarum* (MTCC-1407) and *P. acidilactici* (NCIM-2293) were able to grow well at 13°C and 16°C in the presence of 3% NaCl, 0.12% sodium nitrite and 0.08% sodium nitrate indicating their suitability as starter cultures for production of dry fermented sausages with the blend of Pork and Buffalo meat. Good quality dry sausages were prepared by ripening them at controlled temperature with selected pure bacterial cultures *L. Plantarum* (MTCC-1407) + *P. acidilactici* (NCIM-2293) + *M. roseus* (MTCC-1532) in equal proportion.

Keywords: Fermented Meat Products; Dry Sausages; Pork and Buffalo Meat; Novel Starter Culture; Nitrite; *Micrococcus roseus*; *Lactobacillus plantarum*; *Pediococcus acidilactici*

Introduction

Dry sausages are the fermented meat products which are made with the help of microbial starter cultures. Prior to commercial application of pure cultures and in a traditional way even today, the dry sausage production relies upon natural fermentation which is caused by “in-house microflora”. Natural fermentation leads to development of wild flavors. This necessitates the use of pure or selected starter cultures. The use of microbial culture shortens the fermentation schedule and helps in achieving unique product qualities, consistency and shelf life in these sausages. Primary genera of microorganisms, which are successfully utilized as meat starter cultures are *Lactobacillus* sp., *Micrococcus* sp., *Pediococcus* sp., yeasts and moulds, *Micrococci* are added for their nitrate reduction and catalase activity which help in the development of colour in the meat product. The Lactic Acid Bacteria (LAB) ferment the sugar to lactic acid primarily during the fermentation process, reducing the pH of the product and providing prolonged stability against the food spoilage microorganisms. Lactic acid bacteria are generally employed because they significantly contribute to the flavour, texture, nutritional value and microbial safety of fermented foods [1-6]. The present study was carried out to examine the suitability of *Micrococcus roseus*, *Lactobacillus plantarum* and *Pediococcus acidilactici* starter culture for production of dry fermented sausages.

Materials and Methods

Bacterial strains

Five bacterial strains were used. These included *Micrococcus roseus* (MTCC-1532) and *Lactobacillus plantarum* (MTCC-1407) obtained from the Institute of Microbial Technology, Chandigarh, *Lactobacillus plantarum* (L-89) obtained from the National Dairy Research Institute, Karnal and *Pediococcus acidilactici* (NCIM-2292 and NCIM 2293) obtained from the National Chemical Laboratory, Pune.

Nutrient media

Nutrient broth, MRS agar [7] and M-153 medium for *micrococci* [8] were used.

Growth conditions

The growth of bacterial cultures was examined at different temperatures (3, 13, 16, 25, 45 and 60°C) and different levels of sodium chloride (0, 2, 3 and 4%). Bacterial growth was also evaluated in the presence of sodium nitrite (0.012 and 0.12%) and sodium nitrate (0.08 and 0.8%). The ability of bacteria to ferment sucrose, glucose and lactose at 1% levels was also assessed.

Preparation of inoculums

The actively growing bacterial cultures were inoculated on MRS/M-153 medium in Roux bottles and incubated at 37°C. The bacterial growth was harvested with the help of sterile saline containing glass beads and transferred under aseptic conditions to a conical flask. The optical density of the microbial suspension was measured at 660 nm wavelength and adjusted corresponding to 10⁸ cells/g concentration.

Nitrate reduction

The ability of the bacterial cultures to reduce nitrate was examined by growing the cultures in nutrient broth with 0.08 and 0.8% sodium nitrate. After seven days of incubation, few drops of sulphanilic acid solution and equal amount of L-naphthylamine were added to about 1 ml broth culture. Red colour development indicated positive test.

Results and Discussion

Growth of bacterial cultures at different temperatures

One of the technological requirements for dry sausage production is the ability of the bacterial cultures to grow at between 10°C and 27°C, because both European and American style dry sausages are made at this temperature range. In the present investigations all the cultures under study grew best at 25°C (Table 1). They were also able to grow well at 16°C. *L. plantarum* (MTCC-1407) was unable to grow at 45°C. None of the cultures showed growth at 7°C and 50°C in 48 hours. *M. roseus* (MTCC-1532), *L. plantarum* (MTCC-1407) and *P. acidilactici* (NCIM-2292) showed growth at 13°C and thus evidenced potential to act in the process of ripening of the sausages which is done at around 13°C temperature. Wright and Axelsson [9], Berwal and Dinchev [10] has suggested that ripening temperature in the range of 11 to 15°C is good for production of dry fermented sausages and that lower temperature be preferred if a product of high quality and long shelf life desired.

Bacterial cultures	Temperatures °C					
	7	13	16	25	45	50
<i>M. roseus</i> (MTCC-1532)	-	+	++	+++	++	-
<i>L. plantarum</i> (MTCC-1407)	-	+	++	+++	-	-
<i>L. plantarum</i> (L-89)	-	-	+	+++	+	-
<i>P. acidilactici</i> (NCIM-2292)	-	+	++	+++	+	±
<i>P. acidilactici</i> (NCIM-2293)	-	±	++	+++	+	±

Table 1: Growth of different bacterial cultures at various temperatures.
+++; Very Good Growth; ++; Good Growth, +; Slight Growth, -; No Growth, ±; Growth Variable.

Wheather [11] described that *L. plantarum* grew at 15°C but not at 45°C or 48°C. Luke [12] suggested that fermentation temperatures should be maintained between 15-20°C.

Growth of bacterial cultures in the presence of salt

Sodium chloride is used as a curing ingredient. It provides the desired bind by extraction of myosin and helps in development of flavour and also in preservation. Sodium chloride in dry sausage formulation is used at 2.0 to 3.5% level, so the starter cultures should be able to grow at this concentration. *L. plantarum* (MTCC-1407 and L-89), *P. acidilactici* (NCIM-2292 and 2293) and *M. roseus* (MTCC-1532) were able to grow at 0, 2, 3 and 4% concentration of sodium chloride (Table 2). *P. acidilactici* showed excellent growth in the presence of 2% sodium chloride. *M. roseus* evidenced excellent growth at 4% sodium chloride concentration but there was also slight growth in the absence of sodium chloride. *L. plantarum* and *P. acidilactici* showed very less growth at 4% salt level. Garcia-Diez and Patarata [13], Acton [14] suggested the addition of 3% salt for production of dry sausages containing pork. Bozkurt and Erkmen [15], Palumbo, et al. [16] reported that 3% sodium chloride is needed for proper development of natural lactic microflora to ferment pepperoni and Lebanon bologna type sausages.

Bacterial Cultures	Sodium chloride level (%)				Sodium nitrite level (%)			Sodium nitrate level (%)		
	0	2	3	4	0	0.012	0.12	0	0.08	0.8
<i>M. roseus</i> (MTCC1532)	+	++	++	+++	++	++	+	++	+++	+
<i>L. plantarum</i> (MTCC1407)	+++	++	++	-	+	++	++	+	++	±
<i>L. plantarum</i> (L-89)	+++	++	++	-	++	++	+	++	+	+
<i>P. acidilactici</i> (NCIM2292)	++	+++	++	++	++	+++	++	++	++	+
<i>P. acidilactici</i> (NCIM2293)	+++	+++	++	+	+++	+++	++	++	+++	+

Table 2: Growth of bacterial cultures at various levels of salts.

+++ : Very good growth; ++ : Good growth; + : Slight growth; - : No growth; ± : Growth variable.

Growth of bacterial cultures in the presence of sodium nitrite and sodium nitrate:

Sodium nitrate and sodium nitrites are used in curing process of dry sausages to impart stability, flavour and colour to the product. All the cultures under study were able to grow very well at 0.012% sodium nitrite and 0.08% sodium nitrate (Table 2). One strain of *L. plantarum* (MTCC-1407) and both strains of *P. acidilactici* (NCIM-2292 and 2293) were also able to grow equally well at 0.12% level of sodium nitrite. None of the cultures was able to grow well in the presence of 0.8% sodium nitrate but generally showed slight growth after 48 hours of incubation at 37°C. *M. roseus* (MTCC-1532), *L. plantarum* (MTCC-1407) and *P. acidilactici* (NCIM-2292 and 2293) had the potential to participate in the curing process of sausages which requires 2.5-3% sodium chloride, 0.012% sodium nitrite and 0.08% sodium nitrate for the development of texture, colour and flavour.

Fermentation activity

The bacterial cultures used for making dry sausages should be able to ferment sugars in order to produce lactic acid but the process should be preferably homofermentative. Bacterial cultures were able to ferment sucrose, glucose and lactose at 1% levels in nutrient broth (Table 3). They fermented sucrose and glucose better in comparison to lactose. However, *P. acidilactici* (NCIM-2292 and 2293) were found to be good fermenters of lactose also. None of the strains formed gas during fermentation and were, therefore, treated to be homofermenters. Incze [17] stated that decrease in pH is due to the production of organic acid mainly lactic acid during carbohydrate fermentation.

Bacterial cultures	Sucrose (1%)	Glucose (1%)	Lactose (1%)
<i>M. roseus</i> (MTCC1532)	++	+++	±
<i>L. plantarum</i> (MTCC-1407)	+++	+++	+
<i>L. plantarum</i> (L-89)	+++	++	+
<i>P. acidilactici</i> (NCIM-2292)	+++	+++	++
<i>P. acidilactici</i> (NCIM-2293)	+++	+++	++

Table 3: Fermentation activity of different bacterial cultures. +++: Very good growth; ++: Good growth; +: Slight growth; -: No growth; ±: Growth variable.

This observation has also been supported by the findings of several workers [18,19]. Bassi, *et al.* [20], Wang, *et al.* [21], Nordal and Slinde [22] studied the sugar fermentation using Lactic Acid Bacteria and found that *L. plantarum* fermented sucrose and mainly produced lactic acid.

Nitrate reduction

Sodium nitrate is used in meat products as a source of sodium nitrite and it acts as a reserve for nitrite. The use of sodium nitrate is considered better in fermented sausages because the processing takes almost about a month. Sodium nitrate is reduced to nitrite by bacteria. In the present study none of the bacterial strains except *M. roseus* (MTCC-1532) was able to reduce nitrate to nitrite. *M. roseus* (MTCC-1532) was thus identified as reducing culture for making sausages. *Micrococci* are known to possess catalase activity and ability to reduce nitrate [22]. The catalase positive bacteria of micrococcaceae reduce nitrate to nitrite and further reduce nitrite which helps in elimination of surplus nitrite [12,23,24].

Selection of bacterial starter cultures

A good meat starter culture should have salt tolerance to an extent of 2.5 - 3.5% and should be able to grow in the presence of 0.012% sodium nitrite and 0.08% sodium nitrate, and at 13°C with a range of 10 - 16°C. It should be homofermentative, producing only lactic acid from dextrose and should be inactivated at 50°C [4,25]. *L. plantarum* (MTCC-1407) and *P. acidilactici* (NCIM-2292) fulfilled almost all requirements (Table 4) except for nitrate reduction test.

Starter cultures	NaCl	NaNO ₂	NaNO ₃	Temperature			Fermentation	Suitability
	3%	0.012%	0.08%	13°C	16°C	50°C	Sucrose	
<i>M. roseus</i> (MTCC1532)	+++	++	+++	+	++	-	++	Yes
<i>L. plantarum</i> (MTCC1407)	++	++	++	+	++	-	+++	Yes
<i>L. plantarum</i> (L-89)	++	++	+	-	+	-	+++	No
<i>P. acidilactici</i> (NCIM2292)	++	+++	++	+	++	±	+++	Yes
<i>P. acidilactici</i> (NCIM2293)	++	+++	+++	-	++	-	+++	No

Table 4: Suitability of starter cultures based on performance. +++: Very good growth; ++: Good growth; +: Slight growth; -: No growth; ±: Growth variable.

M. roseus (MTCC-1532) fulfilled all requirements except for pH. Whereas the dry sausages to be manufactured are low pH products in the range of 4.9 to 5.2 but the bacteria from micrococcaceae were not active in this range of pH. Keeping the finding in view, *M. roseus* (MTCC-1532) *L. plantarum* (MTCC-1407) and *P. acidilactici* (NCIM-2292) are most suited for use as starter culture.

Conclusion

It is concluded that *M. roseus* (MTCC-1532) *L. plantarum* (MTCC-1407) and *P. acidilactici* (NCIM-2292) can be used in combination as novel starter cultures for production of dry fermented sausages.

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