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Original Article

Screening of some agri-wastes for economical cultivation of *Candida tropicalis* SS1

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Abstract
The present study was aimed to assess the optimum conditions to cultivate the yeast, *Candida tropicalis*SS1 for the single cell protein (SCP) production employing different fruit's peels. The *C. tropicalis* SS1 was cultivated in media containing 2% pulverized peels of apples, mangoes, water melon and bagasse singly as well as in eleven different combinations. The media were inoculated with 1% (w/v) suspension of 24 h old yeast cultured in nutrient broth. The strain grew best in water melon (WM) at pH 7.0 and 37 °C under non agitation conditions. The proximate analysis revealed that the water melon peels showed the highest amount of crude protein (which was measured by micro Kjeldahl digestion system) followed by bagasse, mango, and apple peels with respective values of as 13.38%, 3.20%, 2.69% and 2.58%. Best growth of *C. tropicalis* SS1 was obtained in aqueous extracts of 2% WM peels and the yeast cells attained up to 33% protein content on dry weight basis. In 2% aqueous extract of WM, the yeast strain yielded maximum biomass up to 6g/L with corresponding absorbance$_{(600\ nm)}$ of 3.49±0.05 after 48 h of incubation at 37 ºC, pH 7.0 and under non aerating conditions. These results indicate important biochemical attributes of the abundantly produced fruit wastes and their efficiency for economic production of *C. tropicalis* SS1 which could be used as SCP supplementing animal feed's formulations.
Key words: Yeast, proximate analysis, fruit waste, single cell protein, submerged fermentation

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INTRODUCTION

Ever increasing human population had accelerated, a few centuries before agricultural and industrial sectors to cope with the demands. Presently, there is a dire need to reconcile the food scarcity and nutritional needs of the ever expanding population with finite natural resources. Continuous increase in food scarcity in the developing countries leads to protein deficiencies in the adults as well as children. Provision of protein to serious protein energy malnutrition is a burning issue being faced by the fast growing populations of the world.

New advances in biotechnological techniques have made potentially effective and economic use of wastes into valuable products including single cell protein (SCP). Increasing concern about pollution caused by the agri-wastes has also stimulated the interest for conversion of these wastes into commercially valuable products. One of the most promising ways is to use such wastes as substrates for the cultivation of yeast for the production of protein rich single cell biomass. The agri-wastes contain cellulose, lignin, and hemicelluloses and can be converted into fermentable sugars which then serve as substrates for growth of different enzymes producing microbes. The biomass of bacteria and yeast is termed as single cell protein (SCP) which is an excellent protein source and might be used as a promising alternative to other conventional protein sources (Kurbanoglu and Algur, 2001; Khan and Dahot, 2010; Qazi *et al.,* 2012). The bioconversion of wastes into SCP is a cheaper and economically feasible way to overcome the protein scarcity (Anupama and Ravindra, 2000). Various fruit wastes being particularly rich in sugars and

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polysaccharides represent very appealing substrates for cultivating microbial (bacteria and yeast) biomass. Rapid growth rate, high protein content and their ability to utilize inexpensive feed stocks as carbon and energy sources for growth have made micro organisms prime candidates for animal as well as animal protein supplements. Yeast biomass is easy to harvest due to larger cell size, high protein content, flocculating ability and less nucleic acid contents as compared to bacterial cells (Wolf *et al.,* 2003). The SCP biomass produced may serve as an ideal supplement for aqua feeds. (Khan and Dahot, 2010). Microbes have 46% of crude protein content possessing large quantities of essential amino acids. (Rao *et al.,* 2003). A wide variety of agri industrial wastes such as peels of orange, mangoes and rice straw, corn straw and sugarcane bagasse have been investigated for SCP production (Nigam *et al.*, 2000; Zhao *et al.,* 2010a).These wastes are present abundantly in nature and may serve as a sole carbon and nitrogen source for the production of SCP by microorganisms. The agro industrial wastes including straw, bagasse, molasses .fruit and vegetable peels are locally produced in higher amounts and increase the pollution level to a significant level. Thus these wastes can be utilized for SCP, thereby causing a reduction in pollution level as well as producing SCP biomass. Hence bioconversion of wastes into SCPis a cheaper and economically feasible way to overcome the protein scarcity (Anupama and Ravindra, 2000).

Pakistan has an agro based economy producing about 50 million tons of the wastes annually which are not properly disposed off and thus causeenvironmental pollution (Khan and Dahot, 2010). Huge amounts of vegetable as well as fruit wastes are produced. Present study was aimed to employ extracts of apple, sugarcane bagasse mango, and water melon peels for cultivation of yeast, *Candida tropicalis*and to produce single cell protein biomassfor obtaining high biologic value protein in an economically feasible and eco-friendly way.

MATERIALS AND METHODS

Preparation of Substrate

Peels of watermelon, mango and apple and sugarcane bagasse were collected from local market of Lahore city, Pakistan. The collections were thoroughly washed using running tap water to make them dust free, cut into small pieces allowing maximum surface area to exposure for drying process. The substrates were initially air dried in sunlight and then placed in an oven at 80 ˚C till consistent weight. The dried peels were ground, sieved through mesh and stored in air tightened plastic jars at room temperature till further use.

Molecular identification of Candida sp.

The yeast strain isolated from soil was identified after 18S rRNA sequencing commercially. The sequence file was BLAST using NCBI BLAST and yeast strain was identified on the basis of highest similarity to the 18S rRNA sequence of classified yeast already submitted to database. The phylogenetic tree - Neighbor joining was constructed by MEGA 5.0 software.

Fermentation technique

The fruits peels media (2% w/v) were prepared in distilled water singly or in combination. The media were then autoclaved followed by filtration .The pH of aqueous extract/ filtrate was set at 7.0 and then again autoclaved for sterilization. The aqueous extracts were inoculated with 1% 24 h old yeast culture incubated at 37 ºC. Growth of select yeast strain was recorded at 600 nm using spectrophotometer. Amongst the media screened, water melon peels supported maximum growth of the select yeast strain therefore it was selected for further study.

Optimization of cultural conditions

The yeast cultivation was optimized for temperature, pH, aeration, inoculum size and medium concentration. Yeast cultivation was optimized for growth by cultivating at different temperatures (30 °C, 37 °C, and 45 °C), pH (3, 5, 7 and 9), inocula sizes (1%, 5%, 10%, 15% and 20% v/v) and medium concentrations (1.5%, 2%, 2.5%, 3%, 3.5% and 4% w/v) under agitation and non agitation conditions. Optimization was carried employing 10ml medium. The yeast growth was measured after 48 h of incubation. Following highest growth, a respective condition was considered optimum. All the experiments were performed in triplicates.

Analytical methods

To study the bioconversion of water melon (aqueous extract) into single cell protein, yeast was cultivated under optimized conditions

in optimized water melon medium (2%) prepared as described earlier. Culture (10 ml) was harvested by centrifugation at 8000 rpm for 8 minutes. The supernatants were discarded. Harvested biomass was washed twice with 10 ml of distilled water and then dried at 60 ºC till consistent weight to assess single cell protein biomass production. The biochemical constituents (crude protein, moisture, ash, crude fiber, crude fat and carbohydrate) of the substrates and yeast biomass were measured following standard procedures (Khan *et al.,* 2011).

Statistical analysis

The data were presented in means±standard error of three replicates. One way ANOVA using Minitab 16 software was used to find out the significant differences. Tukey test was used to compare the means. The effects were declared highly significant at $p<0.001$, very significant at $p<0.01$ and significant at $p<0.05$

RESULTS AND DISCUSSION

The results of the proximate analysis of the fruits waste peels are presented in Table I. The percent composition of all the fruits waste samples was significantly different ($P<0.001$) for all the tested parameters *i.e.* crude protein, crude fat, total carbohydrate, ash and moisture contents The mango peels expressed highest amount of fat followed by apple, water melon and bagasse having values of 4.08%, 1.70%, 1.06% and 1.02% respectively. The comparative study of fruits peels revealed that water melon peels contained highest amount of protein (13.38%) followed by bagasse (3.20%), apple (2.69%) and mango (2.58%). In the present study, the amount of carbohydrate was measured up to 62.66%, 51.37%, 40.28% and 35.58% for apple, mango, bagasse and watermelon, respectively.

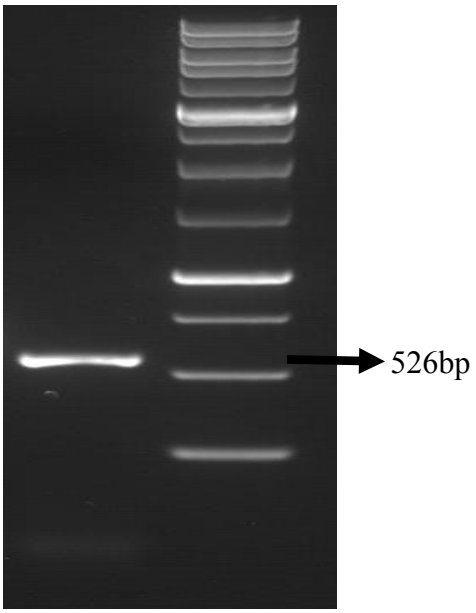

Figure 1. PCR amplified products of ITS of *Candida tropicalis* SS1.

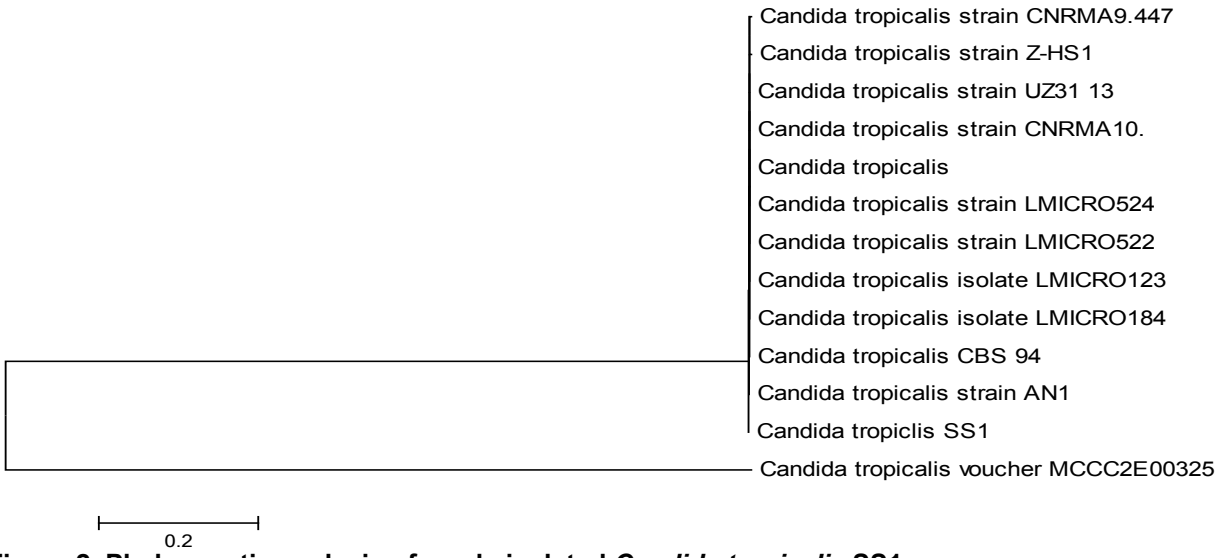

Figure 2. Phylogenetic analysis of newly isolated *Candida tropicalis* SS1.

Table I: Proximate composition of different peels of different fruit wastes

Proximate parameter	Agri-wastes				Significance
	Apple	Bagasse	Mango	Watermelon	
Moisture	17.27^{a}±0.70	12.40^{b}±0.20	17.33^{a}±0.21	16.93^{a}±0.75	P<0.001
Ash	13.32^{b}±0.56	15.62^{b}±0.73	20.35^{a}±0.97	21.34^{a}±1.97	P<0.001
Crude protein	2.69^{b}±0.26	3.20^{b}±0.20	2.58^{b}±0.44	13.38^{a}±0.99	P<0.001
Crude Fiber	2.38^{c}±0.21	27.48^{a}±1.39	4.29^{c}±0.30	11.72^{b}±0.66	P<0.001
Crude Fat	1.70^{b}±0.12	1.02^{c}±0.06	4.08^{a}±0.26	1.06^{c}±0.08	P<0.001
Carbohydrate	62.66^{a}±1.37	40.28^{c}±0.74	51.37^{b}±0.70	35.58^{d}±1.61	P<0.001

Values are means ± SEM of three replicates. One way ANOVA was used to find out the significant differences. Means that do not share a letter in a row are significantly different.

Now a days, people are becoming health conscious and consume large quantities of fruits and fruit juices leading to the accumulation of fruit wastes. The disposal of wastes is a serious problem and their deposition poses health hazard for all living beings. These wastes can be used as a substrate for the growth of yeast which might be used as sources of protein and may be utilized as a feed supplement. Different fruit wastes have been used to cultivate yeast by different researchers.

In the present study, cultivation of yeast, *C. tropicalis* SS1 in 2% aqueous extracts of water melon peels wastes revealed maximum growth O.D. (0.95±0.13) (Table II). Kamel (1979) reported cultivation of yeast employing dates as a potential substrate. Likewise, sweet orange residues have been used for yeast cultivation (Nwabueze and Oguntimein, 1987). Rahmat *et al.* (1995) used apple pomace for yeast cultivation employing *Kloechera apiculata* and *Candida utilis*. Fruit peel extracts contain variable ingredients such as carbohydrates, proteins, fats, minerals which are supposed to be useful for the growth of yeast. In the present study, water melon peel extract contained higher amount of protein (13.38%) which might had favorably affected yeast biomass production.

In the present study, yeast, *C. tropicalis* SS1 grew vigorously in the water melon extract without any supplementation. No supplements such as inorganic nitrogen sources, carbon and glucose sources were used to cultivate *C. tropicalis* SS1 on fruits wastes. Therefore, this process of yeast cultivation became cheaper. Munawar *et al.* (2010) also documented maximum yeast biomass production by utilizing fruit waste extracts. Several researchers in their studies have used inorganic supplements for yeast growth on waste materials.

Table II: Cultivation (48 hrs.) of yeast *Candida tropicalis* SS1 in 2 % fruit wastes based culture media comprising of fruit peels singly and their combinations for the preliminary screening of substrates for optimal growth.

Aqueous extracts of fruit waste medium	Absorbance
Bagasse (B)	0.33^{g}± 0.05
Apple(A)	0.65^{cdef} ± 0.07
Mango (M)	0.67^{bcdef} ± 0.10
Water melon (WM)	0.95^{a} ± 0.13
B+A	0.56^{ef} ± 0.02
B+M	0.52^{fg} ± 0.02
B+WM	0.58^{def} ± 0.09
A+M	0.82^{abc} ± 0.07
A+WM	0.87^{ab} ± 0.02
M+WM	0.78^{abcd} ± 0.02
B+A+M	0.72^{bcdef} ± 0.10
B+A+WM	0.82^{abc} ± 0.10
B+M+WM	0.69^{bcdef}± 0.06
A+M+WM	0.73^{bcde} ± 0.05
B+A+M+WM	0.75^{bcde} ± 0.03
Significance	P<0.001

Absorbance values are means ± SEM of three replicates at 600nm for 48 h of incubation period. One way ANOVA was used to find out the significant differences. Means that do not share a letter in column are significantly different.

Ojokoh and Uzeh (2005) utilized glucose (2% w/v) and $(NH_4)_2HPO_4$ (0.25% w/v) as a nitrogen source supplement for the production of *Saccharomyces cerevisiae* biomass in papaya extract medium. In present study, yeast under optimized conditions showed maximum growth at temperature 37 ºC. pH 7.0, 15% inoculum size (v/v), 2% (w/v) medium concentration under non aerated conditions after 48 h of incubation (Table III).The pH value of culture medium can affect the functions of the cell membrane, the uptake of various nutritional sources and the biosynthesis of metabolites (Menzel and Gottschalk, 1985). In the present study, the initial pH 7 of the culture medium gave the best growth (2.10±0.33).

Table III: Growth conditions optimization of the yeast, *Candida tropicalis* SS1 cultivated in 2% aqueous extract of water melon peels.

Parameter	Magnitude	Growth(O.D 600nm)	Significant Level
	30 ºC	1.48^{a}± 0.14	
Temperature	**37 ºC**	**1.61^{a} ± 0.06**	P<0.001
	45 ºC	0.05^{b} ± 0.01	
	3	0.71^{bc}± 0.03	
	5	1.34^{ab}± 0.44	P<0.01
Initial pH	**7**	**2.10^{a} ± 0.33**	
	9	1.25^{c}± 0.36	
Agitation	Agitation	1.23^{b} ± 0.39	P<0.001
	Non-Agitation	**2.41^{a}± 0.25**	
	1%	1.54^{c} ±0.21	
	5%	3.28^{b}± 0.15	
Inoculum size	10%	4.00^{b} ± 0.25	P<0.001
	15%	**5.62^{a} ± 0.43**	
	20%	5.09^{a} ± 0.61	
Medium concentration	1.5%	2.87^{a} ± 0.37	
	2.0%	**3.49^{a} ± 0.05**	
	2.5%	3.25^{a}± 0.10	P<0.001
	3.0%	2.91^{a} ± 0.47	
	3.5%	1.52^{b} ± 0.14	
	4.0%	0.70^{c} ± 0.05	

Values are mean±SEM of three replicates that represent absorbance (O.D) of the yeast at 600nm of 48 hours incubated cultures (Bold=Optimize conditions). One way ANOVA was used to find out the significant differences. Mean with the same letter in column did not differ significantly.

The biomass of yeast was determined by its cultivation under corresponding optimum growth conditions in water melon peels. In 2% aqueous extract of water melon peels (WM), the select yeast strain yielded maximum biomass up to 6g/L with optical density of (3.49±0.05) at a 37 ºC, pH 7.0 and under non aerating conditions after 48 h of incubation. The proximate composition of yeast biomass produced after cultivation revealed up to 33.44% crude protein (Table IV). Several researchers had reported similar findings (Saquido *et al.,* 1981; Zhao *et al.,* 2010b). Irfan *et al.* (2011) reported highest protein (48%) from *Candida utilis* with initial medium pH of 6.5 using 10% inoculumsize for 72h of fermentation. Dhanasekaran *et al.* (2011) reported the bioconversion of pine apple wastes into single cell protein biomass employing yeast strains *C. tropicalis* and *Saccharomces cerevisiae*. Several different fruit wastes have been uitilized as substrates like dates (Kamel, 1979), sugarcane bagasse (Azin and Moazami,

1989), banana skin (Enwefa, 1991), beet pulp (Ghanem, 1992) and guava peel (Moharib, 2003) for the production of single cell protein.

Table IV: Proximate composition of yeast biomass

Proximate parameters	Yeast cells (%)
Moisture	10.35^{c}±0.250
Ash	8.39^{c}±0.024
Crude protein	33.44^{b}±1.201
Crude Fiber	2.43^{e}±0.079
Fat	5.08^{d}±0.072
Carbohydrate	40.31^{a}±1.430
Significance	P<0.001

Values are mean±SEM of three replicates that represent absorbance (O.D) of the yeast at 600nm of 48 hours incubated cultures. One way ANOVA was used to find out the significant differences. Mean with the same letter in column did not differ significantly.

CONCLUSION

The present study may dictate that yeast, *C. tropicalis*SS1 was able to grow on fruit wasteextract especially water melon peels by utilizing various ingredients available in them. The present work helps in SCP production from inexpensive and cheaper waste materials which will contribute to benefit the poor malnutrition masses through the provision of better protein rich food in both quantitative and qualitative terms in an economically feasible and eco-friendly way. Thus fruit wastes should be exploited properly as substrates for the production of cellular biomass of yeast instead of dumping/wasting them. So they can be used as feed supplement after enrichment with least investment cost.

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