JOURNAL OF PURE AND APPLIED MICROBIOLOGY, September 2013. Vol. **7**(3), p. 2141-2147

Cultural and Microscopic Characteristics of *Trichosporon mycotoxinivorans* and Its Effect on Some Reproductive Aspects in Mice

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(Received: 30 January 2013; accepted: 04 May 2013)

In this research work, the cultural and microscopic characteristics of the yeast, *Trichosporon mycotoxinivorans* were studied using different types of media, e.g., corn meal agar, mycology broth, sabouraud dextrose agar, Czapek dox, yeast mold agar and examined by light microscope. Biochemical characteristics were studied by analytical profile index (API 20 aux strip). Effect of the yeast on the period till fertilization and the duration of pregnancy, number of births, mortality and birth weight rate of pups were determined. The results indicated that the yeast can grow fast in many type of media utilizing simple source like glucose and peptone. The yeast has globose, ovoidal, ellipsoidal, elongate single or chain cell, and produce arthrospores. It has giant septate cells and produce true hyphae. The yeast has ability to metabolize D-glucose, calcium-2-keto-gluconate, L-arabinose, D-xylose, D-galactose, inositol, N-acetyl-glucosamine, D-celiobiose, D-maltose, D-sucrose, D-lactose, D-trehalose, D-melezitose, D-rafinose, methyl-α-D-glucopyranoside and glycerol. However it failed to metabolize adonitol, xylitol and D-sorbitol compounds. It increased the fertility rate in mice female and birth weight of pups, and reduced the mortality rate of pups. The results suggest that this yeast could be used as a probiotic in animal feeding to enhance reproductive characters and survivability of pups.

Key words: Probiotic, Cultural, Microscopic, Biochemical, mycotoxinivorans, fertility.

Trichosporon is frequently isolated from soil, water, vegetables, mammals and birds. It is consided as microflora of Human (skin, mouth and nails). This Genus is classified as basidmycetous yeasts although that teleomorphs is still not known. Genus Trichosporon include 17 species depending on analysis of 26 rRNA gene out of these species 6 species (*T. beigelii, T. asteroids, T. ovoides, T. inkin, T. asahii and mucoideas*) approximately can cause diseases in human (Gueho *et al*., 1998; Sugita *et al.,* 1999).

Middeloven *et al.* (2004) reported that 40 species are now referred to *Trichosporon* based on analysis of genetic sequences. Prillinger *et al.* (1996) isolated a novel spices accepted in the *Trichosporon*. It has been deposited German Collection for Microorganisms and Cell Culture (DSM) with number DSM 14153. That yeast can be used in biological detoxification of several mycotoxins because of its ability to destroy the mycotoxins (biotransformation).

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Molnar *et al.* (2004) have differentiated between *T. loubieri* and *T. mycotoxinivorans* (a new species) based on the difference in phenotypic and genotypic characteristics. *T. mycotoxinvorans* have blocked the detrimental effects of ochratoxin A on various immune properties in broilers (Politis *et al.,* 2005) and were used as a microbial feed additive against mycotoxins due to its ability to biotransform zearalenon (ZON) to a nonestrogenic ZON metabolite (ZOM-1) (Vekiru *et al,.* 2010).

Matos *et al.* (2012) isolated the *T. mycotoxinivorans* from Carabidae gut in the central Amazon rainforest, and concluded that this yeast possessed great biotechnological potential for use in the saccharification of sugarcane bagasse hemicelluosic hydrolyzate and for biomass production with this substrate as carbon source. Also this yeast was isolated from grape marc wastes (Ntougias *et al.,* 2010)

The yeast does not have any negative effect on the compounds and blood constituents; and on liver enzymes in mice blood, moreover it does not have any histological damage in the liver tissue. The ultra-structure of *T. mycotoxinivorans* is found to be similar to most of the basidomycetes yeasts and this yeast increased body weight of mice and reduced the rate of mortality (Khalel *et al.,* 2011; Khalel *et al.,* 2012).

The aim of this research was to study some cultural, microscopic and biochemical characteristics of *T. mycotoxinivorans* and investigate the effect of the yeast on fertility of female mice, as well as on mortality rate,body weight and litter size.

MATERIALS AND METHODS

The yeast

(*Trichosporon mycotoxinivorans* HB 1230) was purchased from Universität für Bodenkultur, Vienna.

Cultural, microscopic and biochemical characteristics

The yeast *T. mycotoxinivorans* was activated at least three times on Mycology Broth (MB) [Difco Laboratories, USA] before study of cultural characteristics. The nature of growth was studied on mycology broth, Malt Extract Broth (MEB) [Amersham] and Nutrient Broth (NB). The morphology of colony was carried out on Corn Meal Agar (CMA) [Oxoid, England], Sabouraud Dextrose Agar (SDA) [Oxoid, England], Malt Extract Agar (MEA) [Amersham], Czapek Dox Agar (CDA agar) [Oxoid, England] and Yeast Mold Agar (YMA) [Oxoid, England]. The cultural characteristics were determined according to Gueho *et al.,* (1998). The microscopic properties were determined by examination under light microscope (feitz, laborlux D, Germany) .The Biochemical Characteristics were identified by analytical profile index (API 20 aux strip) (BioMrieux, France).

Preparation of the yeast suspension

One ml from a cultured cell, which was stored at – 40 ˚C was added to 100 ml of mycology broth (MB) in 500 ml erlenmeyer flask. The flask was plugged with a cotton-wool swap and incubated at 25 ˚C for 3 days. The yeast cells were collected from the exponential phase, by centrifugation at 6000 g for 10 min and washed three times with 10 ml saline solution (0.89 % NaCl). The yeast pellet was resuspended in 5 ml saline solution. The total plate count of suspension was 5×10^6 colony form unit (CFU)/ ml.

Experimental procedures

One hundred twenty adult healthy male and female albino mice *Mus musculus* domesticus (body weight 26 ±1 gm) were used in this study which were made available from experimental animal care center, College of pharmacy, King Saud University. A sixty males (also sixty females) were divided into two equal groups, the first one was maintained on a standard diet as control group, the second group was fed daily standard diet plus 0.250 ml suspension of the yeast (orally). The experiment lasted for four weeks. After the end of the experimental period, mating was allowed in control group (one male with one female) Ten replicates were performed. The same work was replicated with the second group. The period till fertilization and the duration of pregnancy, number of births (litter size), mortality and body weight rates of pups were recorded.

Statistical analysis

The experiment was designed as completed random design (CRD). The data were expressed as means ± standard deviation. Statistical analysis of data was performed by one-way ANOVA using SPSS package 17.0.

RESULTS

Cultural characteristics

The cultural characteristics of *T. mycotoxinivorans* on several media types were studied. In Figure 1(A) it can be seen, that the yeast rapidly grew in MEB, the white sediment and floating layer were observed after the incubation at 22 ºC for 3 days. The same observation was detected on MB and nutrient broth. The yeast was able to grow at a wide range of temperatures (5 to 35 ºC); however, the optimal temperature was found to be 22 ºC. The colony properties on YMA was white, creamy in color with yellow center and a diameter of 12 to 15 mm after the incubation at 22 ºC for 5 days [Figure 1 (B)]. In Figure 1 (C, D, E, F) it can be observed that there were some differences among the colonies CMA, CDA, SDA and MEA, where the growth on CMA and CDA was very slow, and the colonies were small while the growth on SDA and MEA was typical characterized by large, white colonies but they were smaller than on YMA.

Microscopic characteristics

Microscopic characteristics were tested by light microscope (X100) in order to identfy some the microstructures that characterize the yeast. In figure 1(G,H and I) light microscopic micrograph for *T. mycotoxinivorans* shows that it appears as cell ovoidal, globose, ellipsoidal and elongate (single or pairs cells). A septa hyphae, arthoroconidia, and giant cell were observed.

Biochemical Characteristics

The ability of the *T. mycotoxinivornas* to metabolize 19 chemical compounds was studied. Table (1) shows, that the yeast can metabolize D-glucose, calcium-2-keto-gluconate, L-arabinose, D-xylose, D-galactose, inositol, N-acetyl-glucosamine, D-celiobiose, D-maltose, D-sucrose, D-lactose, D-trehalose, D-melezitose, D-rafinose, methyl-α-D-glucopyranoside and glycerol. However it failed to metabolize adonitol, xylitol and D-sorbitol compounds as nutrient sources.

Reproductive aspects

The period till fertilization (service period) and the duration of pregnancy were recorded from

Table 1. Biochemical characteristics of *T. mycotoxinivorans* by API 20 aux strip.

substrate	Resultd after incubation perriod			
	16 hours	24 hours	48 hours	72 hours
Anon	-	-	-	-
D-glucose	+	+	+	+
Glycerol	-	-	+	+
Calcium-2-keto-Gluconate	+	+	+	+
L-arabinose	+	+	+	+
D-Xylose	+	+	+	+
Adonitol	-	-	-	-
Xylitol	-	-	-	-
D-Galactose	+	+	+	+
Inositol	+	+	+	+
D-Sorbitol	-	-	-	-
Methyl_á_D_Glucopyranoside	-	-	+	+
N_Acetyl_Glucosamine	+	+	+	+
D-Celiobiose	+	+	+	+
D-Lactose	+	+	+	+
D-Maltose	+	+	+	+
D-Sucrose	+	+	+	+
D_Trehalose	+	+	+	+
D-Melezitose	-	-	+	+
D_Rafinose	+	+	+	+

API= Analytical profile index.

the day of breeding until the day of birth. Figure 2 (A) shows that the mean period of fertilization and the duration of pregnancy in the treated groups with the yeast was significantly ($p<0.05$) decreased (25.2± 5.1 day) compared to the control group. The mean number of births (pups) was 7.8±0.4 and 6±3.4 in treated and control groups, respectively, this difference was significant ($p<0.05$) . The number of mortality pups was significantly lower by 20% in treated groups than that in the control groups (Figure 2 (3C)). The mean birth weight in the control group (1.9±02 gm) showed a significant decrease ($P<0.05$) compared to the treated group with the yeast (1.4±0.8 gm).

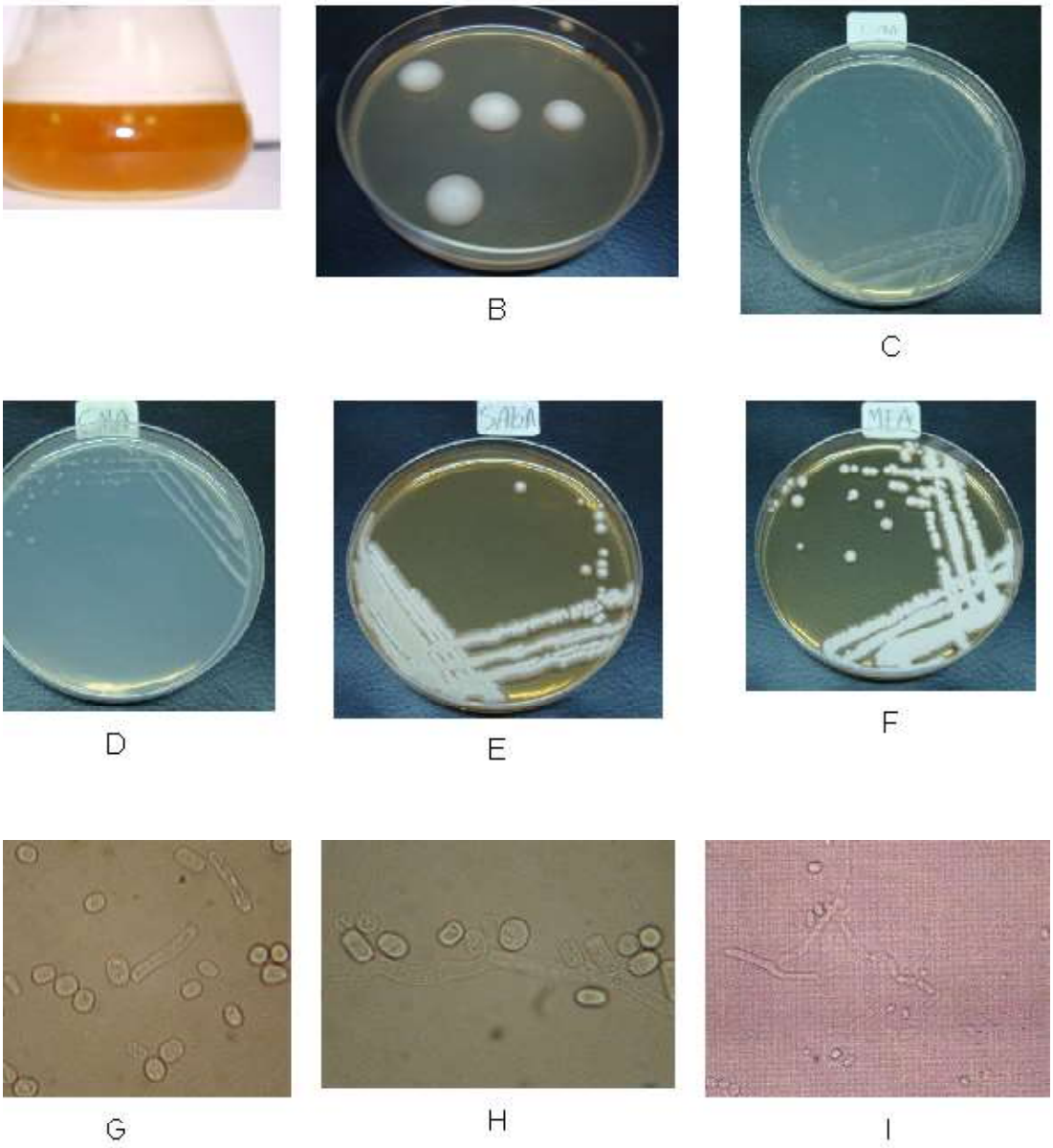

Fig. 1. The cultural characteristics of *T. Mycotoxinivorans* on several media types after the incubation at 25 ºC for 5 days [Malt Extract Broth (A), Yeast Mold Agar (B), Czapek Dox Agar (C), Corn Meal Agar (D), Sabouraud Dextrose Agar (E) and Malt Extract Agar (F)] and the microscopic characteristics (100 x) are shows ovoidal, globose,ellipsoidal and elongate cells (G) septa hyphae and (H) arthroconidia (I)

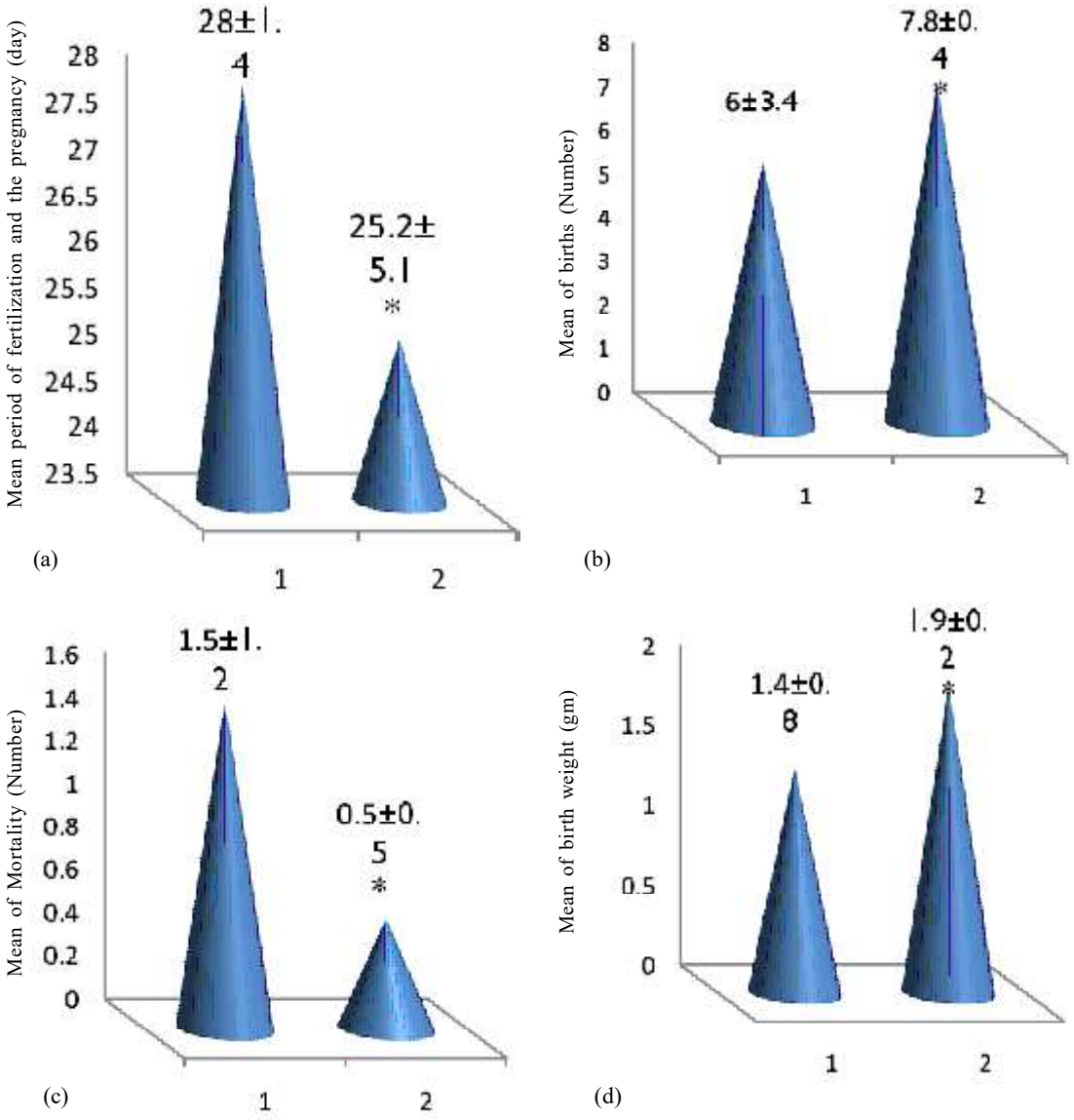

Fig. 2. Means ± S.D of the period of fertilization and the duration of pregnancy (day) (A), of the number births (B), of the mortality (C); and of the birth weight rate *(gm)* (D) after 6 weeks from breeding. Means with star (*) notification are significant different from the control at $P < 0.05$.
1= female and male control groups **2=** female and male treated group with *T. mycotoxinivorans*

DISCUSSION

Growth of *T. mycotoxinivorans* occurred rapidly on MB, MEB and NB media, The turbidity and sediment were observed at the first incubation day. The results indicated that the yeast could grow at 5 to 35 ºC whereas the optimal temperature was found to be in the range of 22 to 25 ºC. The results showed that malt extract and peptone, or yeast extract, peptone and sodium chloride, or soybean extract and glucose could be considered as typical nutrient sources for growth of *T. mycotoxinivorans* (The Oxoid Manual, 1998). The results in this research were inconsistent with those of Molnar *et al.* (2004) in sediment and floating layer. This discrepancy could be due to the use of other

types of media and other strain of yeast. Molnar *et al.* (2004) found that the yeast grew on glucose yeast broth (10 gm glucose and 5 gm yeast extract / liter). Differences in the growth of the yeast on agar media were noticed in YMA, CMA, CDA, SDA and MEA. The growth on SDA and MEA was rapid with medium colonies, also it was rapid with large colonies on YMA while it was slow with small colonies on CMA and CDA. The genus *Trichosporon* grow on SDA as yeast cerebriform and radial surface colonies with white to cream color (Chagas-Neto *et al.*, 2008). The results suggested that *T. mycotoxinivorans* can use sodium nitrate as nitrogen source and it requires simple nutrients as glucose and peptone for growth on agar or broth media.

On the other hand, these results agree with those of Molnar *et al.* (2004)in which the yeast appears under light microscope (100 X) as ovoidal, globose, and elongate single cell or as pairs cells. It has septa hyphae, arthoroconidia, and giant cell. Arthroconidia, blastoconidia, hyphae and pseudohyphae are characteristics of genus of *trichosporon*. Other microscopic structures may help in differentiating between species such as appresoria or macroconidia (Chagas-Neto *et al.,* 2008). *T. mycotoxinivorans* does not form appresoria or macroconidia. Gueho *et al.* (1998) reported that genus *Trichosporon* is characterized by its ability to form true mycelium and arthorospores while it does not form pseudomycelium.

The results indicated that the yeast has the ability to metabolize D-glucose, calcium-2-keto-gluconate, L-arabinose, D-xylose, D-galactose, inositol, N-acetyl-glucosamine, D-celiobiose, D-maltose, D-sucrose, D-lactose, D-trehalose, D-melezitose, D-rafinose, methyl-α-D-glucopyranoside and glycerol. However it failed to metabolize adonitol, xylitol and D-sorbitol compounds. Gueho *et al.* (1998) and Molnar *et al.* (2004) reported that the yeast was not able to accomplish fermentation of the chemical compounds and it had weak ability to metabolize xylitol while the present research found that it would be able to do so. The new chemical compounds were tried in this research including N-acetyl-glucosamine, D-sorbitol and Adonitol. These results could be used as a tool for isolation or identification of this yeast from several environmental sources.

The available literature citing the effect of the *T. mycotoxinivorans* on reproductive performance in mice is scanty. Some commercial products containing *T. mycotoxinivorans* are available in markets as deactivator in broiler. The yeast has ability to destroy some mycotoxins and enhance immune system in animals (Molnar *et al*., 2004; Politis *et al.,* 2005;Hanif *et al.,* 2008; Hanif *et al.,* 2011). Hickey *et al.,* (2009) found that *T. mycotoxinivorans* associated with cystic fibrosis in pneumonia diseases in human. this yeast increases weight of mice and reduces the rate of mortality and did not have any harmful effect on the blood constituents; or liver enzymes in mice, moreover it does not have any histological damage in the liver tissue (Khalel *et al.,* 2011; Khalel *et al.,* 2012). In the present study administration of the yeast reduced period of fertilization and the duration of pregnancy, increased the number of births mice (pups), reduced the rate of morality in pups and increased the birth weight of pups.

The present work suggests that *T. mycotoxinivorans* may possess a positive effect on lessening the service period which might be duo to the increased ability of female mice to accept males for mating which indicated by reasonable degree of estrus manifestations which resulted in decreasing number of services per conception. The increased number of pups at birth could be explained as the yeast might result in an increase in ovulation rate and a decrease in reabsorption of embryos following implantation. The decreased mortality number might be due to the improvement of the immune system caused by administration of the yeast which enable the youngsters to tolerate any environmental harsh conditions. In terms of higher body weight at birth due to gavaging of the yeast, this is might be due to the reasonable feed intake of the dams during pregnancy which led to the availability of enough nutrients necessary for growth around peri-natal period and provision of milk for sukkling.

CONCLUSION

Depending on the previous studies which demonstrated many beneficial effects of the T. mycotoxinivorans such as its ability to detoxify harmful compounds and stimulate immune system,

also it does not have any negative influence on biological parameters in mice.The yeast *T. mycotoxinivorans* could be successfully used as probiotic in numerous animal feeding trials. This research demonstrates further positive effects of the *T. mycotoxinivorans.*

ACKNOWLEDGMENTS

The authors extend their appreciation to the Deanship of Scientific Research at King Saud University for funding the work through the research group project No. RGP-VPP-073.

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