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Antimycotic effect of natamycin on the yeasts and molds obtained from the fresh paneer samples

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Abstract
The paneer samples were analysed for the yeasts and molds counts. The yeasts and molds counts were ranged from 2.32 to 4.22 and Zero to 1.0 $\log_{10}$ cfu/g respectively. Further, all the yeasts isolates were identified to the Rhodotorula species based on the biochemical tests whereas, white, black, Green and Blue coloured molds were identified as Mucor, Rhizopus, Aspergillus and Penicillium based on the colour and structures. The identified yeasts and molds were further, subjected for the antimicrobial activity against the natamycin. The antimicrobial activity of yeasts were ranged from 452-668 sq.mm and Mucor, Rhizopus, Aspergillus and Penicillium produced inhibitory area of 452, 452,138 and 138 sq. mm at 50 ppm natamycin respectively.

Keywords: Natamycin, antimicrobial activity, paneer, yeasts and molds

Introduction
Paneer is an Indian cheese prepared from milk on heating and then acidification. Paneer is one of the popular heat and acid coagulated product, having rubbery consistency, acidic flavour, fat and protein rich milk product. At room temperature, paneer has a limited shelf life and at refrigeration temperature, it can last up to 30 days [Goyal and Goyal, 2016] [1]. The shelf life of paneer is limited due to the presence of spoilage causing microorganisms that enter the product through different means such as water, air, equipments, packaging materials, and handling. Paneer's microflora is dominated by bacteria, yeasts, and molds. Most of the spoilage is caused by yeasts and molds. Natamycin is an antibiotic derived from *Strepmyces natalensis*. Natamycin inhibits yeasts and molds very effectively [Lule *et.al.*, 2016] [3].

Material and methods
Natamycin: Natamycin (E235), liquid concentrate.
Natamycin was procured from Specialities, Savoury ingredients, P.O.box 1, 2600 MA Delft, Netherlands.

Malt extract agar: Prepared as for the standard procedure [Harrigan, 1998] [4]
Malt extract broth: Prepared as for the standard procedure [Harrigan, 1998] [4]
Yeast identification: Sugar fermentation broth, ethanol production and ascospore formation as per the yeast identification key [Harrigan, 1998] [4]

Paneer: Paneer samples were collected from the Experimental Dairy Plant, Dairy Science College, Hebbal, Bengaluru and stored at refrigeration temperature till analysed.

Enumeration of yeasts and molds from paneer sample: The yeasts and molds were enumerated as per the procedure given in the [Harrigan, 1998] [4].

Identification of yeasts: The yeasts colonies obtained were transferred to Malt extract broth and incubated at 30 C. The cultures were tested for various biochemical tests like simple staining, sugars fermentation (lactose, glucose, galactose, sucrose, and maltose), ascospore formation and ethanol production. Based on the results the yeasts were identified to the species name as per the yeast identification key [Harrigan and McCance, 1976] [5].

Identification of mold: The molds colour and microscopic observations were used to identify the molds. The wet mount technique was used to observe the mold under 40 x. [Sangeetha, and Thangadurai, 2013] [6].

Antimicrobial activity test: The yeast antimicrobial activity was done as per the procedure described [Prabha *et al*., 2013] [7]. In case of molds, the Malt Extract Agar (MEA) media of pH 3.5 were smeared with mold growth. Then a 7 mm well was made in the agar medium. Natamycin concentration of 50 ppm was transferred to the well and incubated at 30 C without inverting the plates for 3-5 days. The inhibitory areas around the well were measured using a calibrated scale and the inhibitory areas were calculated.

Results and discussion

Isolation of yeasts and molds from paneer samples: Three fresh paneer samples were analysed for the yeasts and molds counts. Average yeasts and molds counts of 3.31 and 1.0 log $_{10}$ cfu/g were obtained respectively. Further the yeast isolates were identified to their species, from the biochemical tests it revealed that all the isolated yeasts were belongs to the Rhodotorula species. The molds were identified based on the colour and morphology as Aspergillus, Penicillium, Rhizopus and mucor as indicated in the Table 1 and 2.

Singh and Singh [2000] [8] analysed the market samples of paneer collected from Agra city and found comparatively lower Total plate count (6.51 $\log_{10}$ cfu/g), Coliform count (3.05 $\log_{10}$ cfu/g), Yeast and Mold count (2.99 $\log_{10}$ cfu/g), *Enterococci* count (2.73 log10 cfu/g) and *Micrococci* count (2.03 $\log_{10}$ cfu/g) for laboratory made samples against 18.00, 10.39, 7.54, 5.05 and 5.07 $\log_{10}$ cfu/g, respectively for market samples. They concluded that the poor bacteriological quality of market samples was mainly due to the use of poor- quality milk, unhygienic practices during manufacturing, handling and storage of product. Nithin [2018] [9] observed quality of different brands of paneer sold in Delhi City. A total of 80 packets of eight different brands were analysed for their quality. Total bacterial count was observed between 5.40 and 5.61 $\log_{10}$ cfu/g, Yeast and Mold count was observed to be 2.13 and 2.3 log10 cfu/g, coliform count was 1.59 to 1.88 $\log_{10}$ cfu/g and pathogenic organisms like *Staphylococcus aureus* count was 1.04 to 1.34 log10 cfu /g.

Antimicrobial activity of isolated yeasts and molds against the natamycin: The identified yeasts and molds were screened for their antimicrobial activity as per the procedure described by [Prabha *et al*., 2013] [7]. The yeast Rhodotorula produced inhibitory area ranged from 452 to 668 and molds Aspergillus and Penicillium produced the inhibitory area of 432 sq.mm whereas rhizopus and mucor produced 132 sq.mm inhibitory area against 50 ppm natamycin respectively (Table 3 and Fig 1).

Lule *et al*., [2016] [3] reported that yeasts such as *Candida glabrata*, *Rhodotorula rubra*, *Saccharomyces cerevisiae*, *Saccharomyces carlbergensis*, *Saccharomyces* exiguous obtained from Concentrated Orange juice, Yogurt, Apple juice, Brewer's yeast, Soft drink inhibited by natamycin at 4.0, 1.0, 1.5 and 1.0 ppm (MIC). Similarly, molds like *Aspergillus flavus*, *Aspergillus parasiticus*, *Aspergillus fumigatus*, *Aspergillus penicillioides*, *Cladosporium candidum* and *Mucor racemosus* from ducht cheese inhibited by natamycin at 20, 10 and 15 ppm (MIC).

Conclusion

The yeasts and molds identified from the fresh paneer samples produced were inhibited by the natamycin concentration of 50ppm. The natamycin at 50 ppm inhibited the rhodotorula yeast and Aspergillus, Penicillium, Rhizopus and mucor molds obtained from the fresh paneer samples. Hence, natamycin can be effectively used to control the growth of yeasts and molds in the paneer during storage and shelf life of paneer sample can be extended.

Table 1: Enumeration of yeasts and molds from the fresh paneer samples

Paneer sample	Yeasts	Molds
	Viable count log $_{10}$ cfu/g	
FP1	4.22	1.0
FP2	2.86	1.0
FP3	2.32	NIL
Average	3.31	1.0

FP: Fresh Paneer samples
Note: All values are average of three trials

Table 2: Identification of yeasts and molds from fresh paneer samples

Isolate code	Morphology of cell	Colour of colony	Catalase &oxidase	Biochemical tests							Identification
				Lac	Suc	Mal	Glu	Gal	Ascospore	Ethanol	
Y1	Oval	Pink	-	-	+	+	+	+	-	-	*Rhodotorula sp.*
Y2	Oval	Pink	-	-	+	+	+	+	-	-	
Y3	Oval	Pink	-	-	+	+	+	+	-	-	
Y4	Oval	Pink	-	-	+	+	+	+	-	-	
M1		Black									*Rhizopus sp.*
M2		White									*Mucor sp.*
M3		Green									*Aspergillus sp.*
M4		Black									*Rhizopus sp.*
M5		Blue									*Penicillium sp.*

Y= Yeast Isolates M= Mold isolates, -Negative, + Positive
Note: All values are average of three trials

Singh and Singh [2000] [8] analysed the market samples of paneer collected from Agra city and found comparatively lower Total plate count (6.51 $\log_{10}$ cfu/g), Coliform count (3.05 $\log_{10}$ cfu/g), Yeast and Mold count (2.99 $\log_{10}$ cfu/g), *Enterococci* count (2.73 log10 cfu/g) and *Micrococci* count (2.03 $\log_{10}$ cfu/g) for laboratory made samples against 18.00, 10.39, 7.54, 5.05 and 5.07 $\log_{10}$ cfu/g, respectively for market samples. They concluded that the poor bacteriological quality of market samples was mainly due to the use of poor- quality milk, unhygienic practices during manufacturing, handling and storage of product. Nithin [2018] [9], observed quality of different brands of paneer sold in Delhi City. A total of 80 packets of eight different brands were analysed for their quality. Total bacterial count was observed between 5.40 and 5.61 $\log_{10}$ cfu/g, Yeast and Mold count was observed to be 2.13 and 2.3 log10 cfu/g, coliform count was 1.59 to 1.88 $\log_{10}$ cfu/g and pathogenic organisms like *Staphylococcus aureus* count was 1.04 to 1.34 log10 cfu /g. Mouldy surface in paneer mainly due to excessive moisture content in paneer [Kahn 2011 and Kumar 2014] [2, 10, 11].

Antimicrobial activity of isolated yeasts and molds against the natamycin: The identified yeasts and molds were screened for their antimicrobial activity as per the procedure

described by [Prabha *et al.* 2013] [7]. The yeast rhodotorula produced inhibitory area ranged from 452 to 668 and molds Aspergillus and Penicillium produced the inhibitory area of 432 sq.mm whereas rhizopus and mucor produced 132 sq.mm inhibitory area against 50 ppm natamycin respectively (Table 3 and Fig 1).

Table 3: Antimicrobial activity of natamycin on the yeasts and molds from the fresh Paneer

Name of yeast	Inhibitory zone (mm)	Inhibitory area (Sq.mm)
Rhodotorula Sp.	30	668
Rhodotorula Sp.	25	452
Rhodotorula Sp.	25	452
Name of the mold		
Mucor sp.	25	452
Rhizopus sp.	25	452
Aspergillus sp.	15	138
Penicillin	15	138

Note: Natamycin used: 50 µl of 50 ppm
All values are average of three trials

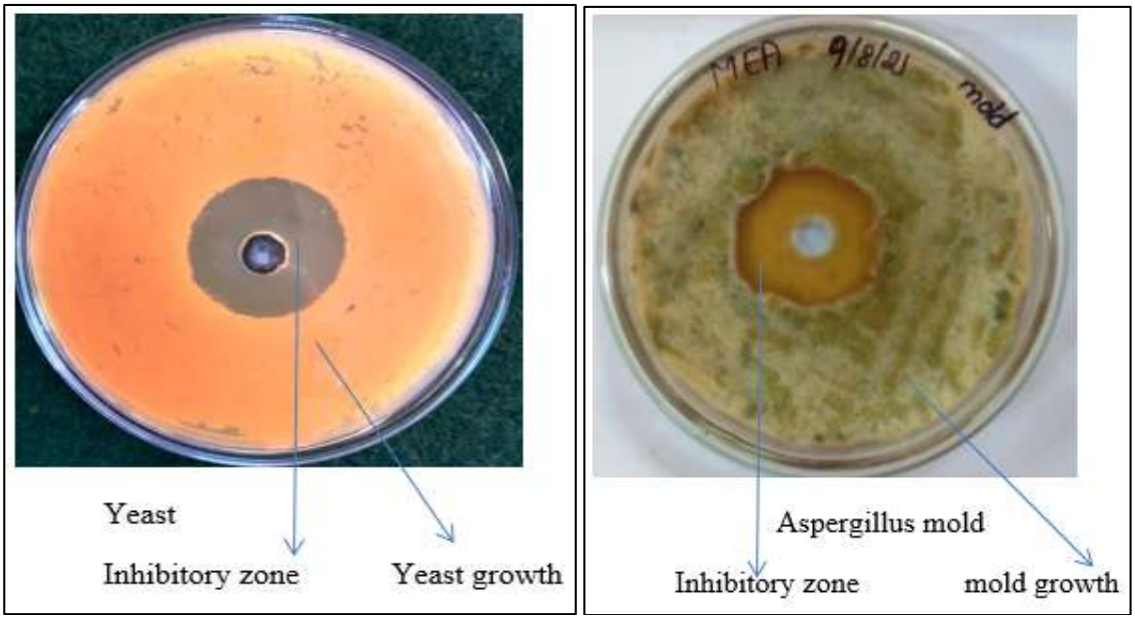

Fig 1: Antimicrobial activity of natamycin against yeast and mold

Lule *et al.*, [2016] [3] reported that yeasts such as *Candida glabrata, Rhodotorula rubra, Saccharomyces cerevisiae, Saccharomyces carlbergensis, Saccharomyces exiguous* obtained from Concentrated Orange juice, Yogurt, Apple juice, Brewer's yeast, Soft drink inhibited by natamycin at 4.0, 1.0, 1.5 and 1.0 ppm(MIC). Similarly, molds like *Aspergillus flavus, Aspergillus parasiticus, Aspergillus fumigatus, Aspergillus penicillioides, Cladosporium candidum and Mucor racemosus* from ducht cheese inhibited by natamycin at 20, 10 and 15 ppm (MIC). Natamycin provided inhibitory effect against selected indicator microorganisms (*Penicillium expansum, Fusarium culmorum* [Hanušová, *et al.*, 2010] [12]. Natamycin effects on yeast and fungi which is used in surface treatment for cheese and dry sausages [Silva, *et al.*, 2016] [13].

Conclusion

The yeasts and molds identified from the fresh paneer samples produced were inhibited by the natamycin concentration of 50ppm. The natamycin at 50 ppm inhibited the rhodotorula yeast and aspergillus, Penicillium, Rhizopus and mucor molds obtained from the fresh paneer samples. Hence, natamycin can be effectively used to control the growth of yeasts and molds in the paneer during storage and shelf life of paneer sample can be extended.

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