J. Agric. Sci. Mansoura Univ., 31 (2),: 999 - 1014, 2006

STUDIES ON THE PRODUCTION OF MICROBIAL β-GALACTOSIDASE

1. OPTIMIZATION OF β-GALACTOSIDASE EXTRACTION FROM *Kluyveromyces fragilis* AND *Streptococcus salivarius* SUBSP. *thermophilus*

El-Sawah, M. M. A.[1]; M. M. Kassem[1]; Aida H. Afify[1]; I. I. Ismail[1] and Azza S. A. Menshawy[2]

[1] Microbiol. Dept., Fac. of Agric., Mansoura Univ., Mansoura, Egypt. melsawah@hotmail.com

[2] Fac. of Agric., Omar El-Mokhtar Univ., El-Bieda, Libya

ABSTRACT

Investigation of β-galactosidase production by three yeasts and five bacterial species of lactose-fermenting microorganisms during different incubation periods was carried out. *K. fragilis* and *S. salivarius* subsp. *thermophilus*, in shaken cultures, proved to be the most potent and produced the highest β-galactosidase activity after 24 h incubation. Toluene was the most effective solvent in releasing β-galactosidase from the microbial cells. β-Glactosidase release was increased with the solvent concentration till it reached its maximum activity at 2.0% (v/v). Higher concentrations were correlated with a great decrease in β-galactosidase activity. Toluene released β-galactosidase in higher yield after 15 min. The higher contact time was correlated with a great decrease in activity. Maximum β-galactosidase activity was extracted at 37°C during suspensions of cells in 0.1-M phosphate buffer at pH 6.8. β-Galactosidase release was increased with the increase of the exposure time for sonication till it reached its maximum after 5 minutes. The maximum release of β-galactosidase was after 6 cycles of freezing and thawing of the cell pellets and cells in buffer. Higher activity was obtained when the pellets were used.

INTRODUCTION

β-D-Galactosidase enzyme is widely distributed in nature and can be found in yeasts, fungi and bacteria (Shukla, 1975). The classical source of bacterial lactase is *Escherichia coli* (Hu *et al.*, 1959); strains of *Streptococcus lactis* 7962 (McFerters *et al.*, 1967). A strain of *Bacillus* was used to produce β-galactosidase enzyme by Ikura and Horikas (1979). This strain produced β-galactosidase inducibly and the induction of the enzyme was faster at pH 10.5 than at pH 7.2. At pH 8.5, intact cells exhibited higher enzyme activity than toluenized cells. At 60°C, pH 10.2 grown cells exhibited higher enzyme activity than pH 7.6 grown cells. Griffiths and Muir (1979) described the properties of the intracellular β-galactosidase of a thermophilic Bacillus. In 1978, Ramana Rao and Dutta reviewed that 62 strains of yeasts, molds, and bacteria were screened for β-galactosidase activity and they found that the molds exhibited the lowest enzyme activity by highest cell yields, while bacteria produced lowest cell yield and maximum enzyme activity. Crude β-galactosidase was produced by lysis of cells of *Streptococcus thermophilus* grown on deproteinized whey by Greenberg and Mahoney (1982). The

enzyme from this source has a neutral pH optimum, a temperature optimum in buffer at 55°C and is more heat stable than the neutral-pH lactases from yeasts, which are available commercially. Also Ramana Rao and Dutta (1977) reported on the production of β-galactosidase from bacteria grown in deproteinized cheese whey. The activity of β-D-galactosidase was studied in 13 strains of *Lactobacillus*. Studies on the enzymes, which hydrolyze lactose in some species of Lactobacilli done by Premi *et al.* (1972) came to the conclusion that in eight strains of *Lactobacillus casei*, no β-D-galactosidase activity was present. De Macias *et al.* (1983) reported that β-D-Galactosidase has been isolated from *Lactobacillus helveticus*, strain isolated from natural starters for the manufacture of Argentine hard cheese and its properties. *Lactobacillus acidophillus* was studied by Nielsen (1987) for the isolation and characterization of lactose hydrolyzing enzyme β-glactosidase. Lactase has been also isolated from several bacterial sources.

The dairy yeast *Kluyveromyces lactis* was first described by Beyerinck in 1889. It is a well known dairy organism used in the production of certain fermented milk products like kumiss and Kurung. The production of biomass and β-galactosidase by the lactose-utilizing yeast *Candida pseudotropicalis* in whey medium was studied by Gomez and Castillo in 1983. According to Klup (1975) the best commercial sources of -galactosidase include: *Escherichia coli*, *Aspergillus niger* and lactose fermenting yeast (*K. fragilis* and *K. lactis*). In 1978, Mahoney and Whitaker provided detailed discussion on a β-galactosidase isolated from *K. fragilis*. Also optimum conditions for β-galactosidase produced by *K. fragilis* were studied by Sonawat *et al.*, 1981. They found that enzyme production reached the maximum after 8-12 h of incubation. Different heat treatments had no effect. The fermentation of lactose was achieved by the yeast *K. fragilis* using an immobilized-cell-tubular-reactor by Glanetto *et al.* (1986). The hydrolysis of lactose solution by whole cells of *K. bulgaricus* and cell-free extracts was compared to the hydrolysis of -nitrophenyl-β-galactoside by Decleire *et al.* (1985). *K. fragilis* is one of the best known sources of lactase (Van Dam et al., 1950; Young and Healey, 1957; Davies, 1964; Feniksova *et al.*, 1971; Wondorff and Amundson, 1971; Uwajima *et al.*, 1972; Kosikowski and Wierzbicki, 1973; Guy and Bingham, 1978; Sanchez and Castillo, 1980; Caputto *et al.*, 1984; Barbosa *et al.*, 1985 and Champluvier *et al.*, 1988). β-Galactosidase was reported also in *K. marxianus* (Goncalves and Castillo, 1982; Champluvier *et al.*, 1988 and Mahoney and Wilder, 1988). Recently, the black yeast *Aureobasidium pullulans* was reported as a new source of β-galactosidase enzyme by Deshpande *et al.*, 1989.

Various methods can be used to release intracellular enzymes such as β-galactosidase depending on their localization inside the cell, intended use, and stability (Engler, 1985 and Kula and Schutta, 1987). Of the several methods reported for the extraction of β-galactosidase: buffer extraction of dried yeast (Wendorff and Amundson, 1971), autolysis with chloroform (Van Dam *et al.*, 1950) and with toluene (Feniksova *et al.*, 1971) and mechanical breakage of the cells with glass beads (Davies, 1964). Stred'ansty *et al.* (1993) published an interesting study of β-galactosidae extraction from *K.*

marxianus cells and pointed out the followings. Mechanical methods are currently preferred for large-scale application, but they require high investment and operational costs. In addition, rather small cell wall fragments are produced, which introduces problems in following solid/liquid separation. Autolysis is not suitable for β-galactosidase extraction from yeast cells because high induction temperatures are used. Cell treatment with acids is also not suitable for β-galactosidase extraction because of the low stability of β-galactosidase. Enzymatic cell disruption is usually not cost-effective on large-scale use and detergents are difficult to remove from the enzyme preparation. About 90% of β-galactosidase activity was released from cells of *K. marxianus* to the extracellular space after 5 h treatment with 1% (v/v) chloroform at 28°C and pH 7.5. Lower concentrations of chloroform were insufficient for β-galactosidase extraction, and higher concentrations were without any effect. About 93% of β-galactosidase was released from cells of *K. marxianus* at pH 10.5, and 0.1 M phosphate concentration in the second hour of the procedure, even at pH 9.5 in 0.5 M phosphate after 1.5 h treatment. Without any solvent addition, 85% of β-galactosidase activity was released from cells of *K. marxianus* after 24 h treatment with 0.5 M phosphate buffer (pH 8.5) at 37°C. Maximal yields of β-galactosidase were reached at 37 °C. At 40-45°C the initial extraction rate was higher, but the yield of enzyme was lower because the total enzyme activity began to fall after 1 h treatment. The extraction of β-galactosidase from cells of *K. marxianus* was higher at 0.5 M than 0.1 M phosphate buffer pH 7.5. A good method for β-galactosidase extraction is solvent treatment. Solvent treatment is a simple and inexpensive method suitable for large-scale application (Mahoney and Whitaker, 1978; Fenton, 1982; Goncalves and Castillo, 1982; and Stredansky *et al.*, 1993).

The objectives of this study were (1) to select the best microbial sources and conditions of β-galactosidase extraction which maximize β-galactosidase yield.

MATERIALS AND METHODES

Microorganisms:

Yeasts:

Kluyveromyces fragilis EMCC DSM 70292 and *Kluyveromyces kefyr* EMCC 75 were obtained from Microbiol. Resource Center, Cairo MIRCEN (CAIM), Ain Shams Univ., A.R.E. *Kluyveromyces lactis* was obtained from Microbiol. Dept., Fac. of Agric, Mansoura Univ., Mansoura, Egypt. The original cultures were maintained at 4°C with monthly transfers to medium containing (w/v): 0.3% malt extract (Difco); 0.5% peptone (Difco), 0.3% yeast extract (Difco); 0.2% lactose (Sigma Chem. Co.) and 2% agar at pH 6.0.

Bacteria:

All cultures, namely, *Lactococcus lactis* subsp. *cremoris* EMCC 10511, *Lactococcus lactis* subsp. *lactis*, *Lactococcus lactis* subsp. *lactis* biovar diacetilactis EMCC 10510, *Streptococcus salivarius* subsp.

thermophilus EMCC 10509 and *Leuconostoc cremoris* were obtained from Microbiol. Resource Center, Cairo MIRCEN (CAIM), Ain Shams Univ., Egypt. The original cultures were maintained at 4°C with monthly transfers to medium containing (w/v): 0.5% yeast extract; 0.5% peptone; 0.5% NaCl; 1.0% glucose and 2.0% agar at pH 7.2-7.4.

Culture media for β-galactosidase production:

Unless otherwise specified, the lactose medium, Goncalves and Castillo (1982), was used as a basal medium for β-galactosidase production. The medium has the following composition (g/L): lactose, 20.0; yeast extract, 3.0; peptone, 5.0 and malt extract, 3.0. The pH was adjusted to 5.5.

Unless otherwise specified, the lactose medium, Goncalves and Castillo (1982) was used as a basal medium for β-galactosidase production. The medium has the following composition (g/L): yeast extract, 5.0; peptone, 10.0;lactose, 5.0 and NaCl, 5.0. The pH was adjusted to 7.0.

Inoculum preparation:

The maintained agar slants were used as propagation for the test organisms. Slants were inoculated with the organisms and after 24 hours incubation at 30°C (yeast) or 37°C (bacteria), slants were stored in refrigerator at 4°C or used directly as working cultures for preparation of seeding material.

Consequently, the growth on the agar slant was scrapped, using 5 ml sterilized tap water then transferred to a 250 ml flask containing 30 ml sterile liquid medium. The resulting cell suspension (after 18 h incubation at 30°C in shaking incubator) were used for inoculating the sterilized experimental (fermentation) flasks.

Cultivation:

Unless otherwise stated, cultivation was made in 300 ml Erlenmeyer flasks each containing 45 ml of sterile medium (pH 6.0 for yeast and 7.0 for bacteria). A 5 ml of an 18-h-old inoculum was transferred to the culture medium. The flasks were incubated at 30°C (yeast) or 37°C (bacteria) with agitation (120 strokes/min) in a reciprocating shaker bath. After incubation for 48 hours, the culture broth from each flask was used for measuring pH and growth. Cells were harvested by centrifugation at 10 000 rpm for 15 min at 4°C.

Enzyme extraction:

The harvested cells were washed twice with 0.067 M potassium phosphate buffer (pH 6.8). Washed cells were suspended in 25 ml of 0.067 M potassium phosphate buffer, pH 6.8, containing 0.1 mM manganese chloride and 0.5 mM magnesium sulfate. 10 ml of the suspension were treated with toluene (2% -v/v- at 37°C for 10 min). Broken cells were removed from the assay mixture by centrifugation at 15 000 rpm for 30 min at 4°C and the precipitated fraction was discarded. The supernatant was used as enzyme source.

In ultrasonic treatment, the microbial cells suspended in buffer were sonicated by MSE ultrasonic disintegrator operated at 200 watt at intervals time period. The samples were kept in cold in an ice bath, the resulting extracts were centrifuged at 10 000 rpm for 10 min at 4°C and the supernatant was used for -galactosidase assay (Sanchez and Hardisson, 1979; Moldoveanu and Kluepfel, 1983 and El-Diwany *et al*, 1986 and 1994).

In freezing and thawing treatment, the microbial cells in buffer were frozen and thawed for 7 days (seven cycles). β-Galactosidase activity was measured periodically in the solution. Cell pellets was also treated for seven cycles, followed by re-suspending in equal volume of the same buffer and -galactosidase activity was measured (Decleire *et. al* 1987 and El-Diwany *et. al*, 1994).

β-Galactosidase assay:

The assay mixture consisted of 2.8 ml of 50 mM sodium phosphate buffer (pH 6.8) and 0.1 ml of 68 mM -nitrophenyl β-D-galactopyranoside (ONPG), Sigma Chem. Co., USA, then 0.1 ml of enzyme source was added. The assays were carried out at incubation temperature at 37°C (yeast) or 45° C (bacteria). After 10 min, the reaction was stopped by addition of 3.0 ml of ice-cold 0.5 M Na_2CO_3. o-nitrophenyl liberated was estimated at 420 nm. One unit of enzyme was defined as activity, which released l mol of -nitrophenyl /ml/min, under the conditions specified above.

Growth measurement:

Yeast and bacteria growth were measured as absorbance of cultures at 650 nm. Before reading, the suspensions always were diluted to give turbidity reading lower than 1.0 E.

Protein determination:

The protein contents of the enzyme extracts were determined by the method of Lowry *et al.* (1951), with bovine serum albumin as standard.

RESULTS AND DISCUSSION

Screening of yeast and bacterial cultures for the production of -galactosidase:

The present experiment aims to investigate the ability of certain local yeast and bacterial species for the production of β-galatosidase. This may be considered as a prelude for selecting the most potent yeast and bacterial species for production of active β-galactosidase. For this purpose, β-galactosidase activities were investigated and evaluated for the yeast and bacterial species listed in Tables 1 & 2. These yeast and bacterial species were cultured using the shaken culture technique. The species were cultured on the basal media described earlier under shaking conditions. Fermentation was lasted for 8, 12, 24, 48, 72 and 96 h. Three yeasts and five bacterial species were investigated. At the end of fermentation process, the growth rate was measured at 650 nm. The culture broth was centrifuged to obtain

the cells and the cell free extract. β-Galactosidase activities and protein concentrations were determined. The results in Table (1) showed that the highest level of β-galactosidase production (12.15 U/ml, $OD_{650\ nm}$ 4.0) was obtained by *Kluyveromyces fragilis* followed by *K. kefyr* (8.5 U/ml, $OD_{650\ nm}$ 3.7) and *K. lactis* (7.0 U/ml, O.D. $_{650\ nm}$ 0.9).

It is also seen from Table (2) that *Streptococcus salivarius* subsp. *thermophilus* proved by far to be the most active producer for β-galactosidase followed by *Lactococcus lactis* subsp *cremoris* as a highly active enzyme producer. The corresponding β-galactosidase activities in U/ml were 37.3 and 15.2, respectively.

Table (1): β-Galactosidase activity of some yeast species.

Yeast	Cultivation Time (h)	Growth rate $OD_{650\ nm}$	Enzyme activity	
			U/ml	U/mg
Kluyveromyces fragilis EMCC DSM 70292	8.0	4.0	10.15	3.6
	12	4.0	12.15	3.7
	24	4.0	12.15	3.9
	48	3.8	6.2	1.5
	72	3.6	5.5	0.9
	96	3.5	5.0	0.5
Kluyveromyces kefyr EMCC 75	8.0	3.5	8.0	1.4
	12	3.7	8.5	1.5
	24	3.7	8.2	1.4
	48	3.4	3.9	0.9
	72	3.3	2.8	0.7
	96	3.0	2.1	0.5
Kluyveromyces lactis	8.0	0.8	5.5	2.3
	12	1.0	6.0	2.3
	24	0.9	7.0	2.5
	48	0.8	5.5	1.5
	72	0.8	3.6	1.0
	96	0.7	1.0	0.3

Optimum β-galactosidase production, in lactose containing media, was obtained by growing the test yeasts for 12 - 24 h. On the other hand, optimum cultivation times observed for bacterial species excreting elevated quantities of the enzyme in shaken cultures are 24h.

Large number of reports in literature on β-galactosidase are concerned with yeast and bacteria as major sources of enzyme, notably *K. fragilis* and *Str. salivarius* subsp. *thermophilus* (Mahoney *et al.*, 1974; Ramana Rao and Dutta, 1977 &1979; Sonawat *et al.*, 1981; Greenberg and Mahoney, 1982; Caputto *et al.*, 1984 and Thomas *et al.*, 1984), also they referred to relationship between the growth rate and β-galactosidase production.

Shortly, the efficient β-galactosidase producing strain is the first step towards development of the production process. Suitable nutrients, including an inducer, environmental conditions and process control strategy must also be chosen. As stated before, the present work aims at studying the microbial -galactosidase activity. The results of this part of the work indicated that *K. fragilis* and *Str. salivarius* subsp. *thermophilus* in shaken cultures at 24 h

incubation proved to be the potent organisms for producing active -galactosidase. These results collectively justified the selection of the last-named microbial species for succeeding work.

Table (2): β-Galactosidase activity of some bacterial species.

Bacteria	Cultivation Time (h)	Growth rate $OD_{650\ nm}$	Enzyme activity U/ml	Enzyme activity U/mg
Lactococcus lactis subsp. cremoris EMCC 10511	8	1.5	11	2.6
	12	1.9	15	2.8
	24	1.95	15.2	3.0
	48	1.9	9.0	1.5
	72	1.7	4.5	0.59
	96	1.55	4.0	0.15
Lactococcus lactis subsp. lactis	8	1.5	4.8	1.5
	12	1.65	5.0	1.9
	24	1.8	4.5	1.9
	48	1.8	2.3	1.0
	72	1.6	1.1	0.75
	96	1.35	0.9	0.5
Lactococcus lactis subsp. *lactis* biovar *diacetilactis EMCC 10510*	8	1.6	6.9	1.6
	12	1.8	12.1	2.1
	24	1.9	14	2.07
	48	1.84	8.2	1.4
	72	1.6	2.9	0.9
	96	1.42	0.2	0.4
Streptococcus salivarius subsp. thermophilus EMCC 10509	8	1.2	25	7
	12	1.5	35	7.7
	24	1.5	37.3	10.2
	48	1.5	27.6	6.9
	72	1.2	23	6.1
	96	1.0	20.1	4.5
Leuconostoc cremoris	8	1.2	5.0	2.3
	12	1.3	8.2	4.5
	24	1.5	9.1	4.8
	48	1.5	9.8	1.9
	72	1.4	2.8	0.8
	96	1.2	1.5	0.3

-Galactosidase Extraction:

β-Galactosidase cannot be extracted from fresh cells of yeasts and bacteria without proir rupturing the cell walls (Mahoney *et al.*, 1974). Ruptures of the cell walls is carried out by several methods, *e.g.*, solvent autolysis and mechanical breakage of the cells (Gunsalus, 1955, Chan, 1971 and Wendorff and Amundson, 1971). In this experiment three treatments of extraction were used to release β-galactosidase from the cells of *K. fragilis* and *Str. salivarius* subsp. *thermophilus*. The used treatments are chemical treatment (Solvent extraction), freezing and thawing treatment and ultrasonic treatment.

Chemical Treatment

Solvent

The effect of 1% (v/v) different organic solvents on β-galactosidase extraction at 37°C for 10 min was studied. The results represented in Table (3) show that the enzyme release varied with the solvent used. Toluene was the most favorable solvent for β-galactosidase release compared with other solvents. Toluene was selected for further studies concerned with solvent concentration. Mahoney *et al.* (1974) reported that toluene autolysis produced more β-galactosidase activity (1.2 U/mg cells) than chloroform (0.45 U/mg cells). Fenton and Dennis (1981) and Fenton (1982) reported the use of organic solvents for extraction of β-galactosidase from *K. fragilis*. They stated that alcohols such as ethanol, methanol, t-butanol and isopropanol are effective solvents in releasing β-galactosidase from yeast cells. They also stated that these solvents prevents losses of β-galactosidase due to microbial degradation, and seems also to prevent proteolytic activity from hydrolyzing β-galactosidase during the subsequent slow extraction with buffer. Several workers used many organic solvents for extraction of β-galactosidase from yeasts and bacteria. Of these workers Selim *et al.* (1983) and Declinr *et al.* (1987) on yeasts and El-Diwany *et al.* (1986) on bacteria.

Table (3): Effect of different organic solvents on β-galactosidase extraction from cells of *K. fragilis* and *Str. salivarius* subsp. *thermophilus*.

Solvent	β-Galactosidase activity (U/ml)	
	K. fragilis	*Str. Salivarius* subsp. *thermophilus*
Toluene	12.25	37.30
Cloroform	12.15	36.05
n-Butanol	9.05	28.50
Benzen	12.00	34.50
Ethanol	5.25	17.22
Methanol	3.32	10.10
Isopropanol	8.51	22.00
2-Mercaptoethanol	9.05	25.11
Acetone	4.62	15.00

Microbial cells were treated with 1% (v/v) of different organic solvents at ~ 37°C for 10 min in 0.5 M potassium phosphate buffer pH 6.8 containing 0.1 mM manganese chloride and 0.5 mM magnesium sulfate.

Solvent concentration

The effect of different concentrations of toluene on the release of intracellular β-galactosidase at 37°C for 10 min was investigated. The results represented in Table (4) indicate that the enzyme release was increased with increasing solvent concentration till it reached its maximum activity at 2% (v/v). The use of higher contact time was correlated with a significant decrease in β-galactosidase activity. These results are in agreement with Mahoney *et al.* (1974) and El-Diwany *et al.* (1994). The first authors found

that the best concentration of toluene was 2% (v/v) for extraction of -galactosidase from *K. fragilis*.

Table (4): Effect of toluene concentrations on β-galactosidase extraction from cells of *K. fragilis* and *Str. salivarius* subsp. *thermophilus*.

Solvent Conc. (v/v)	β-Galactosidase activity (U/ml)	
	K. fragilis	*Str. salivarius* subsp. *thermophilus*
0.0	0.00	0.00
1.0	12.25	37.30
1.5	14.01	40.50
2.0	15.05	43.60
2.5	13.51	42.05
3.0	9.35	35.95

Microbial cells were treated with different concentrations of toluene at ~ 37°C for 10 min in 0.5 M potassium phosphate buffer pH 6.8 containing 0.1 mM manganese chloride and 0.5 mM magnesium sulfate.

Contact Time

The effect of contact time on β-galactosidase release, by 2% (v/v) toluene extraction at 37°C, from yeast and bacterial cells was studied. The results presented in Table (5) show that toluene released β-galactosidase in higher yield after 15 min (15.25 and 43.90 U/ml) in both *K. fragilis* and *Str. Salivarius* subsp. *thermophilus*, respectively). The use of higher contact time was correlated with a considerable decrease in β-galactosidase activity. The results are in agreement with Goncalves and Castillo (1982), Stred ansky *et al.* (1993) and El-Diwany *et al.* (1994). The later authors reported that toluene release β-galactosidase in higher yield after 5 minutes.

Temperature

The effects of temperature on extraction of β-galactosidase from fresh yeast and bacterial cells are shown in Table (6). The results indicate that maximum β-galactosidase activity was extracted after 15 min with 2% toluene (v/v) at 37°C. The results are in line with those of Mahoney *et al.* (1974), Goncalves and Castillo (1982) and Stred' ansky (1983). The first authors found that the most rapid release of maximum β-galactosidase activity of *K. fragilis* was achieved with 2% toluene (v/v) at 37°C. The second authors reported that maximal yields of β-galactosidase from *K. marxianus* were reached between 37°C and 40°C.

pH

The effect of pH of buffer on release of β-galactosidase from yeast and bacterial cells by 2% (v/v) toluene autolysis is presented in Table (7). The results obtained show that the best conditions for β-galactosidase extraction from cells is at pH 6.8. These results confirm previous reports (Wendorff, 1969, Mahoney *et al.*, 1974, Goncalves and Castillo, 1982) that the best condition for -galactosidase extraction is at pH around 7.0. Contrary to the

above results, Stred'ansky *et al.* (1993) found that about 93% of -galactosidase from *K. marxianus* was released at pH 9.5-10.5.

Table (5): Effect of extraction time on β-galactosidase extraction from cells of *K. fragilis* and *Str. salivarius* subsp. *thermophilus*.

Extraction Time (min)	-Galactosidase activity (U/ml)	
	K. fragilis	*Str. salivarius* subsp. *thermophilus*
0	0.00	0.00
5	12.90	37.20
10	15.05	43.60
15	15.25	43.90
20	14.51	41.90
30	13.98	40.50
60	9.75	23.95
90	5.98	20.30
120	5.50	18.00
12 h	5.00	16.95
24 h	4.50	15.05

Microbial cells were treated with 2% (v/v) of toluene at ~ 37°C for different times in 0.5 M potassium phosphate buffer pH 6.8 containing 0.1 mM manganese chloride and 0.5 mM magnesium sulfate.

Table (6): Effect of temperature on β-galactosidase extraction from cells of *K. fragilis* and *Str. salivarius* subsp. *thermophilus*.

Temperature (°C)	-Galactosidase activity (U/ml)	
	K. fragilis	*Str. Salivarius* subsp. *Thermophilus*
25	11.95	13.25
30	12.75	23.85
35	13.50	42.95
37	15.25	43.90
40	11.85	41.59
45	8.05	36.80
50	6.01	33.05

Microbial cells were treated with 2% (v/v) of toluene at 25-50°C for 15 min in 0.5 M potassium phosphate buffer pH 6.8 containing 0.1 mM manganese chloride and 0.5 mM magnesium sulfate.

Buffer concentration

The effect of phosphate concentration of buffer on extraction of β-galactosidase from fresh cells of *K. fragilis* and *Str. salivarus* subsp. *thermophilus* is presented in Table (8). It is obvious from the results that the best concentrations for extraction of β-galactosidase of fresh cells is in 0.1 M phosphate buffer (pH 6.8). These results confirm previous reports (Wendorff, 1969, Mahoney *et al.*, 1974 and Stred' ansky *et al.*, 1993. The last authors reported that under optimal conditions (37°C, pH 9.5-10.5) greater than 90% of the β-galactosidase activity was released into 0.1-0.5 phosphate buffer.

Table (7): Effect of pH on β-galactosidase extraction from cells of *K. fragilis* and *Str. salivarius* subsp. *thermophilus*.

pH of buffer	β-Galactosidase activity (U/ml)	
	K. fragilis	*Str. Salivarius* subsp. *Thermophilus*
5.7	11.25	24.05
6.0	12.30	30.12
6.5	13.50	40.43
6.8	15.25	43.90
7.0	14.50	43.90
7.5	13.50	42.05
8.0	10.03	36.50

Microbial cells were treated with 2% (v/v) of toluene at ~ 37°C for 15 min in 0.5 M potassium phosphate buffer pH 5.7-8.0 containing 0.1 mM manganese chloride and 0.5 mM magnesium sulfate.

Table (8): Effect of phosphate concentration of buffer on β-galactosidase extraction from cells of *K. fragilis* and *Str. salivarius* subsp. *thermophilus*.

Phosphate concentration of buffer (M)	β-Galactosidase activity (U/ml)	
	K. fragilis	*Str. salivarius* subsp. *Thermophilus*
0.001	5.80	19.93
0.01	12.32	39.42
0.05	15.25	43.91
0.1	16.30	48.40
0.5	14.25	42.49
1.0	9.00	39.05
5.0	6.85	24.15

Microbial cells were treated with 2% (v/v) of toluene at ~ 37°C for 15 min in 0.001-5.0 M potassium phosphate buffer pH 6.8 containing 0.1 mM manganese chloride and 0.5 mM magnesium sulfate.

Ultrasonic treatment

The effect of ultrasound on β-galactosidase release was studied. The results represented in Table 9 show that β-galactosidase release was increased with increase of the exposure time. β-Galactosidase reached its maximum activity (12.95 U/ml) at 5 min and (38.99 U/ml) at 4 min in case of *K. fragilis* and *Str. salivarius* subsp. *thermophilus*, respectively. Doulah (1978) pointed out that cells disruption by using ultrasonic treatment is depended upon the mechanical effect of gaseous cavitation in the suspending medium. The mechanical stresses may arise from shock waves and from flows generated by pulsating bubbles. Several investigators used the ultrasound treatments with various power watt and exposure times for -galactosidase extraction (Sanchez and Hardisson, 1979; Chatterjee and Vining, 1982; Moldoveanu and Kluepfel, 1983; El-Diwany *et al.*, 1984; El-Diwany *et al.*, 1986 and Foda *et al.*, 1986).

Table (9): Effect of sonication on β-galactosidase extraction from cells of *K. fragilis* and *Str. salivarius* subsp. *thermophilus*.

Exposure Time (min)	β-Galactosidase activity (U/ml)	
	K. fragilis	*Str. salivarius* subsp. *thermophilus*
1	6.01	22.55
2	7.05	23.95
3	7.55	28.45
4	9.05	38.99
5	12.95	19.48
6	8.95	16.50
7	5.94	15.05
8	5.00	13.60
9	4.51	12.90
10	3.04	12.80
15	2.92	12.05

Physical treatment

The effect of freezing and thawing on β-galactosidase extraction was studied. The results represented in Table (10) show that the maximum release of β-galactosidase was obtained after 7 cycles with the cell pellets and cells in buffer. These results are in line with Decleire *et al.* (1987) and El-Diwany *et al.* (1994). The former authors worked on *Kluyveromyces* - galactosidase and found a higher activity when the pellets were used. The later authors worked on *Candida pseudotropicalis* and found that the maximum release of β-galactosidase was obtained after 5 cycles of freezing and thawing with the cell pellets.

Table (10): Effect of freezing and thawing on β-galactosidase extraction from cells of *K. fragilis* and *Str. salivarius* subsp. *thermophilus*.

Cycle Number	β- Galactosidase activity (U/ml)			
	Cell pellets		Cells in buffer	
	K. fragilis	*Str. salivarius* subsp. *thermophilus*	*K. fragilis*	*Str. salivarius* subsp. *thermophilus*
1	7.55	29.95	7.00	29.85
2	8.25	31.45	7.56	30.00
3	9.11	33.11	7.70	31.05
4	10.50	34.50	8.25	31.50
5	12.76	34.70	8.50	32.50
6	13.45	35.05	9.00	32.70
7	13.01	36.05	7.95	32.80

REFERENCES

Barbosa, M. D. F. S.; Silva, D. O.; Pinheiro, A. J. R. ; Guimaraes, W. V. and Borges, A. C. (1985). J. Dairy Sci., 98 (7): 1618. (C.F. Nijples, H.H., 1980).

Beyerinck, M. W. (1889). Entralblat Bacteriologie, 6: 44. (C.F. Nijples, H.H.,1980).

Caputto, R. L.; Leloir, L. F. and Trucea, R. F. (1984). Lactose and lactose fermentation in *Saccharomyces fragilis*. Enzymologia., 12 : 350.

Champluvier, B.; Kamp, B. and Rouxhet, P. G. (1988). Preparation and properties of β-galactosidase confined in cells of *Kluyveromyces* sp. Enzyme Microb. Technol. 10: 611.

Chan, H. M. C. (1971). Factors influencing the hydrolysis of lactose by β-galactosidase is olated from *Saccharomyces fragilis*. M. Sc. Thesis, Univ of California, Davis.

Chatterjee, S. and Vining, C. (1982). Catabolite repression in *Streptomyces venezuella*. Induction of β-galactosidase, chlorophenicol production, and intracellular cyclic adenosine 3', 5'-monophosphate concentrations. Can. J. Microbiol: 28:31.

Davies, R. (1964). Lactose utilization and hydrolysis by *Saccharomyces fragilis*. J. Gen. Microbiol. 37:81.

De-Macias. M. E. N.; De Nadra, M. C. M. ; de saad A. M. S., De Ruiz Holgado, A.A.P and Oliver, G. (1983). Isolation and properties of -galactosidase of a strain of *Lactobacillus helveticus* isolated from natural whey starter. J. Appl. Biochem, 5: 275.

Decleire, D.; Decat, W. and Van Huynh, N. (1987). Comparison of various permeabilization treatments on *Kluyveromyces* by determining in situ β-galactosidase activity. Enzyme Microbiol. Technol. 9: 300.

Decleire, M.; Van Huyhn, N. and Motte, J. C. (1985). Hydrolysis of lactose solutions and wheys by whole cells of *Kluyveromyces fragilis*. J. Appl. Microbiol. Biotechnol. 21:103.

Deshpande, M. S., Deopurkar, R. I. and Rale, V. B. (1989). Beta-galactosidase from *Aureobasidium pullulans*. Lett. Appl. Microbiol. 9:21.

Doulah, M. S. (1978). Application of the statistical theory of eliability to yeast cell disintegration in ultrasonic cavitation. Biotechnol. Bioeng. 20: 1287.

El-Diwany, A. I ; Hussain, Azhar A. ;Kheiralla, Zeinab H.; and Ashour, Sanaa M. (1994). Studies on β-galactosidase from *Candida pseudotropicalis*. 1- Selection and extraction. Egypt. J. Microbiol. 29(3):231.

El-Diwany, A.I.; Selim, M.H.; Shaker, H.M. and Mohamed, A.A. (1986). Studies on β-galactosidase from Bacillus stearothermophilus. 1-Production, extraction and some properties. Deutsche Lebensmittel-Rundschau 82:11.

El-Sawah, M. M.A.: Kassem, M. M.: Ismail, I. I. and El-Hoseny, M. A. (1991). Optimization of β-galactosidase production by yeasts. 2- Selection of whey supplements and growth conditions. J. Agric. Sci., Mansoura Univ., 16 (2): 2866.

Engler, C. R. (1985). Distribution of microbial cells. In: Comprehensive Biotechnology Vol. 2 (Cooney, C.L. and Humprey, A.E., eds.). Pergamon Press, Oxford, 305.

Feniksova, R. V.; Tikltomirova, A. S.; Kulicova, A. K.; Elpiner, I. E. and Gogolver, M. N. (1971). Preparation of -galactosidase from *Saccharomyces fragilis*. Prikl. Biokhim. Microbiol. 7:34.

Fenton, D.M. (1982). Solvent treatment for β-galactosidase release from yeast cells. Enzyme Microb. Technol. 4:229.

Fenton, D.M. and Dennis, M. (1981). Lactase from yeast cells. J. Appl. Microbiol. 36: 133.

Foda, M.S.; Nassar, F.R.; Mahmoud,S.A.Z.; Ramadan, E.M. and Shata. H.M. (1986). Kinetics and properties of particularly purified yeast β-galactosidase produced in salted whey. Egypt. J. Microbiol., Special Issue, 139.

Glanetto, A.; Berruti,F. ; Glick, B.R. and Kempton, A.G. (1986). The production of ethanol from lactose in a tuluvar reactor by immobilized cells of *Kluyveromyces fragilis*. Appl. Microbiol. Biotechnol., 24 (4): 277.

Gomez, A and Castillo, F. J. (1983). Production of biomass and β-D-galactosidase by *Candida pseudotropicalis* grown in continuous culture on whey. Biotechnol. Bioeng. 25(5):1341.

Goncalves, J. A. and Castillo, F. J. (1982). Partial purification and characterization of β-galactosidase from *Kluyveromyces marxianus*. J. Dairy Sci. 65:2088.

Greenberg, N. A. and Mahoney, R. R. (1982). Production and characterization of β-galactosidase from *Streptococcus thermophilus*. J. Food Sci. 46(6):1824.

Griffiths, M. W. and Muir, D. D. (1979). Properties of a thermostable beta-galactosidase from a thermophilic Bacillus: Comparison of the enzyme activity of whole cells, purified enzyme and immobilised whole cells. J. Sci. Food Agric. 29: 753.

Gruy, E. J. and Bingham, E. W. (1978). Properties of -galactosidase of *Scaccharomyces lactis* in milk and milk products. J. Dairy Sci., 61 : 147.

Gunsalus, I. C. (1955). Extraction of enzymes from microorganisms. In Methods in enzymology. S.P. Colowick and N.O. Kaplan, eds. Academic Press 1:51.

Hu A. S. L.; Wolfe, R. G. and Reithel, F. J. (1959). The preparation and purification of -galactosidase from *Escherichia coli* Ml 308. Arch. Biochem. Biophys. 81 :500.

Ikura, Y. and Horikoshi, K. (1979). β-Galactosidase in alkalophilic β-galactosidase *Bacillus*. Agric. Biol. Chem. 43(6):1359.

Klup, K. (1975). Carbohydrases. Pages 92-97 In " Enzymes in Food Processing " G. Reed. ed. Academic Press, New York. NY.

Kosikowski, F. V. and Wierzbicki, L. E. (1973). Lactose hydrolysis of raw and pasteurized milk by *Saccharomyces lactis* lactase. J. Dairy Sci. 65:146.

Kula, M. R. and Schutta, H. (1987). Purification of protein and the disruption of microbial cells. Biotechnol. Prog. 3:31.

Lowry, O. H.; Rosebrough, N. J.; Farr, A. L. and Randall, R. J. (1951). Protein measurement with the Folin phenol reagent. J. Biol. Chem. 193:265.

Mahoney, R. R. and Widler, T. (1988). Thermostability of yeast lactase *Kluyveromyces marixianus* in milk. J. Dairy Res. , 55 : 423.

Mahoney, R. R.; Nickerson, T. A. And Whitaker, J. R. (1974). Selection of strain, growth conditions and extraction procedures for optimum production of lactose of *Kluyveromyces fragilis*. J. Dairy Sci., 58 (11):1620

Mahoney, R.R. and Whitaker, J.R. (1978). Purification and physicochemical properties of β-galactosidase from *Kluyveromyces fragilis*. J. Food Sci. 65:2088.

McFeters, G.A.; Sandine, W.E. and Elliker, P.R. (1967). Purification and properties of *Streptococcus lactis* β-galactosidase. J. Bacteriol. 93:914.

Moldoveanu, Natalia and Kluepfel, Dieter (1983). Comparison of β-galactosidase activities in different *Streptomyces* strains. J. Appl. and Environ. Microbiol. 46:17.

Nielsen, Wayne, I. (1987). Isolation and characterization of the lactose hydrolyzing enzyme of *Lactobacillus acidophilus*. Abstr. Int. B., 1988, 4 (5): 1458.

Nijples, H. H. (1980). Lactases, Reprint from Developments in Food Carbohydrates-3. Eds. C. K. Lee and M.G. Lindley, Appl. Sci. Pub. LTD, London.

Premi, L.; Sandine, W. E. and Elliker, P. R. (1972). Lactose hydrolysing enzymes of *Lactobacillus* species. Appl. Microbiol. 24:51.

Ramana Rao, M. V. and Dutta, S. M. (1977). Production of beta-galactosidase from *Streptococcus thermophilus* grown in whey. Appl. Environ. Microbiol. 34(2):185.

Ramana Rao, M.V. and Dutta, S.M. (1978). Lactase Activity of Microorganisms. Folia Microbiol. 23: 210.

Ramana Rao, M. V. and Dutta, S. M. (1979). An active beta-galactosidase from *Streptococcus thermophilus*. Indian J. Dairy Sci. 32(2):187.

Sanchez, F. L. and Castillo, F. J. (1980). Produccion, extraccion y caracterizacion parcial de β-D-galactosidase de *Kluyveromyces fragilis* crecida en suero de leche. Acta Cient. Venez. 31:154.

Sanchez, J. and Hardisson, C. (1979). Induction of β-galactosidase in *Streptomyces violacues* . Can. J. Microbiol., 25(7):833.

Selim, M. H.; Foda, M. S.; Ismail, A. A. and Ahmed, K. A. (1983). Genetical studies on β-galactosidase formation in yeast. 1-Characterization of the enzyme in various yeasts. Egypt. J. Microbiol. Special Issue: 131.

Shukla,T.P. (1975). β-Galactosidase technology: A solution to the lactose problem. CRC. Crit. Rev. Food Technol. 5: 325.

Sonawat, H.M.; Grawal, A.A. and Dutta, S.M. (1981). Production of β-galactosidase from *Kluyveromyces fragilis* grown on whey. Folia Microbiol. 26:370.

Stred,ansky, M.; Tomaska, M.; Sturdik, E. and Kremnicky, L. (1993). Optimization of β-galactosidase extraction from *Kluyveromyces marxianus*. Enzyme Microb. Technol. 15:1063.

Streers, E. Jr.; Cuatrecasas, P. and Pollard, H. B. (1971). The purification of β-galactosidase from *Escherichia coli* by affinity chromatography. J. Biol. Chem. 246:196.

Uwajima, T., Yagi, H. and Terada, O. (1972). Purification stabilization and some properties of β-galactosidase from *Saccharomyces fragilis*. Agri. Biol. Chem. 36 : 570.

Van Dam, B.; Revallier-Warfemins, G. and Van Dam- Schermerhorn, L. C. (1950). Preparation of lactase from *Sccharomyces fragilis*. Neth. Milk Dairy J. 4:96.

Wenddorff, W. L. (1969). Studies on the β-galactosidase activity of *Saccharomyces fragilis* and effect of substrate preparation. Ph.D. Thesis , University of Wisconsin.

Wendorff, W. L.; Amundson, C. H. and Olson, N. F. (1970). Nutrient requirements and growth conditions for production of lactase enzyme by *Saccharomyces fragilis*. J. Milk Food Technol. 33 (10): 451.

Wendorff, W.L. and Amundson, C.H. (1971). Characterization of - galactosidase from *Saccharomyces fragilis*. J. Milk Food Technol. 34: 300.

Young, H. and Healey, R. P. (1957). Production of *Saccharomyces fragilis* with an optimum yield of lactase. U.S. Patent 2:776.

دراســـات عـــن إنتـــاج البيتاجلاكتوسيديز الميكروبى

١-الظروف المثلى لإستخلاص البيتاجلاكتوسيديز من خميرة كليفيروميســـز فراجيلـــيز وبكتيريا ستربتوكوكس ساليفاريس تحت النوع ثرموفيلس

محمود محمد عوض الله السواح ، محمد منصور قاسم ، عايدة حافظ عفيفى ، إســماعيل إبراهيم إسماعيل ، عزة سعيد عبد الكافى منشاوى

قسم الميكروبيولوجى ، كلية الزراعة ، جامعة المنصورة ، المنصورة ، مصر .

كلية الزراعة – جامعة عمر المختار – البيضاء – ليبيا .

تم إختبار إنتاج البيتاجلاكتوسيديز فى ثلاثة أنواع من الخمائر وخمسة أنواع مـــن البكتيريـــات ، جميعها تمتلك القدرة على النمو على اللاكتوز كمصدر وحيد للكربون فى بيئة النمـــو ، وذلـــك بإســـتخدام المزارع الميتزة ، وقد وجد أن خميرة كليفيروميسز فراجيليز هى أفضل الخمائر المختبرة ، وأن بكتيريـــا ستربتوكوكس ساليفاريس تحت النوع ثرموفيلس هى أفضل البكتيريات المختبرة .

وجد أن التولوين هو أكثر المذيبات العضوية المختبرة فعالية فى إستخلاص البيتاجلاكتوسيديز من خلايا الخميرة أو البكتيريا ، كما وجد أن زيادة تركيز التولوين بالمحلول المحتوى على الخلايا يؤدى إلـــى زيادة إستخلاص البيتاجلاكتوسيديز حتى تركيز ٢% (حجم/حجم) والذى أعقبه نقص النشاط الإنزيمى ، كما وجد أنه يمكن الحصول على أكبر كمية من الإنزيم بعد ١٥ دقيقة من بداية وقت الإستخلاص بالتولوين ، وقد أدى زيادة وقت الإستخلاص عن ذلك إلى نقص واضح فى النشاط الإنزيمى ، كما وجد أنه يمكن إستخلاص أكبرقدر من البيتاجلاكتوسيديز على درجة حرارة ٣٧ م ، كمـــا وجـــد أن أفضـــل ظـــروف لإســـتخلاص البيتاجلاكتوسيديز من الخلايا تكون عند pH ٦,٨ ، كما وجد أن تركيز ٠,١ مولر لمحلول الفوسفات المنظـــم هو أفضل تركيز لإستخلاص البيتاجلاكتوسيديز من الخلايا .

وجدت زيادة فى نشاط البيتاجلاكتوسيديز مع زيادة وقت التعرض للموجات فوق الصوتية وقـــد بلغت هذه الزيادة أقصاها بعد حوالى خمسة دقائق من بداية التعرض للموجات فوق الصوتية .

وجد أن أقصى إستخلاص للبيتاجلاكتوسيديز من الكرات الخلوية ومن الخلايا المعلقة فى محلـــول منظم يتم بعد ٦ دورات تجميد وتسييح ، كذلك وجد أن النشاط الإنزيمى يكون أعلى فى حالة إستخدام الكرات الخلوية .