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Article

Isolation and Identification of Beta-galactosidase Producing Yeasts From Some Dairy Products

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Abstract: Twenty-two β-galactosidase-producing yeast isolates were isolated from local dairy products. The purified isolates were evaluated for their β-galactosidase activity to identify the most productive isolates for subsequent investigations. The isolates were utilized in fermentation experiments, and β-galactosidase activity was assessed after 48 h. Fermentation experiments results indicated that isolate No. 1 and No. 13, which produced the highest enzymatic activity were selected for studying the optimization of the environmental conditions for the enzyme production. Based on ITs rDNA gene sequence analysis, the two isolates were identified as *Kluyveromyces lactis* and *Pichia kudriavzevii*. The environmental factors for the maximum production of enzymatic activity, such as pH of the medium, temperature, and incubation period were studied. The results demonstrated that the highest production of β-galactosidase under pH 5.0 being 188.12 nmol/ml/min for *Kluyveromyces lactis* and 174.07 nmol/ml/min for *Pichia Kudriavzevii*. The optimal environmental conditions for β-galactosidase production such as the pH, temperature, and incubation period were, respectively, found to be 5 and 30 °C and 48 h for both tested strains.

Keywords: Isolation, Screening, β-galactosidase, Cultural conditions, Yeasts.

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1. Introduction

Lactose, is the principal constituent of milk (approximately 4.8%). Lactose utilization is limited because It's low solubility, lake of sweetness and its laxative effect **(Kretechner, 1972; Simoons, 1973; Sarabana, 1996)**. β-galactosidase (EC.3.2.1.23) is an enzyme that catalyzes the hydrolyzes of lactose into its monosaccharides, glucose, and galactose, it plays an important role to avoid lactose crystallization in concentrated dairy products and solve the problem of lactose intolerance by individuals who are deficient in enzyme. The enzyme is used to avoid the problems associates with whey utilization and disposal **(Gallagher et al., 1974; Sonawat et al., 1981; Hussain et al., 1995; Matioli et al., 2003)**. Also, production of biologically active galacto-oligosaccharides by enzyme of β-galactosidase have been mentioned in the literature **(Boon et al., 2000; Albayrak and Yang, 2002; Dağbağlı and Göksungur, 2008)**.

There are several microbiological sources of β-galactosidase but not all of them are taken or recommended as safe for food usage. Yeasts are thought to be the main source of microbial enzymes for food applications, such as lactase enzyme. The type of strains and the composition of the growth medium affect the enzyme's activity. Both *Kluyveromyces fragilis and*

Kluyveromyces lactis are now preferred sources for the production of the enzyme because they have been the most extensively studied **(Dağbağlı and Göksungur, 2008**; **AL-Jazairi et al., 2014)**.

Our objective consists of two essential points: first is to isolate and screening a yeast strain capable of β-galactosidase production from samples of local dairy products and identify it using morphological and molecular methods; secondly; to study the environmental factors affecting the productivity of β-galactosidase, such as pH of the medium, temperature, and incubation period.

2. Materials and Methods

2.1. Microorganisms used

Yeast isolates were isolated from various dairy products samples including butter milk, cheese e.g., Emmental, karish, Ras, Mozzarella and Cheddar, yogurt and raw milk which collected from local markets in Fayoum Governorate, Egypt.

2.2. Isolation and screening of yeast for β-galactosidase production

The yeast isolates were isolated from dairy samples by serial dilution method using medium which was described by **AL-Jazairi et al. (2014)** (2% lactose, 0.5% yeast extract, 1.0% bacteriological peptone, 2.0% agar and 0.01% chloramphenicol. pH was adjusted at 6.0). The plates were incubated for 3-5 days at 30 °C. The selected yeast colonies were further purified by sub culturing on petri dishes containing the same medium. On yeast malt agar slants, isolates were preserved at 4 °C and monthly subcultured. The purified isolates were screened for enzymatic activity by fermentation experiments and enzymatic productivity in the submerged culture after 48 h to select the most productive isolates ones for further studies. The stock culture of the most two productive isolates were maintained on Yeast Malt agar **(Dağbağlı and Göksungur, 2008)** at 4 °C and were monthly subcultured.

2.3. Morphological and genotypic characterization of the active enzymatic yeast isolates

The selected yeast isolates with the highest enzymatic yields were identified based on morphological traits as described by **Barnett et al. (1990)** and **Nahvi and Moeini (2004)** then conducted using genotypic characterization by determination of Internal Transcribed Spacer (ITS) sequence.

Genomic DNA was extracted utilizing the ABT DNA mini extraction kit (Applied Biotechnology Co. Ltd, Egypt) in accordance with the manufacturer's protocol. A reference for ITS region was classified as complete length if it encompassed the locus of the primers (ITS1 5`- TCCGTAGGTGAACCTGCGG -3` and ITS4 5`- TCCTCCGCTTATTGATATGC -3`) **(White et al., 1990)**.

The PCR products that were amplified were forwarded to Solgent (Solution for Genetic Technologies) Co. Ltd (South Korea) for the processes of gel purification and sequencing utilizing the ABI 3730XL DNA Analyzer. The resultant sequences underwent trimming and assembly within Geneious software (Biomatters). Subsequently, the trimmed sequences were subjected to identification through a search in the Basic Local Alignment Search Tool (BLAST) within GenBank.

The nucleotide sequence data of the isolated Internal Transcribed Spacer (IT_S) regions of ribosomal DNA (rDNA) were subjected to comparative analysis with the available IT_S sequences found in the GenBank database (http://www.ncbi.nlm.nih.gov/Blast), NCBI, Bethesda, MD, (USA) **(Altschul et al., 1997; Benson et al., 1999)**. The IT_S sequences corresponding to all yeast isolates were meticulously aligned with reference sequences extracted from the NCBI database employing the Clustal Omega multiple sequence alignment tool. Utilizing the Clustal Omega multiple sequence alignment program, which serves as a successor to Clustal W, a phylogenetic

dendrogram was constructed **(Thompson et al., 1994).** Phylogenetic trees were generated employing the Unweighted Pair Group Method with Arithmetic Mean (UPGMA).

2.4. Fermentation experiments

For Preparation of inoculum, Erlenmeyer conical flasks (250 ml) that contain 100 ml of specific inoculation medium **(Sarabana, 1996)** (which containing 2.0% lactose and 0.5% yeast extract. pH was adjusted at 6.0) were inoculated and were incubated under shaking (120 rpm) at 3 °C for one day.

One hundred milliliters of the fermentation medium which containing 3.0% lactose, 0.1% yeast extract. 0.1% (NH_4) H_2PO_4, 0.1%, $(NH_4)_2HPO_4$ 0.2 %, K_2HPO_4, and 0.01% $MgSO_4.7H_2O$. pH of the medium was adjusted at 7.0 (**Dağbağlı and Göksungur, 2008**) were dispensed in 250 ml Erlenmeyer flasks, sterilized and inoculated with 10 ml of 24 h old culture for each of the two tested strains. The inoculated flasks were incubated with shaking at 120 rpm for 48 h at 30 °C. At the end of experiments enzymatic activity was determined.

2.5. Factors affecting galactosidase enzyme production

In this study, the effect of various factors on enzyme production, fermentation medium was modified to be appropriate for testing the respective factors as follows, the used fermentation medium pH value was separately adjusted to different pH degrees of 3.0, 4.0, 5.0, 6.0, and 7.0. At the end of the fermentation period the enzymatic activity was estimated. Production of β - galactosidase by *Kluyveromyces lactis* and *Pichia kudriavzevii* using fermentation medium was studied at different temperatures ranging from 25 to 45 °C to investigate the effect of incubation temperature. For determination of the effect of incubation period, both tested strains were incubated at 30 °C on a shaker at 120 rpm and pH 5.0 for different incubation periods ranging from 24 to 120 h. At the end of every incubation period the enzyme activity was determined.

2.6. Determination of enzyme activity

At the end of incubation period the yeast cells were harvested by centrifugation at 4200 rpm for 20 min at 4 °C and the supernatant was discarded. 0.2 gm of cell re-suspended in 25 ml of 0.2 M potassium phosphate buffer pH 6.8 **(El-Diwany et al., 1994)**. The cell suspensions were sonicated on ice in glass tubes using a Pulse 150 Sonic Power Sonicator (150 W, 30 sec with 30 sec cooling periods) for 4 min. Then, they were centrifuged at 4200 rpm for 20 min at 4°C and the supernatant was used for measuring β- galactosidase activity **(Song and Jacques, 1997).** 750µl of substrate solution (ONPG) with pH 6.8 (o-Nitrophenyl-β-D-galactopyranoside (0.01 moles L^{-1}) prepared in a phosphate buffer of pH 6.8 and 250 µl of crude enzyme extract were added in a test tube. 2ml of phosphate buffer (pH 6.8, 0.2 M) was added to the test tube and then incubated at 37°C for 70 minutes. The reaction was quenched by adding 250 µl Na_2CO_3 (0.5 moles L^{-1}). Reaction progress was determined spectrophotometrically at 420 nm against the blank (750 µl ONPG, 2250 µl phosphate buffer of pH 6.8) **(Fernandes *et al*., 2002; Dahal *et al.*, 2020).**

$$\text{Enzyme activity(nmol/ml/min)} = \frac{\text{OD } 420 \times \text{Reaction volume}}{0.0045 \times \text{Enzyme volume} \times \text{Time}}$$

One unit of enzyme activity is the amount of β-galactosidase which forms 1 µmole of o-Nitrophenol in 1 min.

3. Results and Discussion

3.1. Isolation and screening of yeast for β-galactosidase production

In this study, twenty-two yeast isolates were isolated from local dairy products. 4.0 isolates from butter milk, 2.0 isolates from Emmental cheese, 5.0 isolates from karish, 2.0 isolate from Ras cheese,1.0 isolates from Mozzarella cheese, 3.0 isolates from Cheddar cheese, 3.0 isolates from yogurt and 2.0 isolates from raw milk as shown in **Table 1**. The isolates were screened for β-galactosidase activity in order to select the most productive isolates. The isolates were examined in the fermentation experiments and after 48 hours β-galactosidase activity was determined. As

shown in **Table 1**, isolates No. 1 and No. 13 were found to have the highest enzyme activity 170.84 and 155.56 nmol/ml/min, respectively. These two isolates will be used for all the next experiments.

Table 1. The β–galactosidase activity by yeast isolates after 48 h fermentation

Source	Isolate No.	β–galactosidase activity (nmol/ml/min)	Source	Isolate No.	β–galactosidase activity (nmol/ml/min)
Butter milk	1	170.84	**Ras cheese**	12	7.95
	2	52.12		13	155.56
	3	5.07		14	93.81
	4	8.53	**Mozzarella cheese** **Cheddar cheese**	15	107.42
	5	5.85		16	51.66
Emmental chees	6	5.72		17	44.89
Karish cheese	7	122.88		18	11.48
	8	5.95	**Yogurt**	19	62.31
	9	5.68		20	31.05
	10	5.33		21	7.49
	11	11.40	**Raw milk**	22	145.50

3.2. Identification of the most active isolates

The most active isolates were identified based on morphological characteristics as shown in **Table 2**, where the two isolates differed in cells shape, colonies elevation and their colors, while both isolates showed similar behavior towards the Gram test as well as transparency and margins of the colonies.

Table 2. Morphological characters of the studied isolates.

Morphology character	Isolate No. 1	Isolate No. 13
Shape	Circular-large	Ovoid-large
Elevation	Convex	Raised
Margin or edge	Entire	Entire
Color	Creamy	White-creamy
Transparency	Opaque	Opaque
G-stain	G^+	G^+

Results in **Table 3**, clearly revealed that IT_S sequences of the selected isolate No. 1 had 100% genetic similarity with *K. lactis* |OP363173.1| and *K. lactis* |OP363178.1|. Results in **Table 4**, clearly revealed that the IT_S sequences of the selected isolate No (13) had 99.49% genetics similarity with *Pichia kudriavzevii* | MT599316.1|, *Pichia kudriavzevii* |OP526912.1|, and *Pichia kudriavzevii* |ON797477.1|.

To ascertain the taxonomic positions of the selected isolates, ITS sequences were analyzed via NBCI data base as illustrated in **Figures 1 and 2** and **Tables 3 and 4**. As shown in **Figure 1**, isolate No. 1 was in the same cluster with *K. lactis* |KX981203.1|, and *K. lactis* |OP363178.1|,

while isolate No. 13 was in the same sub-subcluster with *Pichia kudriavzevii* |MT599316.1| and *Pichia kudriavzevi* |OP375521.1| **(Figure 2)**.

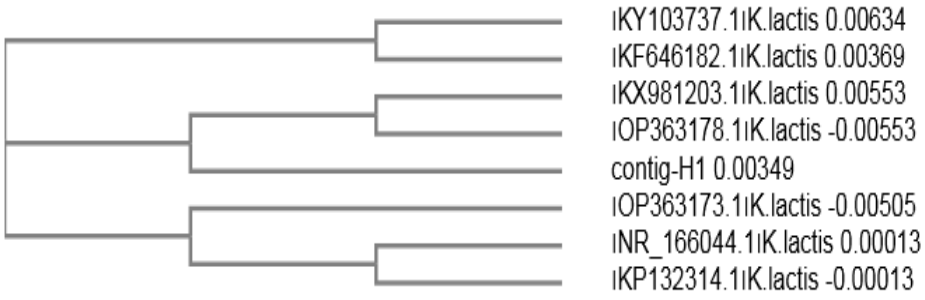

Figure 1. The relationship between our isolate and six representative strains., according to Phylogenetic analysis of bacterial isolates based on IT_S region of geneomic rDNA gene. The tree based on the clustal omegamultiple sequence alignment programs. Rooted phylogentic tree (UPGMA).

Table 3. Genetics similarity percentage of 7 isolate of *Kluyveromyces lactis*, based on IT_S region of genomic IT_S rDNA gene

	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**
1	100							
2	98.97	100						
3	98.97	84.92	100					
4	98.97	82.48	100	100				
5	98.21	97.07	98.37	98.37	100			
6	100	98.89	100	100	97.56	100		
7	100	98.89	100	100	97.56	100	100	
8	97.68	96.59	97.67	97.69	95.60	97.30	97.30	**100**

1*: Kluyveromyces lactis* (H1), 2: |KX981203.1| *K. lactis*, 3: |NR_166044.1| *K. lactis*, 4: |KP132314.1| *K. lactis*, 5: |KF646182.1| *K. lactis*, 6: |OP363173.1| *K. lactis*, 7: |OP363178.1| *K. lactis*, and 8: |KY103737.1| *K. lactis*.

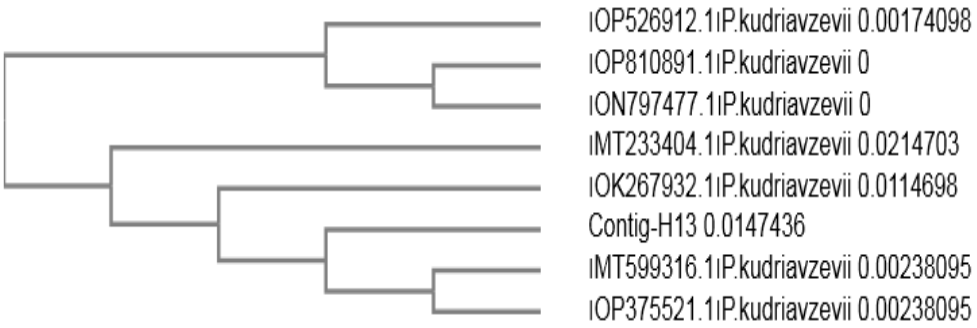

Figure 2. The relationship between our isolate and six representative strains., according to Phylognetic analysis of bacterial isolates based on ITS region of geneomic rDNA gene. The tree based on the clustal ome gamultiple sequence alignment programs. Rooted phylogentic tree (UPGMA).

Table 4. Genetics similarity percentage of 7 isolate of *Pichia kudriavzevii*, based on IT_S region of genomic IT_S rDNA gene

	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**
1	100							
2	99.49	100						
3	89.23	98.49	100					
4	92.56	98.53	97.74	100				
5	99.49	98.41	97.24	99.76	100			
6	94.61	99.52	98.74	98.78	98.33	100		
7	99.49	98.44	97.74	100	99.55	98.57	100	
8	97.43	99.28	97.49	97.07	99.28	96.67	99.28	100

1*: Pichia kudriavzevii* (H13), 2:| MT599316.1| *Pichia kudriavzevii*, 3: |MT233404.1| *Pichi kudriavzevii*, 4: | OP810891.1| *Pichia kudriavzevii*, 5: |OP526912.1| *Pichia kudriavzevii*, 6: |OP375521.1| *Pichi kudriavzevii*, 7:|ON797477.1| *Pichia kudriavzevii*, and 8:|OK267932.1|*Pichia kudriavzevii*.

3.3. Factors affecting β-galactosidase production by the selected yeast strains

3.3.1. pH values

In this experiment, the fermentation medium was adjusted to different pH values 3.0, 4.0, 5.0, 6.0 and 7.0 to study the effect of the pH of the medium on the production of the enzyme by the selected strains of *Kluyveromyces lactis* No. 1 and *Pichia Kudriavzevii* No. 13. The obtained results are graphically illustrated in **Figure 3**.

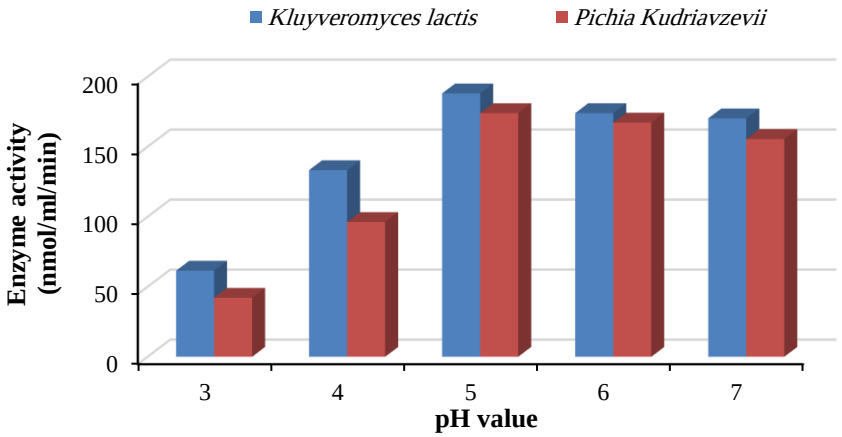

Figure 3. Effect of medium pH on β-galactosidase production by tested strains.

It is clear from the obtained results that the highest β-galactosidase production occurred at pH 5.0 being 188.12 nmol/ml/min for *Kluyveromyces lactis* No. 1 and 174.07 nmol/ml/min for *Pichia Kudriavzevii* No. 13. Also, the results illustrated that the enzyme production were decreased about 7.47% and 9.48% at pH 6.0, 96.09% and 89.37% at pH 7.0 for *Kluyveromyces lactis* No. 1 and *Pichia Kudriavzevii* No. 13, respectively. Similar results were obtained by **Gupte and Nair (2010)** and **Khaled et al. (2016)**. Otherwise, **Kheiralla et al. (1994)** studied the effect of pH on enzyme production by *Candida pseudotropicalis*. They found that the optimum pH was 7.0. Whoever, **Sarabana (1996)** reported that pH 3.0 was the optimum initial pH for enzyme production by *Kluyveromyces fragilis*. While **Afolabi et al. (2022)** reported that maximum β-galactosidase production by *Kluyveromyces marxianus* strain SLDY–005 was at pH 6.0.

3.3.2. Effect of incubation temperature

The fermentation experiments were studied at incubation temperatures of 25, 30, 35, 40, and 45 °C to reveal the most favorable temperature for the maximum β-galactosidase production. As shown in **Table 5**, the optimum incubation temperature was 30 °C for the β-galactosidase production and its amount was 188.12 and 174.07 nmol/ml/min for *Kluyveromyces lactis* No. 1 and *Pichia Kudriavzevii* No. 13, respectively. Data in **Table 5** revealed that enzyme production was decreased about 3.39, 12.88, 61.10, 91.30, 24.70, 8.36, 36.31, and 82.67% for *Kluyveromyces lactis* No. 1 and *Pichia Kudriavzevii* No. 13 at 25, 35, 40 and 45 °C, respectively.

Table 5. Effect of incubation temperature on the β-galactosidase production by tested strains

Temperature (°C)	***Kluyveromyces lactis* No. 1**		***Pichia Kudriavzevii* No. 13**	
	β-galctosidase activity (nmol/ml/min)	**Activity* (%)**	**β-galctosidase activity (nmol/ml/min)**	**Activity* (%)**
25	181.56	96.51	131.04	75.30
30	188.12	100.00	174.07	100.00
35	163.89	87.12	159.51	91.64
40	73.11	38.90	110.68	63.69
45	16.32	8.70	30.17	17.33

*The maximum activity was assumed as the 100%.

Our results supported the findings of **EL-Sawah et al. (1991), Babu et al. (2014), Khaled et al. (2016), Kumari et al. (2019)** and **Afolabi et al. (2022)**, who found that the optimum temperature was 30 °C for maximum β-galactosidase production from *Kluyveromyces fragillis* and *Kluyveromyces marxianus*. While **Decleire et al. (1987)** mentioned that optimum temperature for production of enzyme by *Kluyveromyces fragilis* was 25 °C. On the other hand, **Dağbağlı and Göksungur (2009)** reported that the highest enzyme production by *Kluyveromyces lactis* NRRLY-8279 was 37 °C.

3.3.3. Effect of fermentation period

The fermentation period is very important factor for economic success of the commercial production of enzyme **(Fogarty and Kelly, 1980)**. In these experiments, the fermentation period was prolonged for 120 h and the enzyme activity was determined at 24 h intervals. The results in **Table 6** showed that the highest β–galactosidase activity was obtained after 48 h. The rate of the enzyme production was relatively low during the first 24 h. of fermentation for the two tested strains 68.93 and 63.13% for *Kluyveromyces lactis* No. 1 and *Pichia Kudriavzevii* No. 13, respectively. The majority of the enzyme was synthesized during the second day of the fermentation (31.07 and 36.87% for *Kluyveromyces lactis* and *Pichia Kudriavzevii*, respectively.

Table 6. Effect of incubation period on the production of β-galactosidase by tested strains

Incubation period (h)	***Kluyveromyces lactis* No. 1**		***Pichia Kudriavzevii* No. 13**	
	β-galactosidase activity (nmol/ml/min)	**Activity* (%)**	**β-galactosidase activity (nmol/ml/min)**	**Activity* (%)**
24	128.48	68.93	109.31	63.13
48	186.39	100.00	173.16	100.00
72	171.95	92.30	160.01	92.41
96	103.04	55.30	78.63	45.41
120	70.02	37.60	29.12	16.8

*The maximum activity was assumed as the 100%.

However, increasing the fermentation period over 48 h. the enzyme activity decreased by 7.7, 44.7, and 62.4 % for *Kluyveromyces lactis* and by 7.6, 54.6, and 83.2% for *Pichia Kudriavzevii* at 72, 96 and 120 h. respectively. The decrease in enzyme activity with the extension of fermentation period may be due to the suppression effect of the products from the enzymatic hydrolysis of

lactose. These results are in-agreement with those of **EL-Sawah et al. (1991)**. They found that the optimum incubation time for the production of *β*-galctosidase by *Kluyveromyces fragillis* and *Kluyveromyces lactis* was 48 h, after which the levels begin to decline. Also, **Kassem et al. (1991)** reported that the enzyme yield by *Kluyveromyces fragillis* and *Kluyveromyces lactis* reached maximum after 48 h, the beginning of the stationary phase. While **Paneasar (2008)** and **Dağbağlı and Göksungur (2009)** reported that the maximum enzyme production by *Kluyveromyces marxianus* and *Kluyveromyces lactis* NRRLy-8279 after an incubation period of 24 h. On the other hand, (**Afolabi et al., 2022)** found that *Kluyveromyces marxianus* strain SLDY–005 produced the highest β-galactosidase after an incubation period of 36 h**.**

4. Conclusions

Isolate No. 1 identified as *Kluyveromyces lactis* and isolate No. 13 identify as *Pichia kudriavzevii* were selected as promising β-galactosidase producers. The optimal operating conditions for β-galactosidase synthesis such as pH, temperature, and incubation period were found to be 5 and 30 °C and 48 hours for both tested strains, respectively.

References

Afolabi, F. T., Adewale, O. P., & Adeyemo, S. M. (2022). Isolation, production and optimization of beta-galactosidase by utilizing yeasts isolated from selected dairy products. *Journal of Food Safety and Hygiene*, 8(3), 170–182.

Albayrak, N., & Yang, S. T. (2002). Production of galacto-oligosaccharides from lactose by Aspergillus oryzae β-galactosidase immobilized on cotton cloth. *Biotechnology and Bioengineering*, 77(1), 8–19.

Al-Jazairi, M., Abou-Ghorria, S., & Bakri, Y. (2014). Isolation and identification of a new yeast isolate with high beta-galactosidase activity from Syrian dairy products. *International Food Research Journal*, 21(2), 541–546.

Altschul, S. F., Madden, T. L., Schäffer, A. A., Zhang, J., Zhang, Z., Miller, W., & Lipman, D. J. (1997). Gapped BLAST and PSI-BLAST: A new generation of protein database search programs. *Nucleic Acids Research*, 25(17), 3389–3402.

Babu, M., Raj, S. P., Nirmala, C. B., Deccaraman, M., & Sagadevan, E. (2014). Production of single cell protein using Kluyveromyces marxianus isolated from paneer whey. *International Journal of Biomedical and Advance Research*, 5(5), 255–258.

Barnett, J. A., Yarrow, D. W., & Payne, R. W. (1990). The yeast: Classification and identification (2nd ed., pp. 50–77). Cambridge University Press.

Benson, D. A., Boguski, M. S., Lipman, D. J., Ostell, J., Ouellette, B. F., Rapp, B. A., & Wheeler, D. L. (1999). GenBank. *Nucleic Acids Research*, 8, 12–17.

Boon, M. A., Rite Vant, T. K., & Janssen, A. E. M. (2000). Enzymatic synthesis of oligosaccharides: Product removal during a kinetically controlled reaction. *Biotechnology and Bioengineering*, 70(4), 411–420.

Dağbağlı, S., & Göksungur, Y. (2008). Optimization of β-galactosidase production using Kluyveromyces lactis NRRL Y-8279 by response surface methodology. *Electronic Journal of Biotechnology*, 11, 1–12.

Dağbağlı, S., & Göksungur, Y. (2009). Optimization of β-galactosidase production in stirred tank bioreactor using Kluyveromyces lactis NRRL Y-8279. *Food Science and Biotechnology*, 18(6), 1342–1350.

Dahal, B., Karki, S., Adhikari, N., & Shrestha, U. T. (2020). Characterization of β-galactosidase from lactose utilizing yeast isolated from the dairy sample. *Tribhuvan University Journal of Microbiology*, 7(1), 133–141.

Decleir, D., Decat, W., & Van Hayn, N. (1987). Comparison of various permeabilization treatments on Kluyveromyces by determining in situ β-galactosidase activity. *Enzyme and Microbial Technology*, 9, 300–302.

El-Diwany, A. I., Hussain, A. A., Kheiralla, Z. H., & Ashour, S. M. (1994). Studies on β-galactosidase I: Screening and extraction. *Egyptian Journal of Microbiology*, 29(3), 219–230.

El-Sawah, M. M. A., Kassem, M. M., Ismail, L. L., & Hoseny, M. A. (1991). Optimization of β-galactosidase production by yeast: 2. Selection of whey supplement and growth conditions. *Journal of Agricultural Science, Mansoura University*, 16(12), 2866–2872.

Fernandes, S., Geueke, B., Delgado, O., Coleman, J., & Hatti-Kaul, R. (2002). β-Galactosidase from a cold-adapted bacterium: Purification, characterization and application for lactose hydrolysis. *Applied Microbiology and Biotechnology*, 58, 313–321.

Fogarty, W. M., & Kelly, C. T. (1980). Amylases, amyloglucosidases and related glucoamylases. *Economic Microbiology*, 5, 115–170.

Gallagher, C. R., Molleson, A. L., & Caldwell, J. H. (1974). Lactase intolerance and fermented dairy products. *Research*, 65, 418–419.

Gupte, N., & Nair, J. S. (2010). β-Galactosidase production and ethanol fermentation from whey using Kluyveromyces marxianus NCIM 3551. *Journal of Scientific and Industrial Research*, 69, 855–859.

Hussain, A. A., El-Diwany, A. I., Kheiralla, Z. H., & Ashour, S. M. (1995). Studies on β-galactosidase from Candida pseudotropicalis III: Purification and some properties of the pure enzyme. *Egyptian Journal of Microbiology*, 30(1), 1–17.

Kassem, M. M., El-Sawah, M. M. A., Ismail, I. I., & El-Hoseny, M. A. (1991). Optimization of β-galactosidase production by yeasts: 1. Selection of yeast species and growth media. *Journal of Agricultural Science, Mansoura University*, 16(12), 2859–2865.

Khaled, B., Fethia, F. N., Kemal, G., Matpan, B. F., Omar, A., & Amina, H. (2016). Production of intracellular β-galactosidase using a novel Kluyveromyces marxianus OIV13-247 isolated from an Algerian dairy product. *Research Journal of Biotechnology*, 11(6), 35–43.

Kheiralla, Z. H., El-Diwany, A. I., Hussain, A. A., & Ashour, S. A. (1994). Studies on β-galactosidase from Candida pseudotropicalis II: Production and some properties of the crude enzyme. *Egyptian Journal of Microbiology*, 29(3), 231–245.

Kretchmer, N. (1972). Lactose and lactase. *Scientific American*, 227, 71.

Kumari, S., Panesar, P. S., Kaur, R., & Bera, M. B. (2019). Statistical modeling of β-galactosidase production from novel yeast isolate. *Journal of Scientific and Industrial Research*, 8, 81–85.

Matioli, G., Mores, F. F. D., & Zanin, G. M. (2003). Operated stability and kinetics of lactose hydrolysis by β-galactosidase. *Scientific Health Science Journal*, 25, 7–12.

Nahvi, I., & Moeini, H. (2004). Isolation and identification of yeast strains with high beta-galactosidase activity from dairy products. *Biotechnology*, 3, 35–40.

Panesar, P. S. (2008). Production of β-galactosidase from whey using Kluyveromyces marxianus. *Research Journal of Microbiology*, 3(1), 24–29.

Sarabana, S. S. H. (1996). Production of lactase by different microorganisms [*Master's thesis, Faculty of Agriculture, Cairo University*].

Simoons, F. J. (1973). New light on ethnic differences in adult lactose intolerance. *American Journal of Digestive Diseases*, 2, 826–502.

Sonawat, H. M., Agrawal, A., & Dutta, S. M. (1981). Optimization of β-galactosidase extraction from Kluyveromyces marxianus. *Enzyme and Microbial Technology*, 15, 1063–1065.

Song, D. D., & Jacques, N. A. (1997). Cell disruption of Escherichia coli by glass bead stirring for the recovery of recombinant proteins. *Analytical Biochemistry*, 248, 300–301.

Thompson, J. D., Higgins, D. J., & Gibson, T. J. (1994). CLUSTAL W: Improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. *Nucleic Acids Research*, 22, 4673–4688.

White, T. J., Bruns, T., Lee, S., & Taylor, J. (1990). Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In M. A. Innis, D. H. Gelfand, & J. J. Sninsky (Eds.), PCR protocols (pp. 315–322). Academic Press.

Zhou, Q. Z., & Chen, X. D. (2001). Effects of temperature and pH on the catalytic activity of the immobilized β-galactosidase from Kluyveromyces lactis. *Biochemical Engineering Journal*, 9, 33–40.