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Prevalence of Staphylococcus and Micrococcus in Traditionally Prepared Meat Products

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Aerobic cocci from some of the ethnic meat products of the Eastern and the Western Himalayas were isolated and characterised. A total of 284 micrococcaceae were isolated from 68 samples of traditionally prepared meat products of different pockets of Sikkim and Uttarakhand in India. The occurrence of micrococcaceae was found at the level of 10^5-10^7 cfu/g. The total viable count in all the samples of meat products collected from different places of the Sikkim and Uttarakhand was ranging in between 10^5-10^9 cfu/g. Identification of the isolates revealed that about 91.0 % of the total isolates were identified and characterized as *Staphylococcus* spp. and remaining 9.0 % of the isolates were *Micrococcus* spp. in all the samples analysed.

Keywords: *Staphylococcus, Micrococcus*, FTO, Ethnic Meat Products

Introduction

The association of several species of *Kocuria, Micrococcus* and *Staphylococcus* has been reported from fermented meat and fish products[1,2]. The importance of the microorganisms belonging to the *Micrococcaceae* and *Staphylococcaceae* families in the biochemical processes that take place during the ripening of raw-cured meat products was to develop the colour and flavour of the products[3]. The main microbial groups involved in meat fermentation are LAB[4,5,6,7], and is followed by coagulase-negativestaphylo cocci, micro cocci and enterobacteriaceae[8].The aim of the present work is to determine the predominance of *Staphylococcus* over *Micrococcus* in varieties of meat products consumed in Eastern and Western Himalaya in India.

Materials and methods

Meat Samples

A field survey was conducted in different regions of Sikkim and Kumaun Himalayas. Based on personal observation and interviews with the producers, six types of ethnic meat products from the Sikkim and three major types of meat products from Uttarkhand were documented and collected. The ethnic meat products from Sikkim were *langkargyong, yak kargyong, faakkargyong, langsatchu, yak satchu* and *sukakomasu* and from Kumaun were the *Arjia, Chartayshya* and *Jamma.* Samples were collected aseptically in polythene bags as well as sterile bottles and were, sealed and labelled. Samples were stored at 4°C for microbial and biochemical analyses. Samples were taken out from freeze and analysed until temperature of samples retained to room temperature.

Microbial Analysis

Ten g of sample were homogenised with 90 ml of 0.85 % (w/v) sterile physiological saline in a stomacher lab-blender (400, Seward, UK) for 1 min. A serial dilution (10^{-1} to 10^{-8}) in the same diluent was made. Mannitol-salt Phenol-red Agar (Merck) media were used for the detection of micrococcaceae in the samples following the method of Papamanoli *et al*. (2002)[9]. Inoculated plates were incubated at 30°C for 48 h. Micrococcaceae strains were characterized and identified following the keys described by Sneath *et al*. (1986)[10].Identity of micrococci was further confirmed by growing in FTO (furazolidone) agar[11].Spread plates of Baird Parker agar base (M043, Hi Media) with appropriate additions of Egg yolk tellurite emulsion (FD046, Hi Media) was also used for selective enumeration of *Staphylococcus aureus*. After serial dilution plates were overlaid with the medium and incubated at 37°C for 48 h.

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Results and Discussion

A total of 203 strains of aerobic cocci were isolated from 52 samples of the Sikkim Himalayas (Table 1) and a total of 81 strains were isolated from 16 samples of the Kumaun Himalayas (Table 2).

All the samples were analysed for the microbiological population (Table 3 and 4). The occurrence of micrococcaceae was found at the level of 10^5-10^7 cfu/g. The total viable count in all the samples of meat products collected from different places of the Sikkim and the Kumaun Himalayas was ranging in between 10^5-10^9 cfu/g (Table 3and 4). In all the samples analyzed, the predominance of *Staphylococcus* spp. over *Micrococcus* spp. was recorded. A grouping of all micrococcaceae isolates was done on the basis of cell morphology, gas production from glucose, production of ammonia from arginine, formation of tetrads, growth at 10 % NaCl (Tables 1 and 2)[12].The representative strains were selected from each grouped strains and further grown in furazolidone (FTO) agar. The percentage of prevalence showed that about 91.0 % of the total isolates were identified and characterized as *Staphylococcus* spp. and remaining 9.0 % of the isolates were *Micrococcus* spp. in all the samples analysed. The strains were further confirmed as *Micrococcus* spp. showing growth in furazolidone (FTO) agar and *Staphylococcus* spp. did not grow in

Table 1—Differential characteristics of *Micrococcus* and *Staphylococcus* isolated from meat products of the Sikkim Himalayas

Product[a]	Cell size	Gram stain	Catalase	Arginie hydrolysis	Tetrads	Growth on NaCl 10%	Growth on FTO agar	Grouped strains	% of prevalance	Identity
Lang kargyong (45)	1.5 (0.5-2.4)	+	+	-	+	-	+	4	8.9	*Micrococcus*
	1.1 (0.5-1.6)	+	+	+	-	+	-	41	91.1	*Staphylococcus*
Yak kargyong (30)	1.5 (0.5-2.4)	+	+	-	+	-	+	3	10.0	*Micrococcus*
	1.6 (0.5-1.6)	+	+	+	-	+	-	27	90.0	*Staphylococcus*
Faak kargyong (35)	1.0 (0.5-2.4)	+	+	-	+	-	+	3	8.6	*Micrococcus*
	1.2 (0.5-1.6)	+	+	+	-	+	-	32	91.4	*Staphylococcus*
Lang satchu (28)	1.6 (0.5-2.4)	+	+	-	+	-	+	2	7.1	*Micrococcus*
	1.0 (0.5-1.6)	+	+	+	-	+	-	26	92.9	*Staphylococcus*
Yak satchu (40)	1.6 (0.5-2.4)	+	+	-	+	-	+	3	7.5	*Micrococcus*
	1.2 (0.5-1.6)	+	+	+	-	+	-	37	92.5	*Staphylococcus*
Suka ko masu (25)	1.6 (0.5-2.4)	+	+	-	+	-	+	2	8.0	*Micrococcus*
	1.2 (0.5-1.6)	+	+	+	-	+	-	23	92.0	*Staphylococcus*

[a]Total number of micrococcaceae from each products are given in parenthesis.
FTO, Furazolidone agar

Table 2—Differential characteristics of *Micrococcus* and *Staphylococcus* isolated from meat products of the Kumaun Himalayas

Product[a]	Cell size	Gram stain	Catalase	Arginie hydrolysis	Tetrads	Growth on NaCl 10%	Growth on FTO agar	Grouped strains	% of prevalance	Identity
Chartayshya (32)	1.6 (0.5-2.4)	+	+	-	+	-	+	2	6.3	*Micrococcus*
	1.1 (0.5-1.6)	+	+	+	-	+	-	30	93.7	*Staphylococcus*
Jamma (24)	1.5 (0.5-2.4)	+	+	-	+	-	+	2	8.3	
	1.0 (0.5-1.6)	+	+	+	-	+	-	22	91.7	*Staphylococcus*
Arjia (25)	1.0 (0.5-2.4)	+	+	-	+	-	+	2	8.0	*Micrococcus*
	1.1 (0.5-1.6)	+	+	+	-	+	-	23	92.0	*Staphylococcus*

[a]Total number of Micrococcaceae from each products are given in parenthesis.
Furazolidone (FTO) agar

Table 3—Microbiological populations of meat products collected from different places of the Sikkim Himalayas

Product	Region	Place of collection	Log cfu/g sample	
			Micrococcaceae	TVC
Lang kargyong	North Sikkim	Mangan (n = 3)	6.3 ± 0.9	8.1 ± 0.3
	North Sikkim	Pangthang (n = 3)	5.1 ± 0.4	8.4 ± 0.2
	North Sikkim	Sankalan (n = 2)	5.2 ± 0.2	8.2 ± 0.2
	North Sikkim	Pakshyak (n = 2)	5.1 ± 0.3	8.8± 0.4
Yak kargyong	East Sikkim	Lingtam (n = 2)	5.4 ± 0.4	8.2 ± 0.3
	East Sikkim	Rongli (n = 2)	5.0 ± 0.1	7.7 ± 0.1
	East Sikkim	Zuluk (n = 3)	6.8 ± 0.7	8.8± 0.4
	East Sikkim	Gnathang (n = 2)	5.6 ± 0.1	8.4 ± 0.5
	East Sikkim	Kupup (n = 2)	5.4 ± 0.1	8.3 ± 0.6
	North Sikkim	Lachen (n = 3)	5.9 ± 0.5	7.3 ± 0.6
Faak kargyong	East Sikkim	4th mile (n = 2)	6.4 ± 0.6	8.5 ± 0.5
	East Sikkim	Ranka (n = 2)	6.6 ± 0.4	8.8 ± 0.1
	West Sikkim	Tashiding (n = 2)	5.6 ± 0.1	7.8 ± 0.1
	North Sikkim	Mangan (n = 2)	5.5 ± 0.1	7.8 ± 0.1
Lang satchu	East Sikkim	Ranka (n = 2)	6.2 ± 0.2	7.8 ± 0.1
	East Sikkim	Tadong (n = 2)	7.0 ± 0.1	8.1 ± 0.4
	West Sikkim	Rinchen-pong (n = 2)	6.6 ± 0.1	8.6 ± 0.6
	West Sikkim	Kewzing (n = 2)	6.2 ± 0.7	7.8 ± 0.1
Yak satchu	East Sikkim	Gnathang (n = 2)	6.4 ± 0.6	8.3 ± 0.2
	East Sikkim	Kupup (n = 2)	5.6 ± 0.6	8.6 ± 0.7
	North Sikkim	Lachen (n = 2)	5.4 ± 0.5	7.8 ± 0.1
	South Sikkim	Namchi (n = 2)	5.1 ± 0.1	5.5 ± 0.1
Suka ko masu	East Sikkim	Rongli (n = 2)	4.4 ± 0.1	5.4 ± 0.1
	East Sikkim	Tadong (n = 2)	5.2 ± 0.1	5.4 ± 0.3

Data represents the means (± SD) of number of samples.
n = number of samples. LAB, lactic acid bacteria; TVC, total viable count; DL, Less than detection limit (10 cfu/g).
Micrococcaceae includes species of *Micrococcus* and *Staphylococcus.*

Table 4—Microbiological populations of meat products collected from different places of the Kumaun Himalayas

Product	Region	Place of collection	Log cfu/g sample	
			Micrococcaceae	TVC
Charta-yshya	Dharch-ula district	Pangu (n = 2)	6.9 ± 0.1	9.0 ± 0.1
	Dharch-ula district	Rumjum (n = 2)	5.9 ± 0.1	7.8 ± 0.1
	Dharch-ula district	Marchal (n = 2)	6.9 ± 0.1	7.1 ± 0.1
Jamma	Dharch-ula district	Dharchula (n = 2)	6.0 ± 0.1	9.2 ± 0.1
	Dharch-ula district	Sosa (n = 2)	5.3 ± 0.3	7.5 ± 0.3
	Dharch-ula district	Rumjum (n = 2)	5.3 ± 0.1	7.4 ± 0.1
Arjia	Dharch-ula district	Dharchula (n = 2)	6.5 ± 0.1	9.1 ± 0.1
	Dharch-ula district	Rumjum (n = 2)	6.3 ± 0.1	9.0 ± 0.1

Data represents the means (± SD) of number of samples.
n = number of samples. LAB, lactic acid bacteria; TVC, total viable count; DL, Less than detection limit (10 cfu/g).
Micrococcaceae includes species of *Micrococcus* and *Staphylococcus.*

FTO agar. Non-FTO strains were also identified for *Staphylococcus aureus* by using selective media Baird Parkar Agar (M043 Hi Media). *Staphylococcus aureus*is regularly found in meat and fermented sausages[13,14]. Only few of the samples showed positive to *Staphylococcus aureus* but they are poor competitor at low temperatures, anaerobic conditions and low pH[15].Among micrococcaceae, about 91 % of the isolates were identified as *Staphylococcus* sp. and only 9 % were *Micrococcus* sp. in the 68 samples

analysed, the findings proves that the dominance of *Staphylococcus* spp. over *Micrococcus* spp. *Staphylococcus aureus* was reported in several meat products[16]. There was the predominance of staphylococci over other micrococcaceae in almost all the data reported on the characterization of microbial flora of fermented meats[17]. The predominance of isolates of the *Staphylococcus* genus in comparison with those of *Micrococcus* genus appears as common phenomenon in the majority of studies on the characterization of the microbial flora in fermented sausage[9]. Micrococcaceae species are used to enrich fermentative microorganisms during ageing of the products in order to enhance the colour stability and contribute to flavour development of the cured meat and prevent rancidity[9,18]. Previously we reported the dominance of lactic acid bacteria in fermented meat products of the Western Himalayas[19], however, presence of *Staphylococcus* and *Micrococcus* have also novelty in fermentation of ethnic fermented foods of the Himalayas as in other fermented meat products of the world.

Conclusion

The main objectives of the work were to know the predominance of *Staphylococcus* spp. over the *Micrococcus* spp. among aerobic cocci in a variety of ethnic meat products consumed by the ethnic people of the Himalayas. It was observed that more than 90% of aerobic cocci were *Staphylococcus* spp. In future studies, the technological and safety properties of the aerobic cocci can be investigated in order to determine the role of these species in the manufacture of meat products, with the final purpose of using these strains as starter cultures.

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