Studies on virulence factors of yeasts associated with mastitis in cattle

By

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Abstract

The present study was performed on a total of 200 samples were collected from different dairy farms in middle delta areas. The collected samples included 150 samples from cows suffering from mastitis and 50 samples from apparently healthy ones. The collected samples were subjected for mycological examination and studying the virulence factors of the isolated yeast species. A total number of 26 yeast isolates were isolated from mastitic (22) and (4) from apparently healthy cows milk in a percentage of 84.61% and 15.38% respectively. Also, from the identification of the isolated yeasts from both mastitic and apparently healthy cows milk revealed that, the most common yeasts was *Candida albicans* 6 (23.07%), followed by *Candida parapsilosis* 4 (15.38%) and 3 isolates for each of *C. tropicalis, Geotrichum Candidum Rhodotorula rubra and C. guilliermondii* (11.53%) for each isolate. *Trichosporon cutaneum* 2 isolates (7.69%) and one isolates of *Torulopsis glabrata* and C. rugosa (3.84%) for each isolate.

Germ tube formation, all the isolated *C. albicans in* the present study were germ tube producers, which has an important role in invasiveness, pathogenicity and penetration of host tissue. The Proteolytic activity (proteinase enzyme) nine isolates of yeast species (34.61%) were positive for proteinase enzyme included 6 isolates of *C. albicans*, 2 isolates of *C. parapsilosis* and 1 isolate of *C. rugosa*, and the diameter of proteolytic zone was ranged from 2mm-5mm and Phospholipase activity (phospholipase enzyme) showed that 10 isolates of yeast species (38.46%) were positive for phospholipase enzyme included 5 isolates of *C. albicans*, 2 isolates of *C. parapsilosis* and one isolate for each of *C.guilliermondii*, *C. tropicalis*, and *Torulopsis glabrata*, and *C. tropicalis* showed the highest precipitation zone.

Introduction

Inflammation of mammary glands due to yeast infection was considered as an important problem in bovine dairy herds. As it causes economic losses considered including decrease milk yield, culling of chronic cases and coasts of veterinary services. Such problem may be attributed to treatment with antibiotics and/or their virulence factors which increase their degree of invasiveness and pathogenicity.

The virulence factors of yeast including proteinases, phospholipase secretion, hyphal formation and phenotypic switching of *C. albicans*. Only strains that could produce both filament and yeast forms were capable of

penetrating vital organs and proliferating sufficiently to kill the host cell *Yang (2003)*. Phospholipase activity have an important role for virulence and pathogenicity of *C. albicans* and *Cryptococcus neoformans Odds (1979)* and *Vidotto et al. (1996)*. Aspartic proteinases (SaPs) were considered as a virulence factor for yeasts of genus Candida as *Candida albicans* and *C. tropicalis Zaugg et al. (2001)*. Therefore the presented study was designed to identify yeasts which isolated from mastitic and clinically normal cow's milk and to study their virulence factors.

Material and Methods

Materials:

1. Samples:

Two hundred milk samples, (one hundred and fifty mastitic milk samples collected from cows suffering from mastitis at different localities following treatment with antibiotics and fifty other samples from apparently healthy cows).

2. Media:

2. 1. Media used for isolation and identification were carried out according to (*Cruickshank et al., 1975*) and (*Refai et al., 1969*)

2. 2. Media for detection of virulence factors of yeasts

2. 2. 1. Media for determination of proteinase activity (*Aoki et al., 1990*):

2. 2. 2. Media for determination of phospholipase production (*Polak, 1992*):

3. Biological reagents:

Serum of rabbit *Leslie and Frank (1980)*:

4. Stains: Coomassie blue

5. Solution:_Coomassie blue destaining solution

Methods:

1-isolation and identification of yeasts

1.1. Isolation of yeasts according the technique recommended by *Monga and Kalra (1971)*

1.2. Identification of isolated yeast (*Refai et al., 1969*)

2. Studying the virulence factors of yeast species

2. 1. Determination of proteinase production (*Aoki et al., 1990* and *Vidotto, 1997*): Ten microliters of thick suspension of 24 hours old yeast (in normal saline) were dropped on a horizontally – balanced plate containing 20 ml of the specific medium. The plates were incubated at 37 ^{0}C and examined after 7 days. At the end of incubation, the plates were taken out of the incubator and flooded with Coomassie brilliant blue stain for 5 minutes. After dryness, the plates were flooded with the destaining solution were poured off. After dryness, the plates were examined where the proteinase activity was indicated by unstained area around the yeast growth while the rest of the agar was stained blue. The diameter of both yeast colony and the surrounding unstained zone was measured in millimeters using transparent ruler and enzymatic activity was expressed as Pz as mentioned by *price et al. (1982)*, where Pz= colony width/ colony width+ unstained zone.

2. 2. Determination of phospholipase production (*Polak, 1992* and *Vidotto, 1997*):

Ten microliters of 24 hours- old yeast (thick suspension in normal saline) were dropped on a horizontally- balanced plate containing 20 ml of the

specific medium. The plates were left to be dried at room temperature for 10 minutes, incubated at 37 ^{0}C and examined daily up to 7 days. Production of phospholipase was indicated by opacity (cloudy precipitation) of the agar medium around the yeast growth on the plate surface. Both diameters of yeast growth and cloud precipitation were measured using a transparent ruler. The enzymatic activity was qualitated and identified as Pz *Price et al. (1982)*, where Pz = colony width/ colony width+ precipitation zone.

Results

Table (1): prevalence of yeast isolates from both mastitic milk and apparently healthy cow's milk

Source of milk samples	Yeast isolates	
	No	%
Mastitic animals	22	84.61
Apparently healthy animals	4	15.38

%= the percentages was calculated according to the total numbers of yeast isolates (26).

Table (2): Characterization of yeast isolated from both mastitic and apparently healthy cows milk.

Type of yeasts	Mastitic animals		App.healthy animals	
	No	%	No	%
Candida albicans	6	23.07%	0	0
C. guilliermondii	2	7.69	1	3.84
C. parapsilosis	4	15.38	0	0
C. tropicalis	1	3.84	2	7.69
C. rugosa	1	3.84	0	0
Geotrichum candidum	3	11.53	0	0
Rhodotorula rubra	3	11.53	0	0
Trichosporon cutaneum	1	3.84	1	3.84
Torulopsis glabrata	1	3.84	0	0

The percentage was calculated according to total No of yeast isolates (26)

Table (3): Prevalence of proteolytic activity and germ tube formation.

Types of yeast	Total No of isolates	proteolytic activity		Germ tube formation	
		No of positive	%	No of positive	%
Candida albicans	6	6	23.07%	6	23.07%
C. guilliermondii	3	0	0%	0	0.0
C. parapsilosis	4	2	7.69%	0	0.0
C. tropicalis	3	0	0%	0	0.0
C. rugosa	1	1	3.84%	0	0.0
Geotrichum candidum	3	0	0%	0	0.0
Rhodotorula rubra	3	0	0%	0	0.0
Trichosporon cutaneum	2	0	0%	0	0.0
Torulopsis glabrata	1	0	0%	0	0.0
Total	26	9	34.61%	6	23.07%

% of proteolytic activity was calculated according to total number of yeast isolates (26 isolates).

Table (4) prevalence of yeast species phospholipase activity.

Types of yeast	Total No of isolates	Phospholipase activity	
		No of positive isolates	%
Candida albicans	6	5	19.23 %
C. guilliermondii	6	1	3.84 %
C. parapsilosis	4	2	7.69 %
C. tropicalis	3	1	3.84 %
C. rugosa	1	0	0%
Geotrichum candidum	3	0	0%
Rhodotorula rubra	3	0	0%
Trichosporon cutaneum	2	0	0%
Torulopsis glabrata	1	1	3.84 %
Total	26	10	38.46 %

% of phospholipase activity was calculated according to total No of yeast isolates (26).

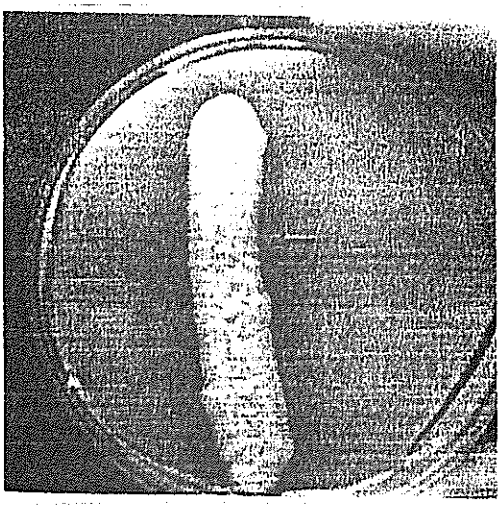

Photo (1): The photo shows testing of the identified yeast isolate for proteinase enzyme production (appeared as clear zone surrounding yeast culture)

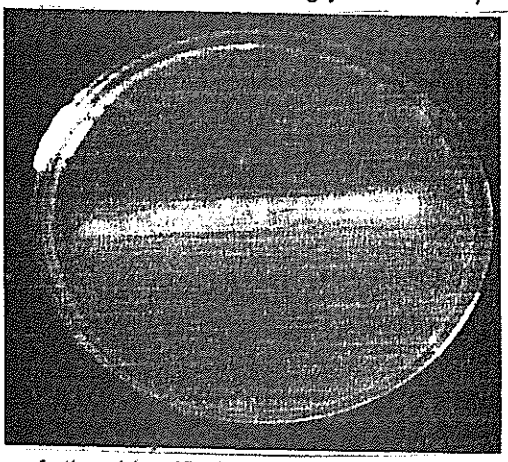

Photo (2): Testing of the identified yeast isolate for phospholipase enzymproduction (appeared as area of opacity surrounding yeast culture).

Photo (2): Testing of the identified yeast isolate for phospholipase enzymproduction (appeared as area of opacity surrounding yeast culture).

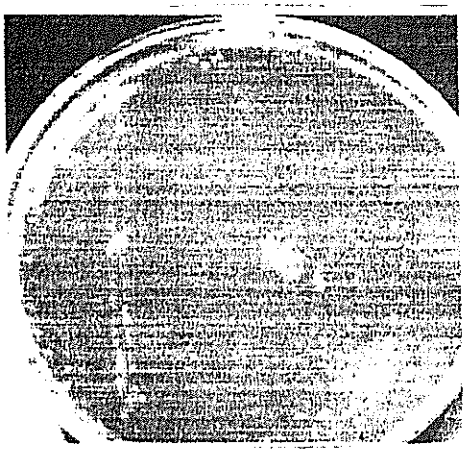

Photo (3): Negative yeast culture showing for phospholipase

Discussion

Mastitis in farm animals is considered to be one of the common and economically important world wide diseases as it leads to lowering in milk production, reduced milk quality and high production costs *(Riffan et al., 2001).*

The results recorded in table (2) revealed that *Candida albicans* was the most common isolates 6 isolates (23.07%) from the milk of mastitic animals, where the obtained results were in agreement with that reported by *Chhabra et al.(1998)* who isolated *C. albicans* (33.3%) and *Moshref(2004)* isolated *C. albicans* with (19.57%). Where *C. krusei* was the most common yeast isolate (40.1%) recovered by *Pengov (2002)* and *(Okamoto et al. (1988)* who stated that *C. tropicalis* was the most common yeast isolate (17.4%).

In the present study, from the table (3), Hyphal formation (germ tube) of *C. albicans* was considered a role of pathogenesis of yeast as mentioned by *(Lo et al. (1997).* The hyphal formation act as a virulence factors of *C. albicans*, and have a role in their pathogenesis and enable it to penetrate vital organs *Yang (2003)*. On the other hand, also the ability of yeast to Proteinase production was considered as virulence factors of yeast species as reported by *Calderon and Fonzi (2001)* and *Naglik et al. (2003*).

Table (3) pointed out that proteolytic activity was (34.61%) of all yeast isolates. And all isolates of *C. albicans* were positive for proteinase production (100%). *Chakrabarti et al. (1991)* recorded that 60% of isolated *C. albicans* were positive for proteinase production. *Kumar et al. (2006)* mentioned that 94.1% of *C. albicans* isolates were positive for secreted aspartyle proteinase (Sap). In the present study, yeast isolates other than *C. albicans* were positive for proteinase production such as *C. rugosa* and *C. Parapsilosis*. Nearly similar finding observed by *Mardegan et al. (2006).*

Phospholipase activity was considered by several authors to be power determinant of virulence factors of yeasts *Ibrahim et al.(1993*) and *Ghannoum (2000*).

From the table (4) phospholipase activity was (38.46%) of total yeast isolates and 5 isolates (83.33%) of *C. albicans* were phospholipase production positive. *Chaves et al. (2003*) mentioned that (46.15%) of *C. albicans* were phospholipase production and *Birinci et al. (2005*) found 61.3% of *C. albicans* were phospholipase positive.

In the present study, we noticed that there were yeast isolates other than *C. albicans* were positive for phospholipase production as *C. tropicalis*, *C. Parapsilosis* and *C. guilliermondii*. Such result was confirmed by *Shimizu (1989*) who detected phospholipase activity in other species of Candida such as *C. parapsilosis, C. tropicalis, C. guilliermondii* and *C. krusei*. On the other hand (*Samaranayake et al. (1984*) and (*Mayser et al. (1996*) detected phospholipase activity only in *C. albicans* and observed that *C. parapsilosis* and *C. tropicalis* did not produce phospholipase. *Birinci et al. (2005)* reported that non *C. albicans* isolates exhibited phospholipase activity. From the above mentioned results it can be concluded that the most common yeasts was *Candida albicans* 6 (23.07%) and the isolates of *Candida albicans* were only germ tube formation positive. On the other hand, Candida species other than *C. albicans* showed proteinase and phospholipase activity as *C. tropicalis*, *C.rugosa* and some isolates of *C.guilliermondii* and *C.parapsilosis* where they had important role in pathogenesis, as well as pathological and immunological changes of the udder.

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الملخص العربي

دراسات على عوامل الضراوة للخمائر المصاحبة للالتهابات الضرع فى الأبقار_

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فى هذة الدراسة تم تجميع ٢٠٠ عينة لبن من الحيوانات الحلابة، ١٥٠ عينة من حيوانات مصابة بالالتهابات الضرع و٥٠ عينة من حيوانات سليمة ظاهريا وتم فحص هذة العينات ودراسة عوامل الضراوة لمعزولات الخمائر . ووجدنا ان الكانديدا البيكانز هى الاكثر المعزولات وجودا وتم عزلها بنسبة (٢٣.07%) وتليها الكانديدا باراباسيلوزيس عدد (٤)عترات بنسبة (15.38%) و٣ عترات لكل من الكانديدا تروبيكاليز الجيوتريكم كانديدم والرودوتروالاربرا و الكانديدا جيلورومندى بنسبة (١١,٥٣%)لكل عترة. و٢ عترة من تريكوسبورون كيوتينيم بنسبة (٧,٦٩%) وعترة واحدة لكل من التوريولوبسيس جلابرتا و الكانديدا ريجوزا بنسبة (٣,٨٤%) لكل عترة.

وبدراسه عوامل الضراوة للمعزولات :

وجد ان كل معزولات الكانديدا البيكانذ كانوا منتجين لانابيب الاستنبات والتى لها دورا هام فى اختراق الميكروب للخلايا وبدراسة مدى قدرتها على افراز انزيمات التحلل (البروتينيز & الفسفوليبيز) وجد ان ٩ معزولات من الخمائر كانوا منتجين لإنزيم البروتينيز بنسبه (٣٤,٦١%) تشمل ٦عترات من الكانديدا البيكانز و٢عترة من الكانديدا باراباسيلوزيس وعترة واحدة من الكانديدا ريجوزا بينمالإنزيم الفسفوليبيز وجد ان ١٠ معزولات من الخمائر كانو منتجين لهذا الانزيم بنسبه(٣٨,٤٦%) وتشمل ٥ عترات من الكانديدا البيكانز و٢ عترة من الكانديدا باراباسيلوزيس وعترة واحدة لكل من الكانديدا جيلورومندى ،الكانديدا تروبيكاليز، التوريولوبسيس جلابرتا وكانت الكانديدا تروبيكاليزهىالأ كثرإنتاجا لإنزيم.