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# BIODIVERSITY OF YEAST MYCOFLORA IN NECTAR OF *BOMBAX CIEBA* AND *CANNA INDICA* FLOWER

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**Abstract**

Sixteen yeast species belonging to 10 genera from nectar of *Bombax cieba* flower and 25 species belonging to 15 genera from nectar of *Canna indica* flower were isolated and identified on the basis of their morphological and biochemical/physiological characteristics. *Pichia lynferdii* and *Zygoascus helinicus* were predominantly isolated from nectar of *Bombax cieba* whereas *Cryptococcus laurentii*, *Debaryomyces hansenii* and *Fibulobasidium inconspicuum* from nectar of *Canna indica* flower.

## Introduction

Nectar of flowers has long been thought as an ideal habitat for yeasts because of its high sugar contents and for many years yeasts were not believed to occur elsewhere. The single cell habit allows yeasts to attain a much wider ecological distribution than mycelial forms. Being devoid of photosynthetic power, yeasts depend strictly on the presence of organic carbon as an energy source. Species of *Saccharomyces* and *Pichia* that assimilate few carbon compounds are abundant in fruit juices and sugary plant exudates such as nectar of flower (Rose & Harrison, 1969, 1987). Lund (1954) isolated 27 species from flower nectar but only two of them viz., *Saccharomyces* and *Hansenula* produced ascospores and other 10 species of *Torulopsis* and 9 of *Candida* being most widespread. *Metschnikowia continentalis, M. hawaiiensis* (Lachance *et al*., 1998b, 1990), *M. drosophilae, M. lochheadii* (Lachance *et al*., 2001a), *M. kamienski* (Miller & Phaff, 1988) and *Candida ipomoeae* (Lachance *et al*., 1998a) occur on morning glories (*Ipomoea* spp.) and insects found on these flowers (Lachance *et al*., 2001b). There are about 100 genera and 700 species of yeasts (Kurtzman & Fell, 1999) of which only 5 genera and 7 species are reported from Pakistan (Mirza and Qureshi, 1978) from Pakistan. In the previous studies we recorded 13 genera and 20 species of yeasts from cultivated and garden soil (Mushtaq *et al*., 2004), 9 genera and 15 species from slime fluxes of trees (Mushtaq *et al*., 2005), 14 genera and 35 species from milk and 9 genera and 16 species from yogurt (Mushtaq *et al*., 2006). In the present study, an effort has been made to isolate and identify yeast mycoflora associated with nectar of *Bombax cieba* and *Canna indica* flower.

## Materials and Methods

Twenty samples of nectar from *Bombax cieba* and 9 from *Canna indica* flower were collected from University of Karachi, Pakistan. Yeasts associated with these samples

were isolated by modified serial dilution method (Harrigan & McCance, 1976). A known amount of sample was diluted up to 10,000 using double distilled sterilized water and inoculated either on malt yeast glucose peptone (YM), malt extract or yeast morphology agar medium and incubated for 5-7 days at 25 ± 1°C. Three isolates of yeasts per plate were selected, as representatives of the yeast mycoflora, from morphologically similar looking growing colonies, which were further purified and maintained on yeast-morphology agar buffered at pH 4.5. All isolated yeasts were primarily classified into 7 different groups *viz*., pink (group A), methanol assimilating (group B), cap-, hat-, saturn- or walnut- shaped ascospore producing (group C), round-, oval-, conical- or reniform shaped ascospore producing (group D), ballistoconidia forming (group E), basidiomycetous (group F) and glucose fermenting (group G). Identification of yeasts up to species level was carried on the basis of standard morphological and physiological/biochemical tests as proposed for each group (Kurtzman & Fell, 1999; Barnett *et al*., 1990).

Shapes and structures of vegetative yeast cells were examined microscopically from 2-3 days old cultures growing on YM (malt-yeast-glucose-peptone) agar, whereas, "Dalmau Plate Culture" method was used to test the ability of yeast to produce pseudo- or true-hyphae and ballisto- or arthro-conidia. Thin layers of sterile corn meal agar were poured in sterilized Petri plates and dried at room temperature for 2 days before streaking with up to 4 cultures per plate. A sterile cover slip was placed over a part of each streak. After 3-5 days of incubation filamentous growth was observed in the aerobic and anaerobic (covered) portions of the streak (Beech *et al*., 1972). To observe ballistoconida formation, malt extract agar (10 to 15 ml) was poured in Petri plates and dried at room temperature for 2 days. The medium was then inoculated with the yeast to be tested in lines along two diameters at right angles. This inoculated plate was inverted over another Petri plate having a sterile microscope glass slide on the surface of medium. The two plates were taped together all round the circumference and the preparation was incubated up to 3 weeks at 20°C. Discharged ballistoconidia that either formed colonies on medium in the lower Petri plate or collected on the slide were examined microscopically (Barnett *et al*. 1990).

Assimilation of carbon and nitrogenous compounds were simultaneously tested in liquid yeast nitrogen base and yeast carbon base medium supplemented with 50mM carbon/nitrogen source to be tested. Growth at different temperatures (25°C, 30°C, 35°C, 37°C and 40°C), in the presence of Cycloheximide (0.1% & 0.01%), and D-glucose (50% & 60%) were also tested in liquid yeast nitrogen base medium used for carbon assimilation. Ability of yeast to grow without added vitamin(s) was tested in liquid vitamin free yeast base. In all tests, media and reagents were prepared in double distilled sterilized water, and filter-sterilized through 0.45µ filter paper using Millipore glass filtration apparatus.

Production of extra-cellular starch-like compounds was observed after a positive growth in liquid medium of a sugar or an alditol. One drop of Lugol's iodine solution was shaken with yeast culture in the tube. A blue, purple or green color indicated that the test result is positive (Cowan & Steel, 1966). Diazonium Blue B (DBB) test was tested on 10-days old culture growing on malt-yeast-glucose-peptone agar. The culture was kept at 55°C for several hours and then flooded with ice-cold DBB reagent. If the culture turned dark red within 2 min at room temperature, the result was recorded as positive (Van der Walt & Hopsu-Havu, 1976).

## Result and Discussion

A total of 15 genera and 32 species were isolated from nectar of *Bombax cieba* and *canna indica* flower (Table 1). Teleomorphic ascomycetous yeasts were identified as *Debaryomyces castellii D. hansenii*, *D. vanrijii*, *Pichia castillae*, *P. dryadoides*, *P. lynferdii*, *P. mississippiensis*, *P. ohmeri*, *Stephanoascus ciferrii*, *Williopsis californica* and *Zygoascus helinicus* (Table 2). Among anamorphic ascomycetous yeasts only 6 species of *Candida* viz., *C. friedrichii*, *C. membranifaciens*, *C. rhagii*, *C. sake*, *C. valdiviana*, *C. versatilis* and a single species of black yeast, *Exophiala salmonis* were isolated and identified (Table 2). Teleomorphic basidiomycetous yeasts viz., *Fibulobasidium inconspicuum*, *Filobasidiella neoformans*, *Mrakia frigida*, *Rhodosporidium toruloides*, *Tremella aurantia*, and anamorphic basidiomycetous yeasts viz., *Bullera megalospora*, *B. pseudoalba*, *B. pyricola*, *Cryptococcus albidus*, *C. flavus*, *C. laurentii*, *Pseudozyma antarctica*, *P. fusiformata* and *Rhodotorula fragaria* were identified (Table 3). All yeast species appeared newly reported from nectar of flower in Pakistan.

**Table 1. Occurrence (%) of yeast mycoflora in nectar of *Bombax cieba* and *Canna indica* flower in terms of mean colony forming units/ml (cfu/ml) sample and range of standard error (SE)[1].**

| No. | Yeast species | *Bombax cieba* | | *Canna indica* | |
|---|---|---|---|---|---|
| | | % | Mean cfu ± SE[2] (Range)[3] | % | Mean cfu ± SE[2] (Range)[3] |
| 1. | *Bullera megalospora* | 5.0 | 0.42 ± 0.42[a] (8.3) | ---- | ---- ---- |
| 2. | *B. pseudoalba* | ---- | ---- ---- | 11.1 | 0.73 ± 0.73[b] (6.6) |
| 3. | *B. pyricola* | ---- | ---- ---- | 22.2 | 1.24 ± 0.82[a] (5.6) |
| 4. | *Candida friedrichii* | ---- | ---- ---- | 11.1 | 0.42 ± 0.42[c] (3.8) |
| 5. | *C. membranifaciens* | ---- | ---- ---- | 11.1 | 0.62 ± 0.62[a] (5.6) |
| 6. | *C. rhagii* | 15.0 | 0.82 ± 0.44[b] (5.2-5.6) | ---- | ---- ---- |
| 7. | *C. sake* | 5.0 | 0.25 ± 0.21[a] (4.2) | ---- | ---- ---- |
| 8. | *C. valdiviana* | 5.0 | 0.39 ± 0.39[a] (7.8) | 22.2 | 1.24 ± 0.82[a] (5.6) |
| 9. | *C. versatilis* | ---- | ---- ---- | 11.1 | 0.86 ± 0.86[b] (7.8) |
| 10. | *Cryptococcus albidus* | ---- | ---- ---- | 77.8 | 5.25 ± 1.09[c] (3.8-8.3) |
| 11. | *C. flavus* | ---- | ---- ---- | 11.1 | 0.62 ± 0.62[b] (5.6) |
| 12. | *C. laurentii* | ---- | ---- ---- | 77.8 | 5.2 ± 1.19[c] (3.8-9.7) |
| 13. | *Debaryomyces castellii* | ---- | ---- ---- | 11.1 | 0.31 ± 0.31[b] (2.8) |
| 14. | *D. hansenii* | 5.0 | 0.24 ± 0.24[a] (4.9) | 33.3 | 2.56 ± 1.32[d] (5.6-9.6) |
| 15. | *D. vanrijii* | ---- | ---- ---- | 11.1 | 0.30 ± 0.30[b] (2.7) |
| 16. | *Exophiala salmonis* | ---- | ---- ---- | 11.1 | 0.71 ± 0.71[b] (5.6-7.6) |
| 17. | *Fibulobasidium inconspicuum* | 5.0 | 0.16 ± 0.16[a] (3.1) | 44.4 | 3.24 ± 1.32[d] (5.8-9.6) |
| 18. | *Filobasidiella neoformans* | 5.0 | 0.28 ± 0.28[a] (5.7) | 11.1 | 0.80 ± 0.80[b] (7.2) |
| 19. | *Mrakia frigida* | ---- | ---- ---- | 22.2 | 1.64 ± 1.08[a] (5.1-9.7) |
| 20. | *Pichia castillae* | 5.0 | 0.20 ± 0.20[a] (3.9) | ---- | ---- ---- |
| 21. | *P. dryadoides* | 5.0 | 0.31 ± 0.31[a] (6.1) | ---- | ---- ---- |
| 22. | *P. lynferdii* | 25.0 | 4.78 ± 3.63[c] (3.9-73.0) | 11.1 | 0.74 ± 0.74[b] (6.7) |
| 23. | *P. mississippiensis* | 10.0 | 0.20 ± 0.14[a] (4.7) | ---- | ---- ---- |
| 24. | *P. ohmeri* | 5.0 | 0.23 ± 0.23[a] (4.7) | 11.1 | 0.74 ± 0.74[b] (6.7) |
| 25. | *Pseudozyma antarctica* | 5.0 | 0.26 ± 0.26[a] (5.3) | ---- | ---- ---- |
| 26. | *P. fusiformata* | ---- | ---- ---- | 11.1 | 0.86 ± 0.86[b] (7.8) |
| 27. | *Rhodosporidium toruloides* | ---- | ---- ---- | 11.1 | 0.64 ± 0.64[b] (5.8) |
| 28. | *Rhodotorula fragaria* | ---- | ---- ---- | 11.1 | 0.62 ± 0.62[b] (5.6) |
| 29. | *Stephanoascus ciferrii* | 5.0 | 0.26 ± 0.26[a] (5.3) | ---- | ---- ---- |
| 30. | *Tremella aurentia* | ---- | ---- ---- | 11.1 | 0.42 ± 0.42[b] (3.8) |
| 31. | *Williopsis californica* | 15.0 | 0.73 ± 0.45[b] (2.4-7.8) | 22.2 | 1.76 ± 1.16[a] (7.8-8.0) |
| 32. | *Zygoascus helinicus* | 25.0 | 2.04 ± 0.81[d] (7.8-8.8) | 11.1 | 0.78 ± 0.78[b] (7.1) |

[1]values of mean cfu/ml, SE and range are in 10,000.
[2]Mean value in each column having different letters are significantly different at $p<0.001$ (Bonferoni test).
[3]Single values in parentheses indicates that yeast species was isolated only from one sample.

**Table 2. Morphological and physiological / biochemical characters of Ascomycetous yeasts (letters in parentheses representing the group of a species as indicated in methods).**

| | Ascomycetous yeast species | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 | 17 | 18 |
| **Character(s) observed / tested** | *Candida friedrichii* (G) | *C. membranifaciens* (G) | *C. rhagii* (G) | *C. sake* (G) | *C. valdiviana* (G) | *C. versatilis* (G) | *Debaryomyces castellii* (D) | *D. hansenii* (D) | *D. vanrijii* (D) | *Exophiala salmonis* (G) | *Pichia castillae* (G) | *P. dryadoides* (C) | *P. lynferdii* (G) | *P. mississippiensis* (C) | *P. ohmeri* (C) | *Stephanoascus ciferrii* (C) | *Williopsis californica* (G) | *Zygoascus helinicus* (C) |
| **Morphological characters** | | | | | | | | | | | | | | | | | | |
| 1 Pseudomycelium | + | + | + | + | + | - | - | - | - | - | - | - | - | + | + | + | - | + |
| 2 Septate hyphae | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| 3 Ballistoconidia | + | + | + | + | + | - | - | - | - | - | - | - | - | - | - | - | - | - |
| 4 Symmetric conidia | + | + | + | + | + | - | - | - | - | - | - | - | - | - | - | - | - | - |
| 5 Ascospores round, oval, conical or reniform | - | - | - | - | - | - | + | + | + | - | - | - | - | - | - | - | - | - |
| 6 Ascospres cap-, hat-, Saturn- or walnut shaped | - | - | - | - | - | - | - | - | - | - | + | + | + | + | + | + | + | - |
| **Carbon assimilation tests** | | | | | | | | | | | | | | | | | | |
| 1 D-Glucose | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| 2 D-Galactose | + | + | + | + | + | + | + | + | v | + | + | - | + | - | + | n | -(+) | n |
| 3 L-Sorbose | + | v | v | + | +(-) | - | v | +(-) | + | v | + | -(+) | +(-) | -(+) | + | + | + | + |
| 4 D-Glucosamine | n | n | n | n | + | n | n | n | n | n | n | - | n | - | n | - | n | + |
| 5 D-Xylose | + | + | v | - | v | + | v | +(-) | + | + | n | - | v | + | v | n | v | n |
| 6 L-Arabinose | - | + | v | - | v | - | - | v | - | v | + | -(+) | -(+) | + | -(+) | + | v | + |
| 7 D-Arabinose | + | + | - | - | v | + | - | -(+) | + | - | - | w/- | -(+) | - | -(+) | v | - | w/- |
| 8 L-Rhamnose | - | - | - | - | - | - | v | v | + | - | + | - | - | - | v | + | - | + |
| 9 Sucrose | + | + | + | + | + | - | + | + | + | + | - | + | +(-) | + | + | + | + | + |
| 10 Maltose | + | + | + | + | + | v | + | + | + | + | + | - | + | n | + | n | + | n |
| 11 α,α-Trehalose | + | + | +(-) | + | + | + | n | + | n | + | + | n | + | n | + | n | + | n |
| 12 Methyl α-D-glucoside | - | - | + | - | + | - | n | + | n | v | - | - | +(-) | v | + | v | + | - |
| 13 Cellobiose | + | + | + | + | + | + | + | + | + | + | + | + | + | +(-) | + | + | + | + |
| 14 Arbutin | + | + | + | - | + | + | n | + | n | + | n | n | + | n | + | n | + | n |
| 15 Melibiose | + | + | - | n | + | n | + | v | + | n | + | n | - | n | - | n | - | n |
| 16 Lactose | - | - | - | - | -(+) | - | + | +(-) | + | - | - | n | -(+) | n | - | - | - | n |
| 17 Raffinose | + | + | + | - | + | + | + | + | + | + | + | + | + | -(+) | + | + | + | + |
| 18 Melezitose | - | v | + | + | + | - | n | + | n | + | - | - | +(-) | +(-) | v | + | - | + |
| 19 Inulin | - | + | + | - | v | + | n | v | n | + | n | n | + | n | v | n | + | n |
| 20 Starch | - | - | - | - | - | - | n | - | n | - | - | - | -(+) | - | v | v | - | +(-) |
| 21 Glycerol | n | n | n | n | n | n | + | + | + | n | n | n | n | n | + | n | n | n |
| 22 Erythritol | + | + | v | - | - | - | - | -(+) | v | v | - | + | + | -(+) | - | + | - | - |
| 23 Xylitol | - | + | v | - | +(-) | + | +(-) | +(-) | + | -(+) | + | - | v | + | - | + | v | v |
| 24 L-Arabinitol | n | n | n | n | n | n | v | v | v | n | n | n | n | n | v | + | n | n |
| 25 D-Glucitol | n | n | n | n | + | - | n | n | n | n | + | + | n | + | n | + | n | n |
| 26 D-Mannitol | + | + | + | +(-) | +(-) | + | + | v | + | + | n | + | v | n | +(-) | n | v | n |
| 27 Galactitol | + | n | n | n | + | n | n | n | n | n | n | n | n | n | n | + | n | n |
| 28 *myo*-inositol | - | n | + | - | + | - | n | - | - | n | n | n | - | n | n | + | n | + |
| 29 2-Keto-D-gluconate | + | + | + | n | n | n | n | + | n | n | n | - | n | v | + | + | n | - |
| 30 D-Gluconate | - | - | - | - | v | - | + | v | + | ' | + | + | -(+) | + | - | + | - | + |

**Table 2 (Cont'd.).**

| | Ascomycetous yeast species | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 | 17 | 18 |
| **Character(s) observed/tested** | *Candida friedrichii* (G) | *C. membranifaciens* (G) | *C. rhagii* (G) | *C. sake* (G) | *C. valdiviana* (G) | *C. versatilis* (G) | *Debaryomyces castellii* (D) | *D. hansenii* (D) | *D. vanrijii* (D) | *Exophiala salmonis* (G) | *Pichia castillae* (G) | *P. dryadoides* (C) | *P. lynferdii* (G) | *P. mississippiensis* (C) | *P. ohmeri* (C) | *Stephanoascus ciferrii* (C) | *Williopsis californica* (G) | *Zygoascus helinicus* (C) |
| 31. D-Glucuronate | n | n | - | n | n | n | n | n | n | n | n | n | n | n | n | + | n | n |
| 32. D-Galacturonate | - | - | - | - | -(+) | - | n | - | n | - | n | n | -(+) | n | v | - | - | n |
| 33. DL-Lactate | + | + | + | - | + | + | +(-) | + | n | + | n | + | + | n | v | n | + | n |
| 34. Succinate | n | n | n | n | n | n | n | n | n | n | n | n | n | n | n | + | n | n |
| 35. Citrate | + | + | - | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| 36. Methanol | - | - | - | - | - | - | - | - | - | - | - | - | -(+) | - | - | - | - | - |
| 37. Ethanol | n | n | n | + | + | n | v | n | + | n | n | n | + | n | n | n | + | n |
| 38. Propane 1,2-diol | n | n | n | n | n | n | v | -(+) | v | n | n | + | n | n | -(+) | n | n | n |
| 40. Butane 2,3 diol | n | n | n | n | n | n | n | - | - | n | n | n | + | - | n | n | n | n |
| **Nitrogen assimilation tests** | | | | | | | | | | | | | | | | | | |
| 1 Nitrate | - | - | - | - | + | + | n | - | - | v | - | + | + | -(+) | + | -(+) | + | v |
| 2 Ethylamine | n | n | n | n | n | n | n | n | n | n | + | + | + | n | n | n | n | n |
| 3 L-Lysine | n | + | + | + | + | + | n | + | + | + | n | n | + | n | + | n | + | n |
| 4 Cadaverine | + | + | + | + | +(-) | - | + | + | + | + | + | + | +(-) | + | + | + | + | + |
| 5 Glucosamine | n | - | - | - | +(-) | - | v | v | v | +(-) | n | n | -(+) | n | v | n | - | + |
| **Fermentation tests** | | | | | | | | | | | | | | | | | | |
| 1 D-Glucose | + | n | + | n | n | + | + | - | w/- | n | - | - | + | + | + | - | + | n |
| 2 D-Galactose | n | n | n | + | n | n | n | + | n | n | n | n | n | n | | n | n | n |
| 3 Methyl α- D-glucoside | + | - | + | - | -(+) | + | v | -(+) | n | + | n | n | v | n | w/- | n | v | n |
| 4 Sucrose | + | - | + | - | -(+) | + | + | v | - | + | n | - | v | - | + | n | -(+) | n |
| 5 Inulin | w/- | w/- | + | + | w/- | w/- | +(-) | -(+) | w/- | w/- | n | n | v | n | w/- | n | -(+) | n |
| **Growth without added vitamin(s)** | | | | | | | | | | | | | | | | | | |
| 1. Without vitamins | -(+) | - | + | v | n | n | + | +(-) | + | n | n | + | n | n | + | n | - | n |
| 2. Without *myo*-Inositol | + | + | + | + | + | + | + | + | + | + | n | n | + | + | + | n | + | n |
| 3. Without Biotin | + | -(+) | + | + | + | + | n | + | n | + | n | n | + | n | + | n | + | n |
| 4. Without Thiamin | + | + | + | + | + | + | n | + | + | +(-) | + | + | + | + | + | + | + | + |
| 5. Without Pyridoxine | + | + | + | + | + | + | n | + | n | + | + | + | + | v | + | + | + | + |
| 6. Without Niacin | n | + | + | + | + | + | + | + | + | + | n | n | + | n | + | n | + | n |
| **Additional tests** | | | | | | | | | | | | | | | | | | |
| 1. With 0.01% (w/v) cycloheximide | - | - | - | + | n | v | n | - | n | n | - | n | n | n | n | n | n | n |
| 2. With 0.10% (w/v) cycloheximide | - | - | - | - | + | - | n | - | + | n | n | n | n | n | - | n | n | N |
| 3. With 1% Acetic Acid growth | - | - | - | - | - | - | v | -(+) | v | - | n | n | - | n | - | n | - | N |
| 4. With 50% (w/v) D-glucose | + | +(-) | - | + | + | + | n | v | + | + | - | - | + | - | + | v | + | - |
| 5. With 60% (w/v) D-glucose | - | - | - | - | - | +(-) | v | v | v | - | - | n | +(-) | n | - | + | - | n |
| 6. Diazonium Blue B reaction | n | n | n | n | n | n | n | - | - | n | n | n | n | n | n | n | n | n |

Responses: + = positive; +(-) = mostly positive with some negative; -(+) = mostly negative with some positive; w/- =weak or negtive, w/+ =weak or positive, - = negtive, n=not determined

**Table 3. Morphological and Physiological / biochemical characters of Basidiomycetous yeast species isolated from nectar of flower (Letters in brackets representing the group of a species as indicated in methods).**

| | Basidiomycetous yeast species | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | **1** | **2** | **3** | **4** | **5** | **6** | **7** | **8** | **9** | **10** | **11** | **12** | **13** | **14** |
| **Character(s) observed** | ***Bullera megalospora* (E)** | ***B. pseudoalba* (E)** | ***B. pyricola* (E)** | ***Cryptococcus albidus* (F)** | ***C. flavus* (B,F)** | ***C. laurentii* (F)** | ***Fibulobasidium inconspicuum* (F, G)** | ***Filobasidiella neoformans* (F)** | ***Mrakia frigida* (B,F)** | ***Pseudozyma antarctica* (G)** | ***P. fusiformata* (B,F)** | ***Rhodosporidium toruloides* (A)** | ***Rhodotorula fragaria* (F)** | ***Tremella aurantia* (F)** |
| **Morphological characters** | | | | | | | | | | | | | | |
| 1. Pseudomycelium | - | + | + | - | - | - | - | v | + | - | - | + | + | - |
| 2. Septate hyphae | + | + | - | - | - | - | - | - | - | + | + | - | - | - |
| 3. Ballistoconidia | + | + | + | - | - | - | - | - | + | - | - | - | - | - |
| 4. Symmetric conidia | + | + | + | - | - | - | - | - | + | - | - | - | - | - |
| **Carbon assimilation tests** | | | | | | | | | | | | | | |
| 1. D-Glucose | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| 2. D-Galactose | + | + | + | + | + | + | + | + | + | + | - | + | + | + |
| 3. L-Sorbose | n | - | - | + | - | +(-) | +(-) | v | + | + | n | + | + | + |
| 4. D-Glucosamine | - | + | - | v | n | - | n | n | + | n | n | + | + | + |
| 5. D-Xylose | + | +(-) | + | v | + | + | -(+) | v | v | v | + | - | + | + |
| 6. L-Arabinose | n | n | + | + | + | +(-) | +(-) | + | v | + | + | + | + | + |
| 7. D-Arabinose | - | n | + | +(-) | v | +(-) | +(-) | v | + | + | n | + | v | + |
| 8. L-Rhamnose | - | + | w/- | v | + | + | + | v | v | + | - | +(-) | - | + |
| 9. Sucrose | + | n | + | + | + | + | + | + | + | + | n | + | + | + |
| 10. Maltose | n | + | + | + | + | + | + | + | - | + | n | + | + | + |
| 11. α,α--Trehalose | n | n | n | n | n | n | n | n | + | n | n | n | n | n |
| 12. Methyl α-D-glucoside | - | + | - | n | n | + | n | n | - | + | n | n | n | n |
| 13. Cellobiose | + | | + | n | n | n | n | n | + | + | n | n | + | n |
| 14. Arbutin | n | n | n | n | + | n | n | n | + | n | + | n | n | n |
| 15. Melibiose | - | + | + | v | + | + | -(+) | -(+) | w/- | + | v | + | - | - |
| 16. Lactose | -(+) | + | + | v | + | + | - | - | v | v | -(+) | v | + | + |
| 17. Raffinose | + | + | + | + | + | + | + | + | + | + | + | v | + | + |
| 18. Melezitose | + | + | +(-) | + | + | + | + | + | +/- | + | + | + | - | + |
| 19. Inulin | - | - | - | n | n | n | n | n | v | n | n | n | n | n |
| 20. Starch | + | n | +(-) | v | + | -(+) | + | + | + | -(+) | + | + | - | + |
| 21. Glycerol | n | n | n | n | n | v | n | n | n | n | n | n | n | n |
| 22. Erythritol | - | - | - | v | + | n | - | v | - | + | + | - | - | - |
| 23. L-Arabinitol | n | + | n | n | n | n | n | n | n | n | n | n | n | + |
| 24. D-Glucitol | n | n | n | + | + | + | n | n | n | n | n | n | n | n |
| 25. D-Mannitol | + | n | + | + | + | + | + | + | + | + | + | + | + | n |
| 26. Galactitol | -(+) | + | +(-) | v | + | + | +(-) | v | + | + | + | -(+) | + | - |
| 27. *myo*-inositol | - | + | + | + | + | + | n | + | n | + | + | n | - | n |

**Table 3. (Cont'd.).**

| | Basidiomycetous yeast species | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | **1** | **2** | **3** | **4** | **5** | **6** | **7** | **8** | **9** | **10** | **11** | **12** | **13** | **14** |
| **Character(s) observed** | ***Bullera megalospora* (E)** | ***B. pseudoalba* (E)** | ***B. pyricola* (E)** | ***Cryptococcus albidus* (F)** | ***C. flavus* (B,F)** | ***C. laurentii* (F)** | ***Fibulobasidium inconspicuum* (F, G)** | ***Filobasidiella neoformans* (F)** | ***Mrakia frigida* (B,F)** | ***Pseudozyma antarctica* (G)** | ***P. fusiformata* (B,F)** | ***Rhodosporidium toruloides* (A)** | ***Rhodotorula fragaria* (F)** | ***Tremella aurantia* (F)** |
| 28. 2-Keto-D-gluconate | + | n | n | n | n | n | n | n | n | + | n | - | n | n |
| 29. 5-Keto-D-Gluconate | n | n | n | n | n | n | n | n | n | n | n | - | n | + |
| 30. D-Gluconate | n | n | n | + | + | + | n | n | n | n | n | n | n | n |
| 31. D-Glucuronate | - | v | + | + | + | + | +(-) | + | + | + | + | -(+) | + | + |
| 32. D-Galacturonate | - | + | n | n | n | n | + | + | - | n | - | - | - | - |
| 33. DL-Lactate | - | + | - | n | n | n | n | n | n | + | n | n | n | n |
| 34. Citrate | + | + | + | -(+) | + | +(-) | - | v | - | v | + | - | + | - |
| 35. Methanol | - | - | - | - | v | - | - | - | v | - | v | - | - | - |
| 36. Ethanol | + | +(-) | + | +(-) | v | + | + | + | + | + | + | +(-) | + | + |
| 37. Propane 1,2-diol | n | n | n | n | n | n | n | n | n | n | n | n | n | n |
| 38. Butane 2,3 diol | n | n | n | n | n | - | n | n | n | n | n | n | n | n |
| **Nitrogen assimilation tests** | | | | | | | | | | | | | | |
| 1. Nitrate | + | - | + | v | - | - | - | - | v | + | + | +(-) | + | - |
| 2. Ethylamine | n | n | - | -(+) | - | + | v | v | v | + | + | +(-) | + | + |
| 3. L-Lysine | - | n | n | + | + | + | n | n | + | n | n | n | n | n |
| 4. Cadaverine | -(+) | + | + | -(+) | - | +(-) | + | v | - | + | + | + | + | + |
| 5. Glucosamine | | + | - | -(+) | -(+) | n | v | v | n | - | +(-) | v | + | + |
| **Fermentation tests** | | | | | | | | | | | | | | |
| 1. D-Glucose | n | n | n | n | n | n | n | n | + | n | n | - | n | n |
| 2. D-Galactose | n | n | n | n | n | n | n | n | n | n | n | n | n | n |
| 3. Methyl α-D-glucoside | n | n | n | n | n | n | n | n | v | n | n | n | n | n |
| 4. Sucrose | n | n | n | n | n | n | n | n | + | - | n | n | n | n |
| 5. Inulin | n | n | n | n | n | n | n | n | w/- | n | n | n | n | n |
| **Growth without added vitamin(s)** | | | | | | | | | | | | | | |
| 1. Without vitamins | n | n | n | - | n | n | n | n | n | - | + | + | + | n |
| 2. Without Biotin | - | n | - | n | n | n | n | n | n | n | n | n | n | n |
| 3. Without Thiamin | n | n | + | -(+) | n | + | + | + | + | - | n | + | + | + |
| 4. Without Biotin & Thiamin | n | + | n | n | n | n | n | n | n | n | n | n | n | n |
| 5. Without Pyridoxine | n | n | n | n | n | n | n | n | n | n | n | + | n | n |
| 6. Without Niacin | n | + | n | n | n | n | n | n | n | n | + | n | n | n |
| 7. Without *P*-aminobenzoate | n | + | + | + | + | + | + | + | + | + | + | + | + | + |
| **Additional tests** | | | | | | | | | | | | | | |
| 1. With 0.01% (w/v) cycloheximide | n | n | n | n | n | n | n | v | n | n | n | n | n | n |
| 2. With 0.10% (w/v) cycloheximide | n | n | n | n | n | n | n | n | n | - | n | - | n | n |
| 3. Starch formation | n | - | - | +(-) | - | + | - | - | - | - | - | - | - | - |
| 4. Diazonium Blue B reaction | + | n | - | - | - | +(-) | v | + | + | - | - | + | - | - |

Responses: + = positive; +(-) = mostly positive with some negative; -(+) = mostly negative with some positive; w/- =weak or negative, w/+ =weak or positive, - = negative, n=not determined

**Table 4. Anova of yeast species isolated from nectar of different plants.**

| Source | Sum of Squares | df | Mean Square | F | Probability |
|---|---|---|---|---|---|
| **Main effects** | | | | | |
| | | | **A. *Bombax cieba*** | | |
| Yeasts (A) | 813.279 | 15 | 54.219 | 532.534 | p<0.0001 |
| Sample (B) | 845.370 | 19 | 44.493 | 437.011 | p<0.000 |
| A*B | 10601.071 | 285 | 37.197 | 365.349 | p<0.000 |
| Error | 32.580 | 320 | 0.102 | | |
| Total | 12614.004 | 640 | | | |
| | | | **B. *Canna indica*** | | |
| Yeasts (A) | 767.734 | 23 | 33.38 | 223.497 | p<0.0001 |
| Sample (B) | 448.338 | 8 | 56.042 | 357.237 | p<0.0001 |
| A*B | 1901.035 | 184 | 10.332 | 69.177 | p<0.0001 |
| Error | 32.26 | 216 | 0.149 | | |
| **Total** | **3854.445** | **432** | | | |

*Candida valdiviana*, *Debaryomyces hansenii*, *Fibulobasidium inconspicuum*, *Filobasidiella neoformans*, *Pichia lynferdii*, *P. ohmeri*, *Williopsis californica* and *Zygoascus helinicus* were commonly isolated from nectar of both *Bombax cieba* and *Canna indica* flower. *Pichia lynferdii* and *Zygoascus helinicus* showed maximum occurrence in samples of nectar from *Bombax cieba*, whereas, *Cryptococcus laurentii*, *Debaryomyces hansenii* and *Fibulobasidium inconspicuum* showed maximum occurrence in nectar of *Canna indica* flower (Tale 1). Univariate ANOVA of yeast species revealed that their occurrence was significantly different at $p<0.001$ in nectars' samples of both plants (Table 4). Bonferroni test also confirmed significant differences among yeast species (Table 1). The total colony forming units (cfu $g^{-1}$) of yeasts ranged from $0.20x10^4$ $g^{-1}$ (*Pichia mississippiensis*) to $4.7x10^4$ $g^{-1}$ (*Pichia lynferdii*) in nectar of *Bombax cieba* flower and $0.3x10^4$ $g^{-1}$ (*Debaryomyces castellii*) to $5.25x10^4$ $g^{-1}$ (*Cryptococcus albidus*) in nectar of *Canna indica* flower (Table 1). It may be noted that distribution of 13 yeast species in *Bombax cieba* and 19 yeast species in *Canna indica* was restricted and isolated only from 1 or 2 samples of nectar flower (Table 1), but showed extensive physiological activities (Table 2 & 3).

In the previous studies we isolated *Bullera pseudoalba*, *B. pyricola*, *Candida valdiviana*, *Cryptococcus albidus*, *Debaryomyces castellii*, *D. hasnsenii*, *Fibulobasidium inconspicuum*, *Filobasidiella neoformans*, *Mrakia frigida*, *Pichia lynferdii* and *Rhodosporidium toruloides* from slime fluxes of *Acacia nilotica*, *Albizzia lebbeck* and *Aralia cachemirica* (Mushtaq *et al*., 2005) and from soil (Mushtaq *et al*., 2004). It is established that transmission of yeast species from nectar of a flower to another or to other substrates such as slime fluxes of trees and soil is carried out by insects including drosophilids, beetles and bees, found on these flowers (Lachance *et al*., 2001b). Hong *et al*., (2003) isolated a novel asexual ascomycetous yeast, *Candida kunwiensis*, which is phylogenetically related to the genus *Metschnikowia* from sweet potato (*Ipomoea batatas*) flowers in Korea and from the body surface of pollinating bumblebees in Germany indicating role of insects in the transmission of yeasts (Hong *et al*., 2001).

Identification of yeasts up to species level was carried out on the basis of their morphological and physiological / biochemical characteristics (Table 2 & 3). None of the anamorphic or teleomorphic ascomycetous yeast produced true septate hyphae (Table 2), whereas, basidiomyceotus *Bullera psedoalba*, *B. pyricola*, *Pseudozyma antarctica* and *P. fusiformata* produced true septate hyphae (Table 3). Among ascomycetes all *Candida* species except *Candidia versatilis* and among basidiomycetes, species of *Bullera* and *Mrakia frigida* produced symmetric conidia, ballistoconidia and pseudomycelium (Table 2 & 3).

All yeast species assimilated D-glucose and positively grow at temperatures viz., 25°C, 30°C, 35°C, 37°C and 40°C. *Cryptococcus flavus*, *Mrakia frigida*, *Pichia lynferdii* and *Pseudozyma fusiformata* assimilated methanol. During assimilation, when carbon source is switched from glucose to methanol (or alkanes) the cells of methylotrophic yeasts become packed with microbodies (peroxysomes) that contain enzyme, alcohol oxidase. This characteristics of methylotrophic yeast strains provides an ideal expression system for the recombinant production of heterologous proteins with sufficient purity and quantity for their functional characterization and/or use in downstream applications. Nowadays, more than 500 proteins have been cloned and expressed using this system (Macauley-Patrick *et al*., 2005), such as production of harmones – somatostatin, tumor necrosis factor and bacterial toxins and their derivatives, with special emphasis on development of recombinant vaccines against the bacterial toxins that are the causative agents of human diseases such as tetanus, botulism and cholera (Byrne & Smith; 2000, Smith *et al*., 2004; Harakuni *et al*., 2005; FitzGerald *et al*., 2004).

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