

IDENTIFICATION OF YEASTS FROM INDIVIDUAL FARM TANK MILK SAMPLES IN BELGIUM

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ABSTRACT

Yeasts from 70 individual milk storage tanks on farms were identified to species level. Well known pathogenic yeasts *Candida kefyri* (13 strains), *Candida rugosa* (4 strains) were isolated from tank milk, which shows that yeast potentially pathogenic for dairy cows are species commonly found as contaminants in tank milk. Moreover, *Pichia kluyveri*, a species until now never isolated from milk was found in numerous milk samples (20 strains) from tanks.

KEY WORDS

Bovine - Mastitis - Yeast - Tank milk.

SAMENVATTING

Gisten geïsoleerd uit 70 individuele tankmelk monsters van telkens verschillende bedrijven werden geïdentificeerd tot op species-niveau. Wel bekende pathogene gisten zoals *Candida kefyri* (13 stammen), *Candida rugosa* (4 stammen) werden geïsoleerd uit tankmelk, gegevens die aantonen dat gisten met een pathogeen vermogen voorkomen als contaminanten in melk. Bovendien, *Pichia kluyveri*, die tot nu toe nog nooit afgezonderd werd uit melk, werd ook nog teruggevonden in talrijke tankmelkstalen (20 stammen).

INTRODUCTION

Bovine mastitis is one of the problems of animal health in dairy herds as it causes milk yield to decline and to affect milk quality and composition. Subclinical mastitis occurs more frequently, and as there are no visible changes in the udder, the causative agents can persist for a longer period leading to considerable economic losses.

Bacteria and fungi, most often yeasts, can act as causal agents, individually or in combination. Clinically mycotic mastitis does not differ from bacterial mastitis. This means that laboratory examinations are necessary for diagnosis.

According to Chermette and Bussieras (1993) yeasts are, in our countries, responsible for about 6 % of the mastitis cases. Yeasts are known to be ubiquitous in the natural surrounding of dairy cattle. Introduction into the gland usually occurs mechanically through the teat canal. *Candida* species are the most common yeasts isolated from milk of the mammary glands (Richard *et al.*, 1980; Bada *et al.*, 1992; Kuo and Chang, 1993). More rarely, *Cryptococcus* sp. appear as the most important ones (Costa *et al.*, 1993). Moreover, many other genera such as *Pichia*, *Trichosporon* and *Debaryomyces* can also be present (Lagneau *et al.*, 1996).

The severity of the infection, however, relates to the yeast species involved, the number of organisms introduced into the gland and the ability of the organism to grow at 40°C (Jensen and Aelbaek, 1994), all this being modulated by the predisposing factors such as an antibacterial treatment, which in most of the cases, pre-

ceeds the appearance of mycotic mastitis (Chermette and Bussieras, 1993). The main purpose of the present study was to isolate and identify yeasts growing in milk as contaminants.

MATERIAL AND METHODS

Sampling

One hundred twenty two samples of 10-15 ml were collected. Seventy samples were from individual dairy farms milk storage tanks. Regarding this bulk tank study, there were many steps in the recovery, the transport and preliminary processing which were not under our observation and opportunities for contamination are probable. Fifty two were from individual quarters of 13 dairy cows from two different dairy herds with mastitis problems. Forty eight are from normal quarters and four from infected ones (one cow). The samples were obtained immediately prior to the automated milking and were always aseptically collected.

Isolation and identification of the yeasts

After homogenization of the sample, five ml were centrifuged (4000 rpm/10 min.). 0.5 ml of the sediment was inoculated onto the surface of a Sabouraud-agar-medium (1 % peptone; 2 % agar; 2 % glucose) supplemented with 0.2% chloramphenicol. The Petri dishes were incubated at 37°C for four to five days. Yeast counts were also made for the samples from individual quarters. A direct examination of the sediment was also

performed for the five samples from infected udders. The yeast species were identified according to Barnett *et al.* (1990).

RESULTS AND DISCUSSION

Pichia kluyveri was the most frequently occurring yeast species in milk tanks (Table 1).

Table 1. Identification of 58 yeast isolates from 70 individual dairy farms milk storage tanks.

Species (number of isolates)
<i>Candida catenulata</i> (1)
<i>Candida kefyr</i> (13)
<i>Candida lipolytica</i> (1)
<i>Candida rugosa</i> (4)
<i>Candida sake</i> (1)
<i>Candida zeylanoides</i> (1)
<i>Cryptococcus curvatus</i> (1)
<i>Cryptococcus laurentii</i> (2)
<i>Debaryomyces hansenii</i> (5)
<i>Debaryomyces maramus</i> (1)
<i>Kluyveromyces lactis</i> (1)
<i>Pichia kluyveri</i> (20)
<i>Pichia minuta</i> (1)
<i>Rhodotorula sp.</i> (2)
unidentified (4)

Twenty individual dairy farms milk storage tanks yielded this species, which, as far as we know, never has been mentioned as a causal agent of bovine mastitis. It is known as an exosaprophytic yeast, isolated from food as olives, fruit, cacao or from plants (Barnett *et al.*, 1990). Among the other yeast species from tanks, we have *Candida kefyr*, a well known pathogenic species (Jensen and Aelbaek, 1994; Lagneau *et al.*, 1996), of which 13 strains were isolated. *Candida rugosa* found four times, is a species that is more and more frequently isolated, not only from milk (Richard *et al.*, 1980; Aelbaek *et al.*; Kuo and Chang, 1993), but also from human samples such as bronchoalveolar lavages.

The four samples collected from infected udders in the dairy farm were positive for bacteria. None of the samples collected from normal quarters, except for one, yielded yeasts. The exception was an isolate of *Trichosporon ovoides*, a well known normal inhabitant of the skin of human beings as well as other mammals (Gucho *et al.*, 1992). Its presence here is most probably accidental. Indeed, unusually large inocula (0.5 ml) of centrifugated sediments, were inoculated on the plates and only six colony forming units (CFU)/ml were pre-

sent in the single quarter milk sample which was found positive.

In conclusion, this inquiry confirms the results obtained two years ago (Lagneau *et al.*, 1996), i.e. that yeasts potentially pathogenic for dairy cows are commonly found as contaminants in bulkmilk. Moreover, milk samples aseptically collected in dairy herds from non-infected glands can be quite negative.

Research on mycotic mastitis performed in Belgium during last years, added to the increasing number of identification requests received at the laboratory of mycology of Antwerp (ITG) shows that it is desirable to act up a network of laboratories studying bovine mycotic mastitis at a national level.

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