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YEAST MYCOFLORA ASSOCIATED WITH SLIME FLUXES OF TREES

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Abstract

A total of 15 yeast species belonging to 9 genera were isolated from 40 slime flux samples collected from *Acacia nilotica*, *Albizzia lebbeck* and *Aralia cachemirica* trees and identified on the basis of their morphological and physiological/biochemical characters. The isolated yeast species belonged to teleomorphic and anamorphic ascomycetous and basidiomycetous fungi and appeared to be new records from Pakistan. *Fibulobasidium inconspicuum* and *Pichia anomala* were predominant and commonly isolated from slime fluxes of all the three trees.

Introduction

Fluxing, the flowing of tree sap from a wound is generally caused by injuries due to boring insects, frost cracks or the breaking of twigs. The flowing sap becomes heavily infected with bacteria, yeasts and protozoa and rarely by filamentous fungi. The tree sap usually assumes a thick and slimy consistency, probably due to microbial capsular polysaccharides formation and for this reason it is usually referred to as slime flux. Tree fluxes are rich and often highly specific habitat for yeasts. Phaff & Knapp (1956) isolated *Endomycopsis javanensis* (Syn. *Arthroascus javanensis*) from slime flux of *Quercus kellogii* in Sierra Nevada, California. A number of *Pichia* species such as *P. fluxuum*, *P. trehalophila*, *P. salictaria*, *P. angophorae*, *P. scutulata*, *P. naganishii*, *P. veronae* and *P. nakazawae* have been reported from slime fluxes of *Abies* sp., *Acer* sp., *Aesculus* sp., *Angophora* sp., *Camellia* sp., *Myoporum* sp., *Populus* sp., *Quercus* spp., and *Salix* sp., from Canada, Japan and USA (Phaff & Knapp, 1956; Phaff *et al*., 1964; Miller & Baker, 1968; Kodama & Kyono, 1974; Kodama, 1975; Phaff *et al*., 1976).

Lodder (1970) for the first time reported several species of *Nadsonia* as well as *Saccharomycodes ludwigii* in slime fluxes of birch and oak in Russia and other countries. On the other hand *Saccharomyces kluyveri* (Phaff *et al*., 1972) and *Sporopachydermia quercuum* (Lachance, 1982) have been reported from fluxes of *Acer*, *Alnus*, *Quercus*, *Ulmus* and several other trees in Pacific north-west of North America and Ontario, Canada. There are about 100 genera and 700 species of yeasts (Kurtzman & Fell, 1999) of which only 5 genera and 7 species have been recorded from Pakistan (Mirza & Qureshi, 1978). In the previous study we isolated and identified 20 yeast species belonged to 14 genera from different soils of Karachi, Pakistan (Mushtaq *et al*., 2004). The aim of this study was the determination and taxonomic characterization of yeast mycoflora associated with slime fluxes of *Acacia nilotica*, *Albizzia lebbeck* and *Aralia cachemirica* in Karachi region of Pakistan.

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Materials and Methods

Fourteen samples each of *Acacia nilotica* and *Aralia cachemirica*, and 12 slime flux samples of *Albizzia lebbeck* were collected from University of Karachi, Karachi, Pakistan. Yeasts associated with these samples were isolated by suspending a known amount of slime flux/gum exudate in sterile distilled water and shaken well to dislodge the adhering cells from residues. Serial dilution was made up to 10,000 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 I), methanol assimilating (group II), cap-, hat-, saturn- or walnut- shaped ascospore producing (group III), round-, oval-, conical- or reniform shaped ascospore producing (group IV), ballistoconidia forming (group V), basidiomycetous (group VI) and glucose fermenting (group VII). Identification of yeasts up to species level was carried on the basis of standard morphological and physiological/biochemical tests proposed for each group (Kurtzman & Fell, 1999; Barnett *et al*., 1990). The data was statistically analyzed using computer-based software, SPSS version 10.

Shapes and structures of vegetative yeast cells and ascospores formation were respectively examined microscopically on YM and acetate agar from 2-3 days old growing cultures. 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). Assimilation of carbon and nitrogenous compounds were simultaneously tested in liquid yeast nitrogen base and yeast carbon base supplemented with 50mM carbon/nitrogen source to be tested. Growth at different temperatures, in the presence of Cycloheximide (0.1 & 0.01%) and at different concentrations of D-glucose (50 & 60%) was also tested in liquid yeast nitrogen base for carbon assimilation. Ability of yeast to grow without added vitamin(s) was tested in liquid vitamin free yeast base. The ability of yeast to ferment sugars was assessed in 150X16 mm test tubes with inserted 50X10 mm Durham tubes. Efficiency of gas collection in the inserted Durham tube was improved by fusing a small globule of glass to the inner tube's rim to keep it away from touching the bottom of the outer tube. The tubes were filled with 10 to 15 ml of yeast extract medium [0.5% (w/v) of a commercially produced dried yeast extract with 50 mM test sugars (sugars were separately filter-sterilized)]. Control test tubes were prepared without sugar (Barnett *et al*., 1990). 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 by shaking one drop of Lugol's iodine solution with yeast culture in the tube. A blue, purple or green color indicated that the test result is positive (Cowan & Stell, 1966). Diazonium Blue B (DBB) test was tested on 10-days old culture growing on YM 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).

Results and Discussion

Studies of slime fluxes of three different trees yielded a total of 15 yeast species belonging to 9 genera, all of which appeared to be new reports from Pakistan. Fourteen slime flux samples from *Acacia nilotica* yielded 4 genera and 7 yeast species, whereas from 12 slime flux samples of *Albizzia lebbeck* 6 genera and 8 species and from 14 samples of *Aralia cachemirica* 6 genera and 6 yeast species were isolated. Data is presented in terms of mean value of colony forming units per gram of sample (cfu g^{-1}) with standard error and range (Table 1). The isolated yeast species were identified on the basis of their morphological and physiological/biochemical characters (Table 2).

Teleomorphic ascomycetous yeast species were found predominant. These included *Debaryomyces castellii* Capriotti, *D. hansenii* (Zopf) Lodder & Kreger-van Rij, *D. yamadae* (van der Walt & Johannsen) van der Walt, Smith & Yamada, *Pichia angusta* (Teunisson, Hall & Wickerham) Kurtzman, *P. anomala* (Hansen) Kurtzman, *P. lynferdii* (van der Walt & Johannsen) Kurtzman, *P. methanolica* Makiguchi and *P. rabaulensis* Soneda & Uchida. Among anamorphic ascomycetous yeast species only *Candida valdiviana* Grinbergs & Yarrow was identified. As compared to ascomycetous yeasts, teleomorphic basidiomycetous species were less encountered and identified as *Fibulobasidium inconspicuum* Bandoni, *Mrakia frigida* (Fell, Statzell, Hunter & Phaff) Yamada & Komagata, and *Sporidiobolus ruineniae* Holzschu, Tredick & Phaff. *Cryptococcus albidus* (Saito) Skinner, *Phaffia rhodozyma* Miller, Yoneyama & Soneda and *Rhodotorula bacarum* (Buhagiar) Rodrigues de Miranda & Weijman were identified as anamorphic basidiomycetous species.

It may be mentioned here that *Fibulobasidium inconspicuum* and *Pichia anomala* were commonly isolated from slime fluxes of all three trees. A Univariate ANOVA revealed that occurrence of yeast species was significantly different at $p<0.001$. Bonferroni test also confirmed the significant differences in occurrence of yeast species (Table 1).

The total colony forming units (cfu g^{-1}) of yeast ranged from $0.9x10^4$ g^{-1} (e.g. *Rhodotorula bacarum*) to 8.4×10^4 g^{-1} (e.g. *Fibulobasidium inconspicuum*). It may be noted that distribution of most of the yeast species was very restricted i.e. isolated only from 1 or 2 samples of slime fluxes (Table 1) but showed extensive metabolic activities (Table 2). The physiological generalization of these yeasts has resulted in ecological specialization, which has been earlier pointed out by McNaughton & Wolf (1979) and Phaff & Starmer (1987). Some of the yeast species isolated from slime fluxes such as, *Candida valdiviana*, *Cryptococcus albidus*, *Debaryomyces castellii*, *D. hansenii*, *D. yamadae*, *Fibulobasidium inconspicuum*, *Phaffia rhodozyma*, *Pichia lynferdii*, *Sporidiobolus ruineniae* have also been found associated with soil from the same area (Mushtaq *et al*., 2004). However, most of the yeast species are known to be widely distributed in tree fluxes especially in European Russia, Japan and the west coast of North America as well as in South Africa, Australia, Indonesia and a few have been reported from South America (Spencer & Spencer, 1997). Several species of *Pichia* and their imperfect forms have been isolated from slime fluxes of deciduous trees and from conifers, which may be transmitted by *Drosophila* species. Basidiomycetous yeasts, such as, *Cryptococcus* and *Rhodotorula* and their teleomorphs, are transmitted by insects from leaf surfaces (phylloplane). Many plants harbor a large population of nitrogen-fixing bacteria on leaf surface, which produce nitrogenous compounds available to these yeasts (Spencer & Spencer, 1997).

Table 1. Occurrence (%) of yeast mycoflora in slime fluxes of different trees*.

No.	Yeast species	*Acacia nilotica*		*Albizzia lebbeck*		*Aralia cachemirica*	
		%	M. cfu±SE** (Range)	%	M. cfu±SE** (Range)	%	M. cfu±SE** (Range)
1.	*Candida valdiviana*	—	—	33.3	1.82±1.15[a] (5.4-5.5)	28.6	2.20±1.42[a] (7.2-8.2)
2.	*Cryptococcus albidus*	—	—	—	—	14.3	0.50±0.50[b] (3.5)
3.	*Debaryomyces castellii*	—	—	16.7	1.23±0.23[b] (7.4)	—	—
4.	*D. hansenii*	—	—	—	—	14.3	0.76±0.76[c] (5.3)
5.	*D. yamadae*	—	—	16.7	1.06±1.06[c] (6.4)	—	—
6.	*Fibulobasidium inconspicuum*	14.3	3.36±2.06[a] (8.4)	16.7	1.23±1.23[b] (7.4)	14.3	0.14±0.14[d] (1.0)
7.	*Mrakia frigida*	14.3	2.96±1.81[b] (7.4)	—	—	—	—
8.	*Phaffia rhodozyma*	—	—	16.7	1.05±1.05[c] (6.3)	—	—
9.	*Pichia angusta*	14.3	3.00±1.84[b] (7.5)	—	—	—	—
10.	*P. anomala*	28.6	2.72±0.69[c] (3.2-3.6)	16.7	1.38±1.38[d] (8.3)	14.3	3.14±3.14[e] (22.0)
11.	*P. lynferdii*	14.3	1.30±0.81[d] (3.3)	—	—	—	—
12.	*P. methanolica*	14.3	0.98±0.60[e] (2.3)	—	—	—	—
13.	*P. rabaulensis*	—	—	16.3	1.38±1.38[d] (8.3)	—	—
14.	*Rhodotorula bacarum*	—	—	—	—	14.3	0.12±0.12[d] (0.9)
15.	*Sporidiobolus ruineniae*	14.3	2.98±1.83[b] (7.3)	16.3	1.05±1.05[c] (6.3)	—	—

*Values of mean colony forming units (M. cfu), Standard error (SE) and range are in 10,000, **Mean values in each column having different letters are significantly different at $p<0.001$ (Bonferoni test).

Table 2. Morphological and physiological/biochemical characters of yeast species isolated from slime fluxes.

No.	Characters	1 *Candida valdiviana*	2 *Cryptococcus albidus*	3 *Debaryomyces castellii*	4 *D. hansenii*	5 *D. yamadae*	6 *Fibulobasidium inconspicuum*	7 *Mrakia frigida*	8 *Phaffia rhodozyma*	9 *Pichia angusta*	10 *P. anomala*	11 *P. lynferdii*	12 *P. methanolica*	13 *P. rabaulensis*	14 *Rhodotorula bacarum*	15 *Sporidiobolus ruineniae*
Group		V	VII	IV	IV	IV	VI	VI	I	II	III	III	II	III	VII	I
Morphological characters																
1	Colony color	wh.cr.	cr.	or.-pi.	wh.cr.	wh.cr.	wh.cr.	wh.cr.	Pi.-red	wh.cr.	wh.cr.	wh.cr.	wh.cr.	wh.cr.	wh.cr.	pi.
2	Shape of cell	gl-ov	gl-ov	r-ov	r-ov	r-ov	sgl-gl.	ov-elo	ov-elo	sph-ov	sph-ov	sph-ov	sph-ov	sph-ov	ov-elo	ov-cy
3	Splitting cells	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
4	Pseudomycelium	+	-	-	-	-	-	+	-	-	-	-	-	+	-	-
5	Septate hyphae	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+
6	Arthroconidia	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
7	Ballistoconidia	+	-	-	-	-	-	+	-	-	-	-	-	-	-	-
8	Symmetric conidia	+	-	-	-	-	-	+	-	-	-	-	-	-	-	-
9	Ascospores round, oval, conical or reniform	-	-	+	+	+	-	-	-	-	-	-	-	-	-	-
10	Ascospres cap-, hat-, Saturn- or walnut shaped	-	-	-	-	-	-	-	-	+	+	+	+	+	-	-

Table 2 (Cont'd.)

Assimilation of carbon compounds																
1	D-Glucose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
2	D-Galactose	+	+	+	+	+	+	+	-(+)	+	v	+	n	+	v	+
3	L-Sorbose	+(-)	+	**v**	+(-)	+	+(-)	+	+	v	+(-)	+(-)	+	+	+	+
4	D-Glucosamine	+	**v**	n	n	+	n	+	-	-		n	-	n	-	n
5	D-Xylose	**v**	**v**	**v**	+(-)	+	-(+)	v	+	+	V	v	+	+	-	+
6	L-Arabinose	**v**	+	-	**v**	n	+(-)	**v**	+	v	-(+)	-(+)	n	w/-	-	n
7	D-Arabinose	**v**	+(-)	-	-(+)	n	+(-)	+	**v**	v	-(+)	-(+)	n	w/-	-	n
8	L-Rhamnose	-	**v**	**v**	**v**	+	+	**v**	+(-)	v	-	-	-	v	-	n
9	Sucrose	+	+	+	+	+	+	+	+	+	+	+(-)	+	+	+	+
10	Maltose	+	+	+	+	+	+	-	+	+	+	+	v	n	+	+
11	α,α-Trehalose	+	n	n	+	n	n	+	+	+	+	+	+	n	+	n
12	Methyl α-D-glucoside	+	n	n	+	n	n	-	+	n	+(-)	+(-)	v	-	n	n
13	Cellobiose	+	n	+	+	+	n	+	+	+	+(-)	+	+	+	+	n
14	Arbutin	+	n	n	+	n	n	+	v	n	+(-)	+	n	n	-	+
15	Melibiose	+	**v**	+	**v**	-	-(+)	w/-	**v**	n	-	-	n	-	-(+)	+(-)
16	Lactose	-(+)	**v**	+	+(-)	+	-	v	-(+)	n	-	-(+)	n	n	-	+(-)
17	Raffinose	+	+	+	+	**v**	+	+	+	-	+	+	n	+	+	+
18	Melezitose	+	+	n	+	+	+	+/-	+	n	+(-)	+(-)	n	+	+	-(+)
19	Inulin	**v**	n	n	v	n	n	**v**	-	n	-	+	n	n	n	n

Table 2 (Cont'd.)

0	Starch	-	v	n	-	-	+	+	+	n	+(-)	-(+)	+	-	+	n
1	Glycerol	n	n	+	+	+	n	n	n	n	n	n	n	n	n	+
2	Erythritol	-	v	-	-(+)	-	-	-	-	+	+	+	+	-	+	-
3	Xylitol	+(-)	n	+(-)	+(-)	-(+)	n	n	-	n	-(+)	v	n	+	n	n
4	L-Arabinitol	n	n	v	v	+	n	n	n	n	n	n	n	n	n	+
5	D-Glucitol	+	+	n	n	n	n	n	n	n	n	n	n	+	n	n
6	D-Mannitol	+(-)	+	+	v	+	+	+	+	+	+(-)	v	+	n	+	n
7	Galactitol	+	v	n	n	+	+(-)	+	-(+)	+	n	n	+	n	-	+
8	*myo*-Inositol	+	+	n	-	v	n	n	n	-	n	-	n	n	-	-
9	2-Keto-D-gluconate	n	n	n	+	n	n	n	+	n	-	n	-	-	-	-
0	5-Keto-D-Gluconate	n	n	n	n	n	n	n	n	n	n	n	n	+	n	n
1	D-Gluconate	v	+	+	v	-	n	n	-	+	v	-(+)	+	n	-	n
2	D-Glucuronate	n	+	n	n	n	+(-)	+	-	n	n	n	-(+)	n	-	-
3	D-Galacturonate	-(+)	n	n	-	-	+	-	-(+)	n	-(+)	-(+)	n	n	-	n
4	DL-Lactate	+	n	+(-)	+	n	n	n	+	n	+	+	n	n	n	n
5	Citrate	+	-(+)	+	+	+	-	-	-(+)	+	+	+	v	+	-	+
6	Methanol	-	-	-	-	-	-	v	-	+	-	-(+)	+	-	-	-
7	Ethanol	+	+(-)	v	n	+	+	+	+	n	n	+	n	n	+	+
8	Propane 1,2-diol	n	n	v	-(+)	v	n	n	n	n	n	n	n	n	n	n
9	Butane 2,3 diol	n	n	n	-	n	n	n	n	n	n	+	n	n	n	n

Table 2 (Cont'd.)

siml. of N comp.															
1	Nitrate	+	v	n	-	n	-	v	-	+	+	+	-	-	+	+
2	Ethylamine	n	-(+)	n	n	n	v	v	+	+	+	+	+	n	+	n
3	L-Lysine	+	+	n	+	n	n	+	+	n	+	+	n	n	n	n
4	Cadaverine	+(-)	-(+)	+	+	v	+	-	+	n	+(-)	+(-)	n	-	+	+
5	Glucosamine	+(-)	-(+)	v	v	+	v	n	-(+)	n	v	-(+)	n	n	+	n
rmentation																
1	D-Glucose	n	n	+	-	v	n	+	n	+	+	+	+	n	n	n
2	D-Galactose	n	n	n	+	+	n	n	n	-(+)	n	n	w/-	n	n	n
3	Methyl α-D-glucoside	-(+)	n	v	-(+)	n	n	v	-	n	+(-)	v	n	n	n	n
4	Sucrose	-(+)	n	+	v	-	n	+	-	n	+(-)	v	n	n	n	n
5	Inulin	w/-	n	+(-)	-(+)	-	n	w/-	w/-	n	w/-	v	n	n	n	n
owth without vitamin(s)																
1	Without vitamins	n	-	+	+(-)	-(+)	n	n	+	n	+	n	n	n	-	+
2	Without *myo*-Inositol	+	n	+	+	+	n	n	+	n	+	+	n	n	n	n
3	Without Biotin	+	n	n	+	n	n	n	+	n	+	+	n	n	n	+
4	Without Thiamin	+	-(+)	n	+	+	+	+	+	+	+	+	+	+	n	+
5	Without Biotin & Thiamin	n	n	n	n	n	n	n	n	n	n	n	n	n	n	+
6	Without Pyridoxine	+	n	n	+	n	n	n	+	n	+	+	n	-(+)	+	n
7	Without Niacin	+	n	+	+	+	n	n	+	n	+	+	n	n	n	n
8	Without *p*-aminobenzoate	n	+	n	n	n	+	+	+	+	n	n	n	n	n	n

Table 2 (Cont'd.)

Growth at different temperatures																
1	At 25°C	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
2	At 30°C	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
3	At 35°C	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
4	At 37°C	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
5	At 40°C	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Additional tests																
1	With 0.01% (w/v) cycloheximide	n	n	n	-	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	n	n
3	With 1% Acetic Acid growth	-	n	v	-(+)	v	n	n	-	n	-	-	n	n	n	n
4	With 50% (w/v) D-glucose	+	n	n	v	+	n	n	+	n	+(-)	+	v	-	n	n
5	With 60% (w/v) D-glucose	-	n	v	v	+	n	n	-	n	-	+(-)	n	n	n	n
6	Starch formation	n	+(-)	n	n	-	-	-	+(-)	n	n	n	n	n	-	n
7	Diazonium Blue B reaction	n	-	n	-	-	v	+	+	n	n	n	n	n	-	n

Colony color: wh=white; cr=cream, yl=yellow; gr=gray; cr-tan= cream to tan; or=orange; pi=pink; t.wh=tanish white
shape of cell: r=round; ov=oval; gl=globose; sgl= sub globose; sph=spherical; elo=elongated; eli=eliptical; cyl=cylindrical
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. Univariate ANOVA of yeasts, isolated from slime fluxes.

Source	Sum of squares	df	Mean square	F	Probability
Acacia nilotica					
Main effects					
Yeasts (A)	9.359	6	1.560	545.138	p<0.001
Sample (B)	134.17	6	22.362	7815.418	p<0.001
A*B	329.771	36	9.160	3201.537	p<0.001
Error	0.14	49	2.86E-03		
Total	**549.085**	**98**			
Albizzia lebbeck					
Main effects					
Yeasts (A)	5.580	7	0.797	318.833	p<0.001
Sample (B)	319.822	5	63.964	25585.767	p<0.001
A*B	372.128	35	10.632	4252.890	p<0.001
Error	0.120	48	2.50E-03		
Total	**854.220**	**96**			
Aralia cachemirica					
Main effects					
Yeasts (A)	105.997	5	21.599	14920.632	p<0.001
Sample (B)	201.950	6	33.658	23250.805	p<0.001
A*B	870.354	30	29.012	20041.039	p<0.001
Error	6.08E-02	42	1.45E-03		
Total	**1290.442**	**84**			

Even though the total number of yeast species encountered in slime fluxes were less but they included opportunistic human pathogen such as *Pichia anomala* (Chakarbarti *et al*., 2001), airborne type of contaminant like *Sporidiobolus ruineniae* (Phaff & Starmer, 1987) as well as biotechnologically important yeasts, such as *Debaryomyces hansenii*, *Pichia angusta*, *P. methanolica* and *Phaffia rhodozyma*. The osmotolerant yeast, *Debaryomyces hansenii* and the pink yeast, *Phaffia rhodozyma* were isolated from *Albizzia lebbeck. D. hansenii* is known to produce polyhydroxy alcohols that act as compatible solutes and permit the cell to survive and grow under conditions of high osmotic tensions (Phaff *et al*., 1979). *P. rhodozyma* is known to form astaxanthin, a red pigment chemically closely related to β-carotene. It is valued as a food additive in fish farming, as it gives red color to the flesh of trout and salmon. A significant point in this connection is that this yeast species grows at approximately 26°C (Barnett *et al*., 1990), which is a disadvantage. During this study isolates of this species were successfully grown at high temperatures of up to 40°C probably due to adaptive ability to prevailing high temperatures in southern parts of Pakistan including Karachi. There is possibility to establish this industry in Pakistan since under laboratory conditions thermophilic yeasts can only be produced by genetic engineering, which is not always feasible. *Pichia*

angusta and *P. methanolica* are well known methylotrophic yeasts and ideal vehicles for the production of heterologous proteins such as hormones – somatostatin, tumor necrosis factor and many others. Under natural conditions such as slime fluxes, they utilize methanol liberated from pectins of plants' cell wall that serves to produce energy for generating yeast biomass. Methanol oxidation to CO_2 via formaldehyde provides the energy, whereas, fixation of formaldehyde via the xylulose monophosphate pathway provides glyceraldehyde phosphate from which cell constituents are built (Beradi, 1997).

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