

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 where as, 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; 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 [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 [2]. Natamycin is an antibiotic derived from *Streptomyces*

natalensis. Natamycin inhibits yeasts and molds very effectively [3].

MATERIALS 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 [4]

Malt extract broth: Prepared as for the standard procedure [4]

Yeast identification: Sugar fermentation broth, ethanol production and ascospore

formation as per the yeast identification key [4 and 5]

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 [3].

Identification of yeasts: The yeasts colonies obtained were transferred to Malt extract broth and incubated at 30 C. The cultures were tested for various bicochemical tests like simple staining, sugars fermentation (lactose, glucose, galactose, sucrose, maltose), ascospore formation and ethanol production. Based on the results the yeasts were identified to the species name as per the yeast identification key [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 [6].

Antimicrobial activity test: The yeast antimicrobial activity was done as per the procedure described [7]. In case of molds, the Malt Extract Aagr (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 log₁₀ 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 Tables 1 and 2.

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	-	-	+	+	+	+	-	-	<i>Rhodotorula</i>
Y2	Oval	Pink	-	-	+	+	+	+	-	-	<i>sp.</i>
Y3	Oval	Pink	-	-	+	+	+	+	-	-	
Y4	Oval	Pink	-	-	+	+	+	+	-	-	
M1		Black									<i>Rhizopus sp.</i>
M2		White									<i>Mucor sp.</i>
M3		Green									<i>Aspergillus sp.</i>
M4		Black									<i>Rhizopus sp.</i>
M5		Blue									<i>Penicillium sp.</i>

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

Singh and Singh [8] analyzed 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 ($2.73 \log_{10}$ 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 [9] observed quality of different brands of paneer sold in Delhi City. A total of 80 packets of eight different brands were analyzed 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 $2.3 \log_{10}$ 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 $1.34 \log_{10}$ cfu/g. Mouldy surface in paneer mainly due to excessive moisture content in paneer [10 and 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 [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. [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).

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)
<i>Rhodotorula Sp.</i>	30	668
<i>Rhodotorula Sp.</i>	25	452
<i>Rhodotorula Sp.</i>	25	452
Name of the mold		
<i>Mucor sp.</i>	25	452
<i>Rhizopus sp.</i>	25	452
<i>Aspegillus sp.</i>	15	138
<i>Penicillin</i>	15	138

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

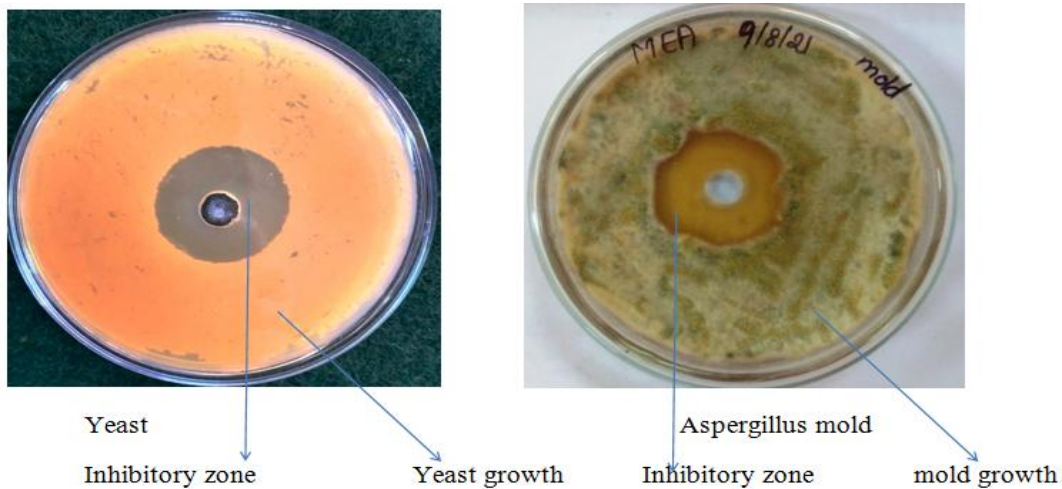


Fig. 1. Antimicrobial activity of natamycin against yeast and mold

Natamycin provided inhibitory effect against selected indicator microorganisms (*Penicillium expansum*, *Fusarium culmorum*) [12]. Natamycin effects on yeast and fungi which is used in surface treatment for cheese and dry sausages [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.

COMPETING INTERESTS

Authors have declared that no competing interests exist

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