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Isolation and Identification of Yeast Strains with High Beta-galactosidase Activity from Dairy Products

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Abstract: In this study of twenty-five samples which were collected from dairy producing centers in the city of Isfahan, 30 yeast strains were isolated using Malt Extract Broth (MEB) containing 0.1 gl^{-1} chloramphenicol and Yeast Extract Glucose Chloramphenicol Agar (YGCA). Of the isolated strains, 11 with the ability to lactose fermentation were isolated (M1-M11). These strains were identified by morphological and physiological properties. From these 11 strains, six were identified as *Kluyveromyces lactis*, four as *Kluyveromyces marxianus* and one as *Candida versatilis*. In the next step, the amount of produced single cell protein, SCP, from whey; whey including nitrogen supplementation and whey with mixed yeast cultures in these strains was measured. Among these strains, M2 that was identified as *K. lactis* had the most SCP production from whey with 11.79 gl^{-1}. It was found that the ammonium sulfate as a nitrogen source has a significant effect on biomass yield so that amount of produced biomass of the M11 strain *(K. marxianus)* increase from 11.54 gl^{-1} (from whey without nitrogen supplementation) to 15.75 gl^{-1} (in the presence of nitrogen source). The co-cultures of yeast strains with *Saccharomyces cerevisiae* were evaluated. In the mixed yeast cultures of these yeast strains and *S. cerevisiae*, the M2 strain which was identified as *K. lactis* had the most biomass production, 22.38 gl^{-1}. ONPG assay of beta-galactosidase in yeast strains found that a strain of *K. lactis* (M2) has the highest enzyme activity (8103 Eu ml^{-1}). These strains can be used for removal of whey pollutant, SCP & ethanol production and treatment of lactose in dairy food.

Key words: *Candida versatilis*, co-culture, beta-galactosidase, *Kluyveromyces lactis*, *Kluyveromyces marxianus*, SCP, Whey

INTRODUCTION

Whey is the aqueous fraction of milk generated as a by-product of cheese manufacturing which is produced in large amounts. The main solute in cheese whey is lactose, present at a concentration of about 4.5-5%[1,2]. Other components are protein, salts and vitamins that are present in minor amounts. The low concentration of these components makes their recovery uneconomical. Because of it's high organic content, dumping directly to the environment causes serious contamination problems. As a solution, bioconversion of whey into single cell protein, SCP or ethanol has been performed in several countries[3,4,5,6]. SCP, could be produced from whey with the employing of yeasts from different species including *Kluyveromyces, Candida* and *Trichosporon,* as they are naturally able to metabolize lactose[7-11]. This protein, SCP, can be used as a dietary supplement in feeding domestic fowl and farm animals[12].

In this study, several yeast strains with the ability of lactose fermentation from dairy products were isolated and characterized. The ability of the strains were evaluated for consumption of lactose and beta-galactosidase activity from dairy product.

MATERIALS AND METHODS

Sampling and Isolation of yeast strains: Twenty-five samples including whey, yogurt and cheese were collected from dairy producing centers in the city of Isfahan in sterile, 200 ml bottles and brought to the laboratory in a cooler box. For enrichment of yeast in these samples, 5mL (or 5g) of samples were inoculated in 50mL of Malt Extract Broth (MEB) containing 0.1gl^{-1} chloramphenicol and incubated at 25°C for 24 hours with constant shaking of 180 rpm. After incubation, the existence of yeast cells in the media was examined using light microscopy. The media containing yeast cells were then used for isolation of the yeast strains.

The yeasts were isolated on spread plates of Yeast Extract Glucose Chloramphenicol Agar (YGCA) after making serial dilutions. The plates were incubated at 25°C for 72 hours. Colonies with distinct morphological differences were selected and purified by streaking on potato-dextrose agar

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(PDA). The purified isolates were stored on PDA slants at 4°C.

Identification of yeast strains: To isolate the yeast strains capable of lactose fermentation, the isolated yeasts were cultured in Durham tubes containing 2% (w/v) solution of lactose. Then the positive yeast strains for lactose fermentation were identified using the standard taxonomic key outlined by Kutzman and Fell[13]. This identification was based on different chemical tests including the fermentation of sugars, liquid assimilation of carbon compounds, liquid assimilation of nitrogen compounds, growth at 37°C and 40°C and growth in 50% Glucose and Urase activity.

Fermentation of Sugars: Fermentation of lactose, glucose, galactose, sucrose, maltose, raffinose and trehalose were tested in Durham tubes containing 2% (w/v) solution of sugars, except for raffinose where 4% was used. The fermentation basal medium consisted of 4.5g yeast extract, 7.5g peptone and 20g sugar (40g for raffinose) in 1 liter of distilled water. The final working medium was made by the addition of 4mL of Bromothymol blue stock solution (50mg/75mL in distilled water) which was added to the 100mL fermentation basal medium. The pH of the medium was adjusted to 7-8. At this pH, the solution had a dark greenish color.

The medium was dispensed into Durham tubes (4mL in each tube) and sterilized at 121°C for 10 min. The tubes were inoculated with yeasts from a culture which was 48 hours old and incubated at 25°C for up to 28 days.

The tubes were inspected at frequent intervals. Positive results were indicated by the accumulation of gas in the Durham tubes and change of color in the indicator from dark green to yellow.

Liquid assimilation of carbon compounds: A 10-fold concentrated solution of the nitrogen base medium was prepared by dissolving 6.7g of Bacto Yeast Nitrogen Base and a carbon source equivalent to 5g of glucose in 100ml of distilled water. The medium was filter sterilized. In the test tubes, 0.5 ml of the medium was diluted aseptically with 4.5 ml of sterile water. Tubes were then inoculated with cells from a culture which was 24-48 hours old and incubated at 25°C for 2-3 weeks. A positive reaction was detected by visual inspection for an increase in the turbidity of the solution. The carbon compounds tested were galactose, sucrose, maltose, cellulose, trehalose, melibiose, raffinose, inuline, D-xylose, L-arabinose, D-ribose, L-rhamnose, glycerol, D-mannitol, citrate and inositol.

Liquid assimilation of nitrogen compounds: The procedure for liquid assimilation was similar to the assimilation of carbon compounds test except that the yeast nitrogen base was replaced by yeast carbon base (Difco). The nitrogen compounds tested were nitrate and L-lysine.

Growth in 37°C and 40°C: For determining the ability of growth at 37°C and 40°C, the yeast strains were cultured in a medium containing 2% (w/v) Glucose-Peptone-Yeast extract Broth. The medium was made of 20g glucose, 10g peptone and 5g yeast extract in 1 liter of distilled water. This solution was dispensed into tubes and sterilized at 121°C for 15 min. The tubes were inoculated with actively growing yeast culture and incubated at 37°C and 40°C for 1-2 weeks. The tubes tested were shaken at 180 rpm. A positive reaction was detected by observation of turbidity in the solution.

Growth in 50% glucose and Urea hydrolysis tests: The growth ability of the isolated yeast strains at high concentration of sugar was tested by growth on agar media containing $500gl^{-1}$ Glucose, $10gl^{-1}$ Yeast extract and $13gl^{-1}$ Agar. This medium was sterilized at 110°C for 10 min and dispensed into plates. The plates were inoculated with an actively growing yeast culture and incubated at 25°C for 3-4 days. Hydrolysis of urea was examined by using commercially produced Christensen's urea agar base (Merck, Germany). The slant was inoculated from an actively growing yeast culture and incubated at 25°C for 4 days. The ability of hydrolysis of urea was detected by development of a pink color in the slant medium

Production of SCP: The production of SCP was measured on the basis of the weight of dry biomass (gl^{-1}) in triplicate. For this purpose fresh whey from the dairy producing factory of Isfahan was collected in sterile bottles and brought to the laboratory.

pH of whey was adjusted to 4.5 and then boiled at 100°C for 15 min. Whey was cooled and sedimented proteins were collected by filtration. 1 liter of the limpid liquid obtained (greenish yellow) was sterilized at 115°C for 10 min and then inoculated by an actively growing culture. The medium was incubated at 25°C for 48 hours with constant shaking at 180 rpm. After the incubation, the biomass of the yeast cells was prepared by centrifugation at 4000 rpm. The biomass was dried and then weighed. The effect of nitrogen supplementation in amount of SCP production was also studied by applying ammonium sulfate ($0.8\ gl^{-1}$) as the nitrogen source. The

best yeast strains were selected and applied as co-culture with *Saccharomyces cerevisiae* for treatment of cheese whey and SCP production.

Measurement of Enzyme activity: ONPG assay was applied for the calculation of enzyme activity in yeast strains. For this purpose, the strains were inoculated in YEPD medium and cheese whey and then incubated in 25°C for 10-12 hour. The OD_{600}nm was recorded and then 1ml of yeast culture was spun out. The yeast cells were washed with 1 ml cold Z buffer (0.06 M Na_2HPo_4, 0.04M NaH_2Po_4, 0.01M KCl and 0.001M $MgSo_4$). For permeabilization of cells, 10 µl 0.1% SDS and 20 µl chloroform was added and incubated at 30°C for 10 min. 200 µl ONPG solution (4mg of ONPG in 1ml potassium phosphate buffer) was added in tubes and the time was recorded. The reaction was allowed to run until the solution had turned yellow. The reaction was stopped with the addition of 400 µl of 1M Na_2CO_3 and the time was recorded. The cells were spun out, OD_{420} nm of supernatant was read and Miller units were calculated (Units=1000×OD_{420}/volume (1ml)×time (min)×OD_{600}). The enzyme activity of M2, M5 and M11 strains which had high enzyme activity in YEPD medium was measured in deproteinized cheese whey as culture medium.

RESULTS

In this study 30 different yeast strains were isolated by using Malt Extract Broth (MEB) containing 0.1gl^{-1} chloramphenicol and Yeast Extract Glucose Chloramphenicol Agar (YGCA) (Table 1). The strains were examined for lactose fermentation ability. Among them, 11 strains were found to be capable of lactose fermentation (M1-M11) (Table 1). These strains were identified by morphological and physiological properties using the standard taxonomic key outlined by Kutzman and Fell[13]. These strains were identified using several chemical tests including the fermentation of different sugars, liquid assimilation of carbon and nitrogen compounds, growth at 37°C and 40°C, growth in 50% glucose and Urase activity.

As illustrated in Table 2, six isolates were identified as *Klyveromyces lactis*. These were taken from six different dairy sources. In addition to glucose, lactose and galactose, these strains were capable of fermenting sucrose and raffinose. However, five of the strains (M1, M2, M6, M7 and M10) were unable to ferment maltose. All the strains were negative for utilization of nitrate as a nitrogen source. Moreover, the utilization of L-lysine as a nitrogen source was a variable characteristic in *K.Lactis* strains; five (M1, M2, M5, M6 and M7) were negative and one (M10) positive (Table 2). These strains lacked assimilation of melibiose, L-arabinose, D-ribose and citrate. The ability of assimilation of inuline was variable in these strains, also, one being positive (M1) and five negative (M2, M5, M6, M7 and M10). Among these strains, three (M2, M6 and M10) were unable to grow in 37°C and five (M2, M5, M6, M7 and M10) in 40°C. None of these strains showed Urase activity.

Table 1: The yeasts isolated from dairy products in the city of Isfahan.

Samples	Number isolated	Yeasts that fermented lactose
1	1, 2	-
2	3	3 (M1)[1]
3	4	-
4	5, 6, 7	5 (M2)
5	8	8 (M3)
6	-	-
7	9	-
8	10	10 (M4)
9	11	11 (M5)
10	12, 13	12 (M6)
11	14	-
12	15, 16	-
13	17	-
14	18	18 (M7)
15	19, 20, 21	19, 20 (M8 and M9)
16	22	-
17	23	-
18	-	-
19	-	-
20	24, 25	-
21	-	-
22	26	-
23	27	27 (M10)
24	28	-
25	29, 30	30 (M11)

1.Conventional name of yeast.

Four strains were identified as *Klyveromyces marxianus* (Table2). These were found in four different sources. These strains were found to be negative for maltose and trehalose fermentation but capable of utilizing L-lysine as a nitrogen source. Nitrate could not be used as a nitrogen source by these strains. They were unable to assimilate maltose, melibiose, L-rhamnose and inositol. The ability of assimilation of cellobiose, trehalose, L-arabinose, D-ribose and D-mannitol were variable in these strains. All of the strains were able to grow at 37°C, but only three strains (M3, M4 and M8) were able to grow at 40°C. All were found negative for Urase activity (Table 2).

Among the strains identified, one was classified as *Candida versatilis*. This strain was capable of fermenting glucose, lactose, galactose, sucrose and raffinose. This strain was also positive for utilization of L-lysine as a nitrogen source. However, this strain was negative for the assimilation of assimilate inuline, L-rhamnose and inositol. Furthermore, this strain was also unable to grow at 37°C and 40°C, but able to grow in 50% Glucose. This strain lacked Urase activity (Table 2).

Table 2: Identification of yeasts isolated from dairy producer centers in city of Isfahan.

	Assimilation																						Fermentation						
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29
K.lactis	-	+	+	+	-	-	-	-	+	+	-	-	-	+	+	+	-	+	+	-	+	+	-	+	-	+	+	+	M1
K.lactis	-	-	-	+	-	-	-	-	+	+	-	-	-	+	-	+	-	+	+	+	+	+	-	+	-	+	+	+	M2
K.marxianus	-	+	+	ND	+	-	-	-	-	+	-	-	+	+	+	+	-	+	+	-	+	+	-	+	-	+	+	+	M3
K.marxianus	-	+	+	ND	+	-	-	-	+	+	-	-	+	+	+	+	-	-	+	-	+	+	-	+	-	+	+	+	M4
K.lactis	-	-	+	+	-	-	-	-	+	+	-	-	-	-	-	+	-	+	+	+	+	+	-	+	+	+	+	+	M5
K.lactis	-	-	-	+	-	-	-	-	+	+	-	-	-	-	-	+	-	+	+	+	+	+	-	+	-	+	+	+	M6
K.lactis	-	-	+	+	-	-	-	-	+	+	-	-	-	+	-	+	-	+	+	-	+	+	-	+	-	+	+	+	M7
K.marxianus	-	+	+	ND	+	-	-	-	+	+	-	-	+	+	+	+	-	-	-	-	+	+	-	+	-	+	+	+	M8
C.versatilis	-	-	-	+	+	+	-	-	+	+	-	+	-	-	-	+	-	+	+	+	+	+	-	+	-	+	+	+	M9
K.lactis	-	-	-	-	+	-	-	-	+	+	-	-	-	-	-	+	-	+	+	-	+	+	-	+	-	+	+	+	M10
K.marxianus	-	-	+	ND	+	-	-	-	-	+	-	+	-	+	+	+	-	-	-	-	+	+	-	+	-	+	+	+	M11

1 Urase Activity 2 Growth in 40 3 Growth in 37 4 Growth in 50% Glucose
5 l-Lysine 6 Nitrate 7 Inositol 8 Citrate
9 D-Mannitol 10 Glycerol 11 L-Rhamnose 12 D-Ribose
13 L-Arabinose 14 D-Xylose 15 Inuline 16 Raffinose
17 Melibiose 18 Trehalose 19 Cellobiose 20 Maltose
21 Sucrose 22 Galactose 23 Trehalose 24 Raffinose
25 Maltose 26 Sucrose 27 Galactose 28 Glucose
29 Conventional name of Yeast

Table 3: The amount of SCP production

		Biomass yield (g/liter) in whey as culture medium		
Name of isolated	Yeast Species	Without supplementation	With ammonium sulfate (0.8 g/liter)	Co-culture of yeast strains & S.cerevisiae + ammonium sulfate
M1	*K. lactis*	7.31	ND	ND
M2	*K. lactis*	11.79	15.35	22.38
M3	*K. marxianus*	8.10	ND	ND
M4	*K. marxianus*	7.62	ND	ND
M5	*K. lactis*	11.09	15	17.11
M6	*K. lactis*	10.95	14.34	16
M7	*K. lactis*	9.41	12.83	ND
M8	*K. marxianus*	10.00	13.5	ND
M9	*C. versatilis*	10.19	13.69	ND
M10	*K. lactis*	8.73	12.1	ND
M11	*K. marxianus*	11.54	15.75	19.58

Table 4: ONPG assay of beta-galactosidase in yeast strains.

		Enzyme activity (EU/ml)Yeast SpeciesName of isolated	
Name of isolated	Yeast species	Using of YEPD medium	Using of whey as culture medium
M1	*K. Lactis*	2113	ND
M2	*K. Lactis*	8103	5266
M3	*K. Marxianus*	3530	ND
M4	*K. Marxianus*	3858	ND
M5	*K. Lactis*	5487	5020
M6	*K. Lactis*	4040	ND
M7	*K. Lactis*	1889	ND
M8	*K. Marxianus*	4481	ND
M9	*C. Versatilis*	3301	ND
M10	*K. Lactis*	2494	ND
M11	*K. Marxianus*	5357	4642

The single cell protein, SCP, production by the isolated yeast strains was next examined (see above). After preparation of cheese whey, inoculation of yeast strains and incubation in suitable temperature; the biomass of yeast cells was collected and dried. The dry biomass was weighed (Table 3). Among the 11 yeast strains which were tested, the strain M2, which was identified as *K. lactis* (Table 2), was found to have the most SCP production, 11.79gl^{-1}. In the next step, the effect of ammonium sulfate as nitrogen supplementation on biomass yield was evaluated. In the presence of the nitrogen source significant increase in produced biomass was observed (Table 3). In this condition, the M2 (was identified as *K. lactis*) and M11 (was identified as *K.marxianus*) strains, were found to have the most biomass yield, 15.75 gl^{-1} and 15.35 gl^{-1}, respectively. The

co-cultures of M2, M5, M6 and M11 strains with *Saccharomyces cerevisiae* were evaluated. The mixed yeast culture of M2 strain (*K. lactis*) and *S. cerevisiae* had the most biomass production, 22.38 gl^{-1} (Table 3).

Enzyme activity of beta-galactosidase in yeast strains was measured (Table 4). Among 11 yeast strains, the M2 strain (*K.lactis*) was found to have the highest enzyme activity, 8183 unit/ml. Also the M5 strain (*K.lactis*) and M11 strain (*K.marxianus*) showed high enzyme activity, 5487 and 5357 unit/ml, respectively. In cheese whey as culture medium, enzyme units of the M2, M5 and M11 strains were measured at 5266, 5020 and 4642 unit/ml, respectively.

DISCUSSION

Eleven yeast strains were identified in this study. The predominant species with lactose fermentation ability were *Kluyveromyces lactis, (six* strains) and *Kluyveromyces marxianus,* (four strains) but only one strain was identified for the other species, *Candida versatilis.* Compared to other studies, it seems that *K.lactis, K.marxianus var. marxianus* and its anamorph, *C.kefyer,* are some of the most predominant and important yeast species in milk[8,11,14]. As for the other yeast strains isolated and identified in this study, *Candida versatilis* has been reported in yogurt, cheese and other dairy products[15,16,17,14]. *K. marxianus and K. Lactis* are used in different biotechnology applications[3,18,2]. The thermotolerance of *K. marxianus* which was also identified among the yeasts isolated in the present study, could be used in very rapid processes of ethanol production which might compensate for it's lower tolerance to ethanol compared to *S. cerevisiae. Kluyveromyces fragilis* (*K.marxianus)* strains are the yeasts that have been most widely studied for the production of yeast biomass from whey[19-21,7] and large-scale processes for producing *Kluyveromyces* biomass have been in operation for several years[7,12]. *K. Lactis* has been used for its industrial potential in the production of beta-galactosidase enzyme which can be used to reduce the lactose content of milk[23]. In this study, the strains M2 and M6 that were identified as *K.lactis* had the most SCP production. The amount of SCP production can be improved by adding ammonium sulfate as a nitrogen supplementation or peptone supplementation for high biomass production with low ethanol production. The produced biomass in the presentation of ammonium sulfate, 0.8gl^{-1}, that was measured showed significant increases (Table 3). In this condition, the M11 (*K. marxianus*) and M2 (*K. lactis*) strains were found to have the highest biomass yield, 15.75 and 15.35 gl^{-1}, respectively. In order to increase the biomass yield, mixed culture of yeasts have been studied[23,24]. Mixed yeast cultures of the M2, M5, M6 and M11 yeast strains and *Saccharomyces cerevisiae* were used. The biomass yield of *S.*cerevisiae and the M2 strain (*K. lactis*) co-culture showed a significant increase, 22.38 gl^{-1}. The biomass of *Kluyveromyces* species can be used as a dietary supplement in feeding domestic animals[12,4].

Compared to other studies it seem that the enzyme activity from *Kluyveromyces* species, is high[25]. Among the 11 studied yeast strains, the M2 strain (*K. lactis*) was found to have the highest enzyme activity, 8183 unit/ml. The M5 strain (*K. lactis*) and M11 strain (*K. marxianus*) also showed high enzyme activity, 5487 and 5357 unit/ml, respectively. In cheese whey, as culture medium the enzyme units of these three yeast strains were measured and found to be 5266, 5020 and 4642 unit/ml, for the M2, M5 and M11 strains, respectively. This study showed that isolated *K. lactis* (M2) is the best yeast strain for the removal of whey pollutants, treatment of lactose intolerance with the reduction of lactose content of milk, SCP and beta-galactosidase production from whey.

REFERENCES

1. Rohm, H., F. Eliskases-Lechner and M. Brauer, 1992. Diversity of yeasts in selected dairy products. J. Applied Bacteriology, 72: 370-376.
2. Zadow, J.G., 1992. Whey and lactose processing. Elservier Applied Science, London and New York.
3. Gonzales, S.M.I., 1996. The biotechnological utilization of cheese whey. A review. Biores. Technol., 57: 1-11.
4. Irvine, D.M. and R.M. Hill, 1985. Cheese technology in: Moo-young, M. editor. Comprehensive Biotechnology, Pergman, Oxford, pp: 523-265.
5. Marshall, V.M., 1987. Fermented milks and their future trends: Microbiological aspects. J. Dairy Res., 57: 559-574.
6. Mawson, A.J., 1994. Bioconversions for whey utilization and waste abatement. Biores. Technol., 47: 195-203.
7. Castillo, F., 1990. Lactose metabolism by yeast. In Verachtert, H. and De Mot, R. editors. Yeast: Biotechnology and Biocatalysis. Marcel Dekker, New York, pp: 297-320.
8. Fleet, G.H., 1990. Yeast in dairy products-A review. J. Applied Bacteriology, 68: 199-211.
9. Fleet, G.H. and M.A. Main, 1987. The occurrence and growth of yeasts in dairy products. International Journal of food Microbiology, 4: 145-155.

10. Galvez, A., M.J. Ramires and M. Garcia-Garibay, 1990. Chemical composition of mixture of Single-Cell-Protein obtained from kluyveromyces fragilis and whey proteins. Arch-Latinoam-Nutr., 40: 252-262.
11. Seiler, H. and M. Busse, 1990. The yeasts of cheese brines. International Journal of Food Microbiology, 11: 289-304.
12. Litchfiedld, J.H., 1983. Single-cell proteins. Science, 219: 740–746.
13. Kurtzman, C.P. and J.W. Fell, 1998. The Yeasts: A taxonomic study. Elservier: Amsterdam.
14. Gadaga, T.H., A.N. Mytukumira and J.A. Narvhus, 2000. Enumeration and identification of yeasts isolated from Zimbabwean traditional fermented milk. Inter. Dairy J., 10: 456-466.
15. Suriyarachchi, V.R. and G.H. Fleet, 1981. Occurrence and growth of yeast in yogurts. Appled and Environmental Microbiology, 42: 574-579.
16. Deak, T. and L.R. Beuchat, 1996. Handbook of food spoilage yeasts. Boca Raton, FL: CRC Press.
17. Jakobsen, M. and J. Narvhus, 1996. Yeasts and their possible beneficial and negative affects on the quality of dairy products. Inter. Dairy J., 6: 755-768.
18. Klaus, W., 1996. Nonconventional yeasts in biotechnology, A Handbook. Springer-Verlag Berlin Heidelberg New York, pp: 139-201.
19. Beausejour, D., A. Leduy and R.S. Ramalho, 1981. Batch cultivation of *Kluyveromyces fragilis* in cheese whey. Can. J. Chem. Eng., 59: 522-526.
20. Moresi, M., M. Patete and A. Trunfio, 1989. Trunfio, Scaling-up of a batch whey fermentation by *Kluyveromyces fragilis*. Appl. Microbiol. Biotechnol., 31: 495-501.
21. Moresi, M., A. Trunfio and M. Patete, 1990. Kinetics of continuous whey fermentation by *Kluyveromyces fragilis*. J. Chem. Tech. Biotechnol., 49: 205-222.
22. Suarez, F.L., D.A. Savaiano and M.D. Levitt, 1995. The treatment of lactose intolerance; Aliment. Pharmacol. Ther., 9: 589-597.
23. Carlotti, A., F. Jacob, J. Perrier and S. Poncet, 1991. Yeast production from crude sweet whey by a mixed culture of C*andida kefyr* LY496 and *Candida valida* LY497. Biotechnol. Lett., 13: 437-440.
24. Cristiani-Urbina, E., A.R. Netzahuatl-Munoz, F.J. Manriquez-Rojas, C. Juarez-Ramirez, N. Ruiz-Ordaz and J. Galindez-Mayer, 2000. Batch and Fed-batch cultures for the treatment of whey with mixed yeast cultures. Process Biochemistry, 7: 649-657.
25. Günther, E. and E. Bürger, 1982. A method of manufacturing lactose-hydrolyzed yoghurt by means of beta-galactosidase, in Dupuy, P. (ed.), Use of enzyme in Food Technology, Lavoisier, paris, 1982.