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MOLECULAR IDENTIFICATION AND CHARACTERIZATION OF YEAST SPECIES ISOLATED FROM FERMENTATION FOOD

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Abstract:

The Molecular identification and characterization of yeasts isolated from fermentation food stuffs in Sari, north Iran, were investigated. The yeasts involved in the fermentation food stuffs were found to consist of Saccharomyces spp. almost exclusively. Of yeast species isolated investigated, 27.4% were identified as Saccharomyces cerevisiae, whereas more than half of the isolates [72.6%] had physiological properties of other yeast for example K. lactis، K. marxianus P. manshurica، P. membranifaciens، P. occidentails، P. kudriavzerii، Z. paradaii، C. pseudoasrei، C. paraugosa، T. delbrueckii been reported. Identification of Species Saccharomyces utilizes diagnostic tests of yeasts such as the growth of yeast colonies in culture media enriched [YPD, SDA] and CHROM agar and tests differential diagnosis of Urease test, germ tube test and corn meal agar with Tween 80 were culture. Both ITS PCR- RFLP and Sequencing strongly indicated that these isolates were related to S. cerevisiae, regardless of their phenotypic characteristics. Besides, identity the genus and species on the basis of Coverage Query and Max is done, that highest percentage of samples similar to sequences in the gene bank title is.The results showed that all isolates had individual profiles, although some profiles the spontaneous fermentation by a variety of strains of S.cerevisiae were closely related. Such variations from fermentation food stuffs might be attributed to local deviations in the production, among which starter cultures should be selected.

Keywords: *Fermentation, S.cerevisiae, YPD, SDA, CHROM agar, PCR RFLP, Sequencing*

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INTRODUCTION:

Today the idiom fermentation process that under the influence the activity of enzymes or microorganisms forward. [1] The use of fermentation in order to that increase the shelf life of food stuffs have long been about used by humans, Whereas fermentation process in food stuffs processing, in order to achieve optimal quality of is essential.[2] Now in developed countries, the industrial yeasts to produce food products, biochemical and recombinant proteins are used.[3] Of course, often food products fermented by activity of lactic acid bacteria and fungi particularly yeasts and to a lesser degree molds are produced. [4] Yeasts, fungus true that in terms of the morphology a single-celled and reproducing by budding or that about the genus *Shizosaccharomyces* carried by division direct. [5] Although the yeast shape appearance of simple, but this is likely to be more evolved than simple fungi. Their natural habitats mostly in nutrient-rich environments like the nectar of plants, plant secretions, rotting fruit and animal body fluids.[6] A major study about the classification of yeast by Rij Van-Kreger describes about 500 different species, which are divided into 60 geniuses.[7] Despite of there are a large variety of yeasts and yeast-like fungi, But only a relatively small number of them usually participate in food stuffs production and microbial fermentation.[8] All yeast used in the preparation of fermented food stuffs or belong to Ascomycetes or members of genus Candida yeast. [9] Saccharomyces yeasts the most important industrial yeast for the production of fermented food stuffs is, and species *Saccharomyces* most commonly yeast used in the preparation of beverages and food stuffs is produced by fermentation of fruits and vegetables. [10] Fungi are everywhere organisms and to the depth and breadth of ecological activities are an important component of most eukaryotic microorganisms. [11] Fungi during many years as an important source of industrial enzymes have been used and today half of these enzymes derived from a fungus. [12] In recent years, several new tools provided to identify yeast species. For example, by using of method PCR and sequencing genes Relevant, Rapid diagnosis and accurate many of the yeast possible is made. [13-14] therefore purpose of current study, determination of methods molecular for the isolation of yeasts and identification of Saccharomyces species of traditional food fermentation.

MATERIALS AND METHODS:

Choosing Of location and sampling

In this study cross - sectional, with the aim of isolates and identifies the species of *Saccharomyces* from food stuffs fermentation, the samples randomly selected from city of Mazandaran province by using of a sterile syringe 5 ml were collected. This traditional products Contains 15 samples of Bread dough, 15 samples of yogurt, 20 samples of milk, 10 samples of cheeses, 25 samples of kefir and Cambojia and 15 samples of pickles, Respectively. Samples of fermentation Food stuffs by maintaining the cold chain were transported to the laboratory.

Enriched culture medium for the isolation of Saccharomyces

In laboratory of samples for fifteen minutes in round 1500 were centrifuged and then by using of pipette pasteur sterile 2 ml samples of were taken from Super Natant; then 0.1 ml of a dilution 1/100 on the plate Contain medium Sabouraud dextrose agar *[SDA]* plus antibiotics penicillin and streptomycin after 24 hours of incubation at laboratory temperature 25 ºC for isolation of Fungi our cultivated and also 0.1 ml of a dilution 1/100 on the plate Contain yeast extract peptone dextrose medium *[YPD]* after 3 days of incubation at laboratory temperature 35 ºC for isolation of yeasts our culture and with respect to the period of light - dark was incubated. Also mass culture plates isolates to micro tubes 1.5 ml sterile that containing medium Tryptic Soy Broth *[TSB]* is transferred and stored at -80 ° C freezer.

Urease Test

Each of the isolates with two times repetitions was cultured in tubes test containing Urea Broth. One of tubes Urea Broth at temperature 37 ° C was incubated and every the half hour was studied. Other tubes at temperature 25 °C were incubated and every six hours was studied and the results were presented based on the color changes to yellow.

Germ tube test

Each of the isolates inside the tube containing fresh human serum at temperature 37 ° C incubated and after 2 to 3 hours the formation of germ tubes were studied.

Culture Chrome agar medium

A plate containing Chrome agar medium prepared and each of the isolates cultured linearly and a plate containing the same medium but without the isolation is placed on it, after 24 hours, the plates were incubated at room temperature and colonies grow out of any on the plate which was previously uncultivated about the were studied.

Culture corn meal agar medium + Tween 80

A plate containing Corn Meal Agar medium has prepared and Cultured isolates to linearly and a plate containing the same medium that is placed in the

non-isolated after 24 hours, the plates were incubated at room temperature and colonies grow out of any on the plate which was previously uncultivated about the were studied.

Isolation of method molecular of yeast species

To confirm the diagnosis of yeast strains isolated from plates containing Sabouraud dextrose agar and yeast extract peptone dextrose, with extraction DNA was performed. Initial screening using gene region primers *ITS1, ITS4* isolates were amplified and finally the suspected samples by specific primers *SC1* and *SC1*d and *PCR-RFLP* method was performed by enzyme *Msp1*.

Statistical analysis

Statistical analysis was performed by using the *REST* software and Sequencer Device. Analyze data utilizes based on statistics descriptive frequency and linear regression was performed. Data collection tools software *SPSS -16* is used.

RESULTS:

The results of the present study on 100 samples of traditional fermentation food that containing 15 samples Bread dough, 15 samples yogurt, 20 samples milk, 10 samples cheese, 25 samples Cambojia and kefir and 15 samples pickles shows that a total of 24 samples yeast colonies Saccharomyces species were isolated.

Results Mycology and molecular analysis are as follows:

Urease test

The isolated yeast species were studied in terms of urease test, all of which Urease were negative. [Fig. 1]

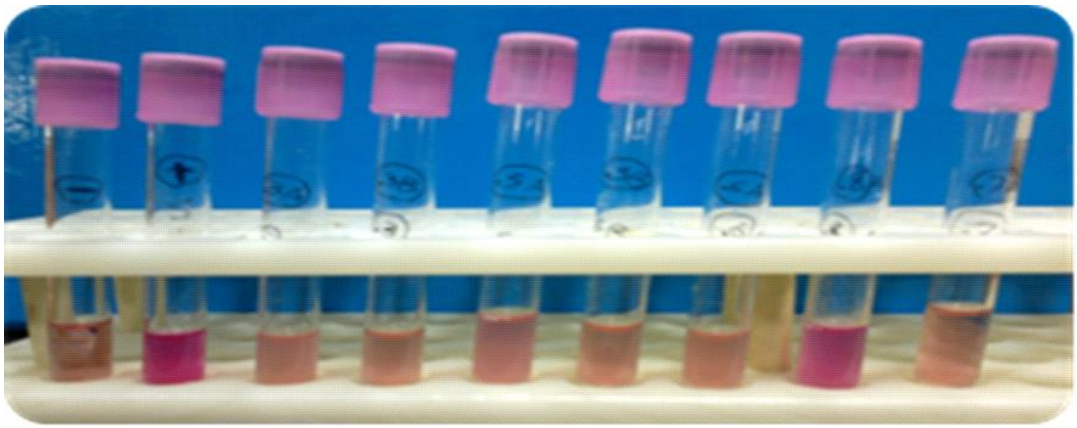

Fig. 1: Urea test species isolated with two samples control positive Cryptococcus

Production Chlamydocanidia in corn meal agar medium

A total of 100 samples referred to Saccharomyces and other fungi at corn meal agar medium were studied for the presence of chlamydocanidia in all samples blastoconidia was observed [Figure 2].

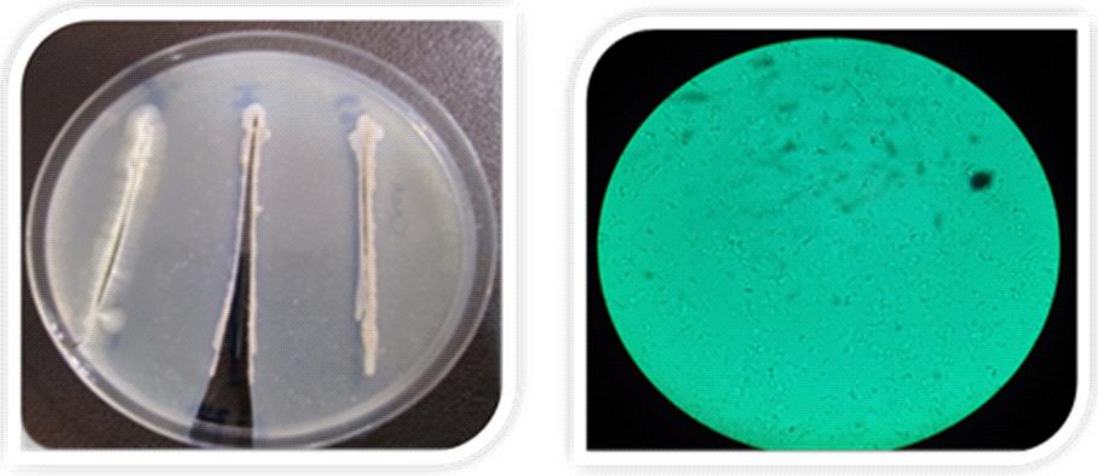

Fig. 2: Corn meal agar culture and production blastoconidia

Culture CHROM agar medium

All 100 samples suspected of Saccharomyces species and other fungi in the medium *CHROM agar* were created color Pink to purple and Candida albicans positive control samples were used in the tests. [Figure 3]

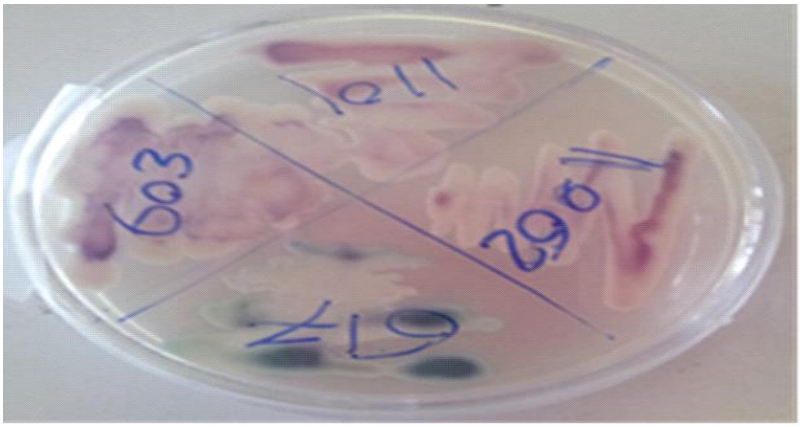

Fig.3: Culture of Saccharomyces strains isolated in alongside samples of Candida albicans on the medium *CHROM agar*

Germ tube test

All 100 samples suspected of Saccharomyces species and other fungi in terms of Germ tube test were negative.

Molecular studies on isolates

The results of comparing sequences are performed For 100 samples with primers *ITS1, ITS4* by using of gene region *ITS1-5.8s-ITS2* is as follows, respectively.

A total of 100 samples referred to *Saccharomyces* and other fungi, 24 cases *[27.4%] Saccharomyces cerevisiae, 15 cases [13.27%] Kluyveromyces lactis, 12 cases [10.67%] Pichia manshurica, 7 cases [7.83%] Pichia occidentails, 6 cases [6.83%] Candida pararugosa, Pichia kudriavzevii, 5 cases [5.3%] Pichia membranifaciens, Candida stellimalicola and* of each of *4 cases [3.5%] Torulaspora delbrueckii, Zygosaccharomyces parabailii ,Kluyveromyces marixianus,* has been seen *[Table - 1].*

Table 1: fungi identified from fermented materials

Isolation Species												Suspect samples	number of samples	samples	Row
T.delbrueckii	*k. lactis*	*P. membranifaciens*	*P.occidentails*	*C.stellimalicola*	*K. marxianus*	*P.manshurica*	*C. pseudoaaseri*	*C. pararugosa*	*Z .parabail*	*P. kudriavzevii*	*S. cerevisiae*				
2	-	-	-	-	-	6	-	-	-	-	7	15	15	Bread dough	1
-	10	2	-	1	1		1	1	-	2	2	20	20	milk	2
-	2	-	2	-	1	-	2	-	2	-	6	15	15	yogurt	3
-	3	-	1	1	-	-	-	-	1	1	1	10	10	Cheese	4
1	-	2	2	3	-	4	-	3	-	2	4	25	25	Kafir-cambojia	5
1	-	1	2	-	2	2	1	2	1	-	3	15	15	pickles	6
4	15	5	7	5	4	12	4	6	4	6	24	100	100		Total

Results of PCR with primers *ITS1, ITS4*

Samples Suspected by using of specific primers, *ITS1 ITS4* are created by based on different species of **500-800** bp. [Figure 4].

Results of PCR with specific primers *SC1r, SC1d*

Samples suspected and isolated the specific primers *SC1r, SC1d* products were created by **300**bp. [Figure 5]

Results of *PCR-RFLP* by the enzyme *Msp1*

The results of restriction enzyme pattern *Msp1* that *PCR* product with **800** bp to 2 the band, **716** bp and **124** bp have been broken. [Figure 6].

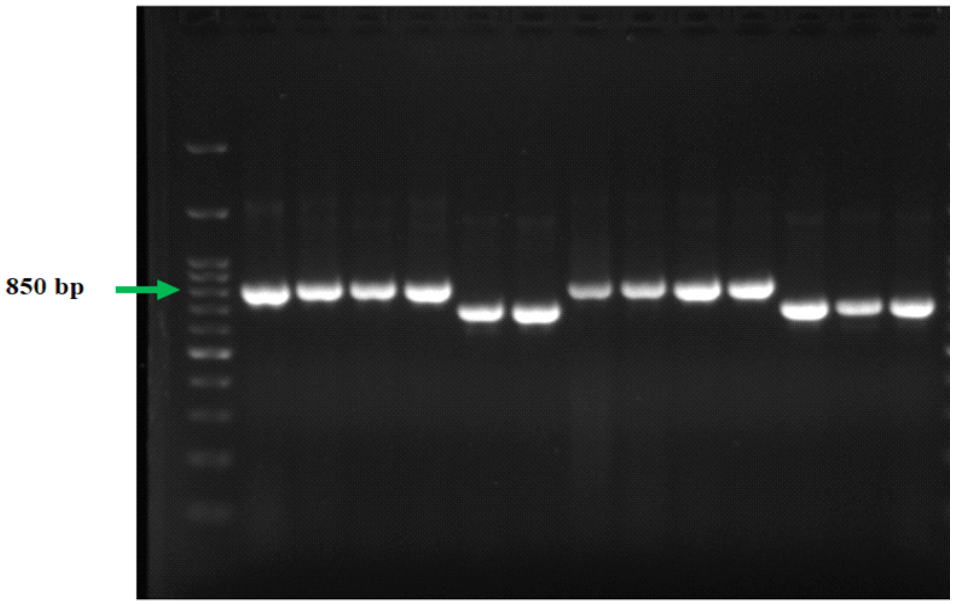

Fig.4: Electrophoretic analysis of the species Saccharomyces [bp 850] and non-Saccharomyces samples

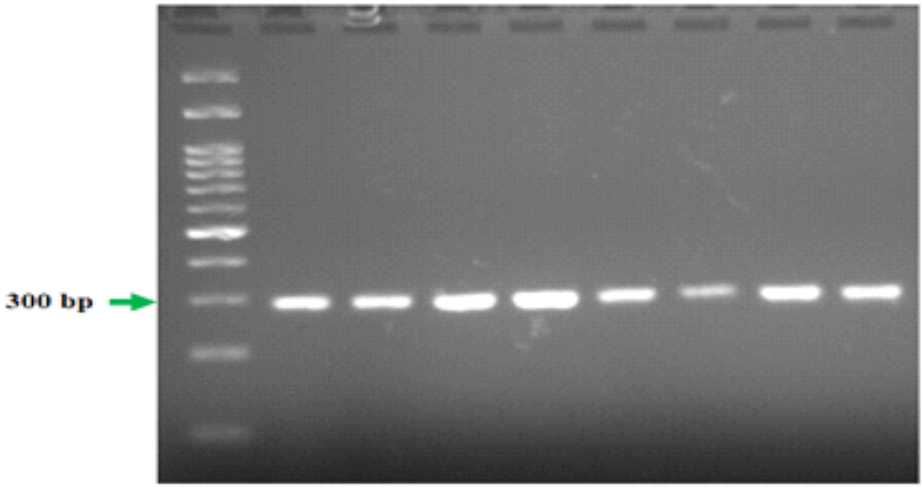

Fig.5: Electrophoretic analysis of the Saccharomyces cerevisiae with specific primers [bp 300]

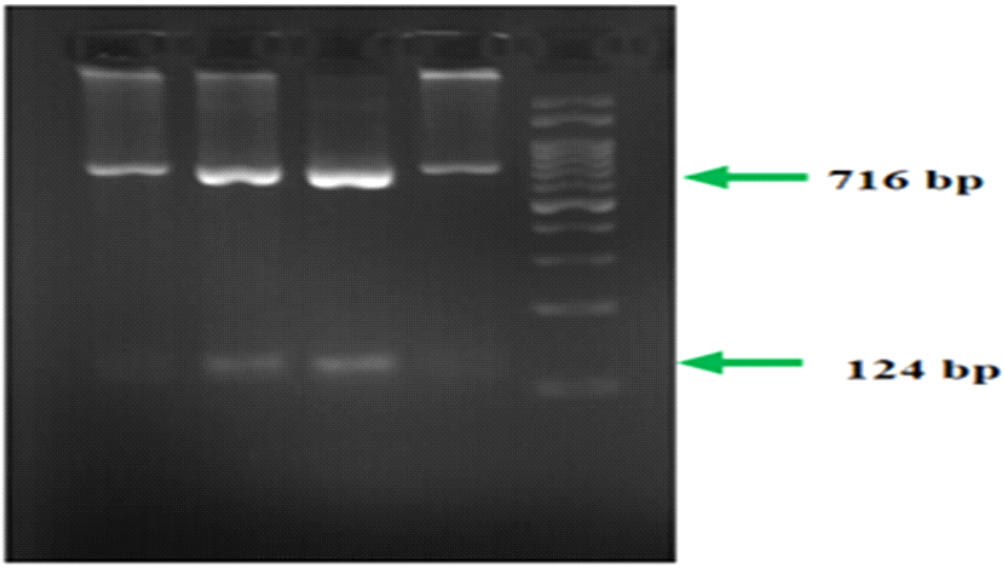

Fig.6: Electrophoretic analysis of the Saccharomyces cerevisiae by enzymes *Msp1*

Results Analysis of sequenced samples

After sending the results of sequencing by the Korean company, for chromatogram files by using of special software *Chromas 2* observed and was corrected manually if needed. **[Figure 7].**

Then sequences stored as files *fasta* and Through the *NCBI* site by using of the software *BLAST*, The obtained sequences with sequences in the *Gen Bank* were compared. The genus and species based on Coverage Query and Max identity takes place that the highest percentage of samples similarities to sequences in the gene bank as is. **[Figure 8].**

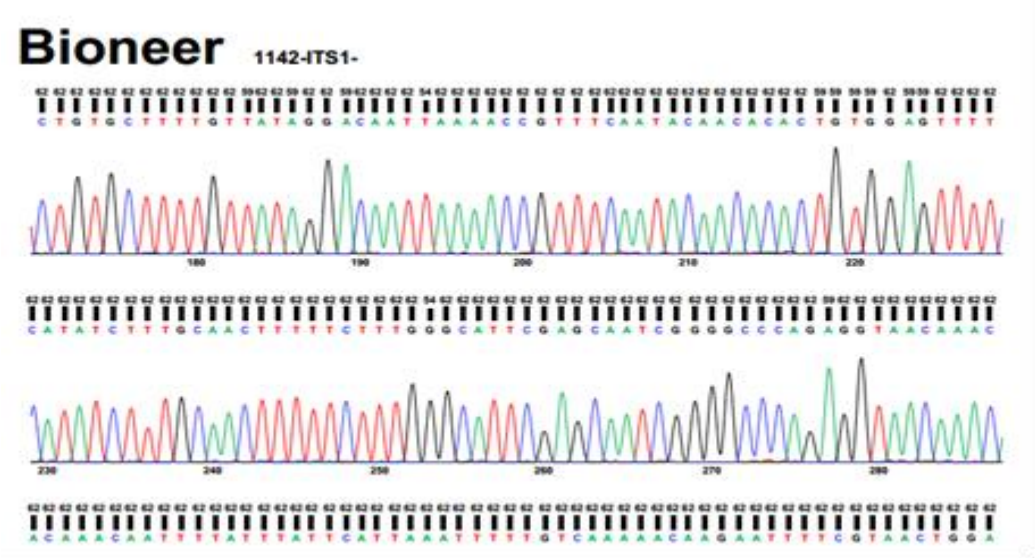

Fig.7: Sequence chromatogram pattern of using software *Chromas 2*

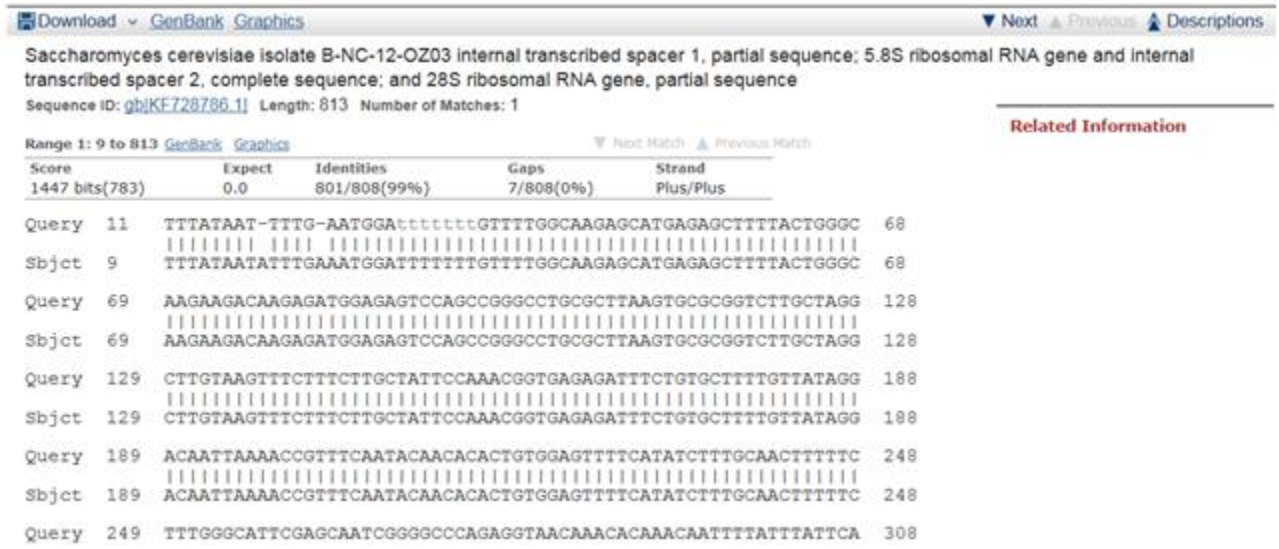

Fig.8: *BLAST* page related to the test sequence has the highest percentage of sequence similarity with the strain available in *Gen Bank*

DISCUSSION:

Fungi during many years as an important source of industrial enzymes have been used in food stuffs fermentation and today also half of these enzymes derived from a fungus. [11] Perhaps this saying often about molds true, but now gradually yeasts can have been a variety of reasons in the spotlight. [7] Today, yeasts [single-celled fungi] in terms of volume of production and economic aspects, in biotech crops from other industries have surpassed microorganisms. Among the causes of yeasts in the production can be to maintain and easy culture and non-viscous, environmental needs and simple food, High cell densities in the production environment at a low cost, ability to carry out post-translational processes, lack of endotoxin wall and can be mentioned viral DNA.[15] In the among been less attention to environmental species, Environmental species than species food stuffs fermentation and species of animal origin and human diversity and have more capabilities because interactions and environmental conditions far more complicated, more difficult and more variable environment almost is defined Food and body. [16] In the present study, the highest many of yeast strains isolated by using of method *PCR-RFLP* of *Saccharomyces cerevisiae* more of kefir and Cambojia and then bread dough was isolated. Since this organism is opportunistic in people with certain conditions, such as patients with weak immune systems may be risky according to various reservoirs of *Saccharomyces* species in the nature carefully at similarity between isolates from different environments, to learn more about the ecology of the species fungi, in order to determine the risk to people is necessary. Based on the studies conducted in around the world clearly known about yeast to human activity. In studing the different continents of Europe, Australia, Africa, *Saccharomyces cerevisiae* in different geographical areas were identified using DNA sequences and came to the conclusion that biological diversity Saccharomyces in habitats that population structure or human had hand, more have been . [6] The studies which had been about Cambojia in America Showed that most yeast Z. *kombuchaensis*, *B. bruxellensis* and *C. stellate* was isolated. In the case of kefir grains most yeast, *I. occidentalis*, *S.cerevisiae*, *S.unisporus* and *K.marxianus* were isolated. Whereas yeast species in our study *P.membranifaciens*, *P.occidentalis*, *P.stellimalicola*, *P.maushrica*, *P.pararugosa*. *P.kudriavzevii* and *S.cerevisiae* was isolated. Of course, isolation of the yeast with the high performance of industrial food has been proven in various global environmental strains but wild strains with the ability the inherent protein secretion in this respect and as a candidate for producing enzymes are highly valued.

CONCLUSION:

It is important to note that because of the various reservoirs saccharomyces species in the nature and because of the similarity between isolates from the environment different world in studies further understanding of the ecology of this fungus and its relationship with different species of animals and plants in order to determine the risk for the general population is essential.

According to various studies done in other areas of the world that with the help of molecular techniques, to express the similarity of isolates from patients and

their environment, On the basis of information obtained from this study can be used in future for larger studies that to determine method molecular and epidemiological relationship of between environmental isolates done.
Also with the help of molecular techniques such as *MALDI-TOF* and *MLST* Phylogenetic relationship of isolates can be determined.

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