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Research Article

SELECTION OF STARTER CULTURES FOR PRODUCTION OF DRY FERMENTED MEAT PRODUCT (SAUSAGES)

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ABSTRACT
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 13ºC and 16ºC 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.

Keywords: *Fermented Sausage, Starter Culture, Nitrite, Micrococcus Roseus, Lactobacillus Plantarum and 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. Jeevaratnam *et al.,* (2005) reviewed and stated that Lactic acid bacteria and their anti microbial metabolites have potential as natural preservatives to control the growth spoilage and pathogenic bacteria in foods.

Lactic acid bacteria are generally employed because they significantly contribute to the flavour, texture, nutritional value and microbial safety of fermented foods (Caplice and Fitzgerald, 1999; Coretti, 1977a). 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 (De-Man.*et al.,* 1960) and M-153 medium for *micrococci* (MTCC, 1994) were used.

Growth Conditions: The growth of bacterial cultures was examined at different temperatures (3, 13, 16, 25, 45 and 60ºC) 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 37ºC. 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 wave length and adjusted corresponding to 10^8 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- nephthylamine were added to about 1ml 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 10ºC and 27ºC, 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 25ºC (Table 1). They were also able to grow well at 16ºC. *L. plantarum* (MTCC-1407) was unable to grow at 45ºC. None of the cultures showed growth at 7ºC and 50ºC in 48 hours. *M. roseus* (MTCC-1532), *L. plantarum* (MTCC-1407) and *P. acidilactici* (NCIM-2292) showed growth at 13ºC and thus, evidenced potential to actin the process of ripening of the sausages which is done at around 13ºC temperature. Berwal and Dinchev and Berwal (1993) has suggested that ripening temperature in the range of 11 to15ºC 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. Wheather (1995) described that *L. plantarum* grew at 15ºC but not at 45ºC or 48ºC. Luke (1988) suggested that fermentation temperatures should be maintained between 15-20ºC.

Table 1: Growth of Different Bacterial Cultures at Various Temperatures

	Temperatures ºC					
Bacterial Cultures	**7**	**13**	**16**	**25**	**45**	**50**
M .roseus (MTCC-1532)	-	+	++	+++	++	-
L. plantarum (MTCC-1407)	-	+	++	+++	-	-
L. plantarum (L-89)	-	-	+	+++	+	-
P. acidilactici (NCIM-2292)	-	+	++	+++	+	±
P. acidilactici (NCIM-2293)	-	±	++	+++	+	±

+++ Very good growth, ++ Good growth, + Slight growth, - No growth, ± Growth variable

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. Acton (1976) suggested the addition of 3% salt for production of dry sausages containing pork. Palumbo *et al.*, (1976) reported that 3% sodium chloride is needed for proper development of natural lactic microflora to ferment pepperoni and Lebanon bologna type sausages.

Table 2: Growth of Bacterial Cultures at Various Levels of Salts

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
M.roseus (MTCC1532)	+	++	++	+++	++	++	+	++	+++	+
L.plantarum (MTCC1407)	+++	++	++	-	+	++	++	+	++	±
L. plantarum (L-89)	+++	++	++	-	++	++	+	++	+	+
P.acidilactici (NCIM2292)	++	+++	++	++	++	+++	++	++	++	+
P.acidilactici (NCIM2293)	+++	+++	++	+	+++	+++	++	++	+++	+

+++ 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 grew 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 37ºC. *M. rosues* (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 (1992) stated that decrease in pH is due to the production of organic acid mainly lactic acid during carbohydrate fermentation. This observation has also been supported by the findings of several workers (Biswas *et al.*, 2006; Klettners and List, 1980). Nordal and Slinde (1980) 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 (Nordal and Slinde, 1980). The catalase positive bacteria of micrococcaceae reduce nitrate to nitrite and further reduce nitrite which helps in elimination of surplus nitrite (Luke, 1988; Berwal and Rajkumar, 1998).

Table 3: Fermentation Activity of Different Bacterial Cultures

Bacterial Cultures	Sucrose (1%)	Glucose (1%)	Lactose (1%)
M. roseus (MTCC1532)	++	+++	±
L . plantarum (MTCC-1407)	+++	+++	+
L.. plantarum (L-89)	+++	++	+
P. acidilacitci (NCIM-2292)	+++	+++	++
P. acidilacitci (NCIM-2293)	+++	+++	++

+++ Very good growth, ++ Good growth, + Slight growth, - No growth, ± Growth variable

Table 4: Suitability of Starter Cultures Based on Performance

Starter Cultures	NaCl	$NaNO_2$	$NaNO_3$	Temperature			Fermentation	Suitability
	3%	0.012%	0.08%	13ºC	16ºC	50ºC	Sucrose	
M. roseus (MTCC1532)	+++	++	+++	+	++	-	++	**Yes**
L. plantarum (MTCC1407)	++	++	++	+	++	-	+++	**Yes**
L. plantarum (L-89)	++	++	+	-	+	-	+++	No
P.acidilactici (NCIM2292)	++	+++	++	+	++	±	+++	**Yes**
P .acidilactici (NCIM2293)	++	+++	+++	-	++	-	+++	No

+++ Very good growth, ++ Good growth, + Slight growth, - No growth, ± Growth variable

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 13ºC with a range of 10-16ºC. It should be homofermentative, producing only lactic acid from dextrose and should be inactivated at 50ºC (Diebel, 1974).

L. plantarum (MTCC-1407) and *P. acidilactici* (NCIM-2292) fulfilled almost all requirements (Table 4) except for nitrate reduction test. *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 micrococaceae 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 starter cultures for production of dry fermented sausages.

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