Effect of Natamycin on the survival of Prototheca zopfii during manufacture and storage of Feta cheese

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Abstract

The effect of Natamycin on the survival of *P. zopfii* using different concentrations of Natamycin (0.2%, 0.4%) in Feta cheese which was laboratory prepared, inoculated with the previously isolated and identified *P. zopfii* to yield a concentration of 2 x 10^8 c.f.u./ml or gm at room temperature (25 ± 2ºC) and refrigerator temperature (5 ± 2ºC). *P. zopfii* counts were demonstrated and PH value were determined every twelve hours. *P. zopfii* couldn't be detected after 24h and after curd in the samples of Feta cheese containing Natamycin of 0.2%, 0.4% in storage at room and refrigerator temperature, respectively. The public health significance of the organism and the precautions, which should be taken to control this organism in dairy industry as well as the recommended sanitary measures, were also discussed.

Introduction

Prototheca species are ubiquitous unicellular yeast-like colorless microalgae Rodringues, 2003). The genus prototheca was first referred by Wilhelm Kruger in 1894 (Kruger, 1894). *P. zopfii* and *P. wickerhamii* are pathogenic for humans and other animals (Linares et al., 2005 and Inci and Ataken, 2006) and are considered zoonotic agents (Lee et al., 2008).

Widespread acquisition of antimicrobial resistance of prototheca zopfii was reported by many researches (Costa et al. 1996; Buzzini et al. 2004).

So, there is a need for natural preservatives which posses antimicrobial activity but cause no problems to the handlers and consumers. In this respect, Natamycin is a natural substance also Management) research laboratories in 1955. It is widely used in the known as pimaricin, is a polyene macrolid produced by bacterium *Streptomyces natalensis* in the DSM (Disaster Science and food industry as a preservative to prevent fungal contamination of cheese. It has been approved as a food additive in over 40 countries, and used in other products such as yoghurt (Koontz and Marcy, 2003). It acts by binding the membrane sterols, primarily ergosterol, the principal sterol in fungal membranes, distorting the selectivity of the membrane permeability leading to cell death and is thus active against fungi but not against bacteria (bacteria do not contain ergosterol) (Aparicio et al., 1999). Natamycin is applied in liquid sprays and dips or as powder on whole, shredded, and soft cheese considered to be a GRAS (Generally Recognized As Safe) for human. Milk and milk products, when contaminated with Prototheca spp. represent a potential means of transmission of this zoonosis, considering all the public health significance of Prototheca species as well as its economic losses, the present study was planned to deal with The effect of Natamycin on the survival of *P. zopfii* during manufacture and storage of Feta cheese.

Methods

Collection of samples

A total of 50 locally manufactured Damietta cheese samples were collected from Qena city markets. 10 grams of each cheese sample were mashed thoroughly in a sterile morter, then 90 ml solution of sodium citrate were added to obtain a dilution of 1:10.

Enrichment procedure

Enrichment of *Prototheca* species was adopted using one ml of the prepared milk or cheese samples and inoculated into a sterile test tube containing 10 ml of *Prototheca* Enrichment (PE) broth, then incubated at 30ºC for 72h.

Isolation and Identification of *Prototheca* species

Isolation was done using surface spreading technique on *Prototheca* Isolation Medium (PIM) according to (Pore 1973). The plates were incubated for 72 hours at 30˚ C. Typical *Prototheca* colonies appear soft, wet, yeast like white-to-light-tan colonies. Presumed colonies were transferred to nutrient agar slants, and incubated at 25 – 37ºC for 48h for further identification.

Prototheca isolates were identified microscopically according to (Pore 1998). The biochemical procedures for *Prototheca* identification involved, Urease test (Edwards and Ewing 1972), Nitrate reduction test (Park et al. 1984), Carbohydrate and alcohol assimilation (APHA 1985) and Arginine dehydrolase (Harrigan and MacCance 1976) were also checked.

1) Culture preparation:

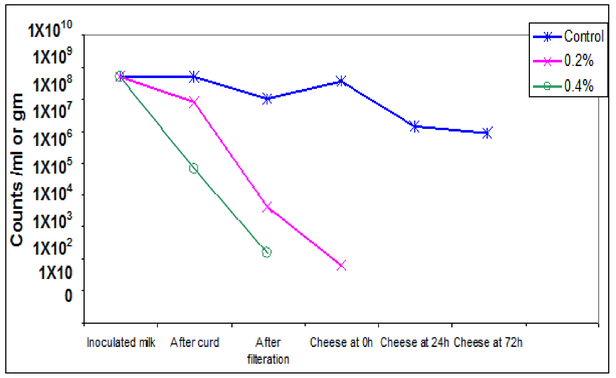
P. zopfii strain used in this study was previously isolated from Damietta cheese (Abdel Hameed in press). The organism was propagated in Prototheca enrichment broth and incubated at 30ºC for 72h. One milliliter of the culture was serially diluted in 1% peptone water to attain the desired inoculum levels.

2) Manufacture and treatment of Feta cheese:

Feta cheese was prepared in the laboratory of Assiut Dairy Plant from pasteurized milk at 78ºC for 15 second. Enough broth culture of *P. zopfii* was added to the warmed (40ºC) pasteurized milk to provide approximately 2 x 10^8 c.f.u./ml.

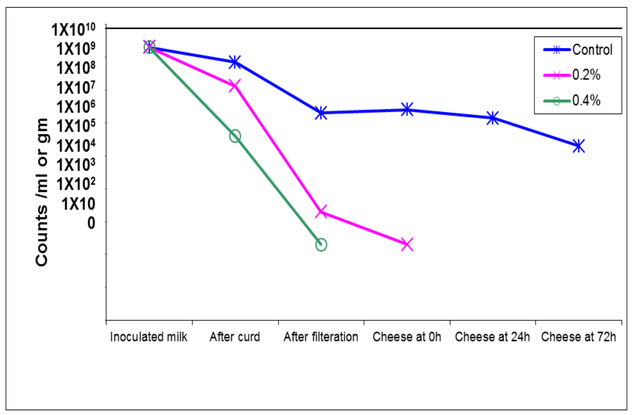
The inoculated pasteurized milk was salted to provide a concentration of 4% of rennet and calcium chloride (0.02%) and Glucorodelta lactone (GDL) 2.5% were added and mixed continuously. Addition of Natamycin (Natamax) to pre cheese after mixing, Natamycin provided by Danisco (Als. Dk-7200 Grindsted, Denmark) was added in concentration of 0.2% and 0.4% (WHO, 1976). Cheese was manufactured and divided into three sections to be tested in this part of the study. Two concentrations of natamycin (Piramycin) (0.2% and 0.4%) in aqueous suspension in which cheese is immersed before the ripening. The third cheese section was left without natamycin suspension and kept as control. Each section was divided into two portions, the first was kept at room temperature (25 ± 2ºC) and the other was stored at refrigerator temperature (5 ± 2ºC). The effect of natamycin aqueous suspension on the growth of *P. zopfii* was determined for inoculated milk, curd and after filtration, as well as, at zero time day of cheese manufacture and every 24h. Control samples were tested for pH values according to standard methods of A. P. H. A. (1978) with a pH meter (Orion Model 201) equipped with standard combination electrodes which was inserted into the sample for at least 45 sec. then the pH value was recorded directly.

Results



Milk and cheese samples (Storage time)

Figure1. Effect of Natamycin on the growth of P. zopfii during manufacture and storage of Feta cheese at refrigerator temperature (5 ± 2ºC).



Milk and cheese samples (Storage time)

Figure2. Effect of Natamycin on the growth of P. zopfii during manufacture and storage of Feta cheese at refrigerator temperature (5 ± 2ºC).

Conclusion

P. zopfii couldn't be detected after 24h and after curd in the samples of Feta cheese containing Natamycin of 0.2%, 0.4% in storage at room and refrigerator temperature, respectively.

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