African Journal of Biotechnology Vol. 9(44), pp. 7550-7559, 1 November, 2010
Available online at http://www.academicjournals.org/AJB
DOI: 10.5897/AJB10.644
ISSN 1684–5315 © 2010 Academic Journals

Full Length Research Paper

Characterization of immobilized alkaline cyclodextringlycosyltransferase from a newly isolated *Bacillus agaradhaerens* KSU-A11

Abdelnasser S. S. Ibrahim*, Mohamed A. El-Tayeb and Ali A. Al-Salamah

Department of Botany and Microbiology, College of Science, King Saud University, P.O 2455 Riyadh 11351, Kingdom of Saudi Arabia.

Accepted 12 October, 2010

Alkaliphilic bacteria were isolated from soil and water samples obtained from Egyptian soda lakes (Wadi Natrun area, Egypt). Screening for cyclodextrin glycosyltransferase (CGTase)-producing alkaliphilic bacteria resulted in isolation of 10 positive strains. Strain KSU-A11 was selected as the best CGTase producer (2.1 U/ml). 16S rDNA sequence analysis identified the KSU-A11strain as *Bacillus agaradhaerens*. CGTase was partially purified using starch adsorption technique. The partially purified CGTase was immobilized on chitin by covalent binding tecnique using cross linking reaction with high immobilization yield (85%). The properties of the free and immobilized CGTase were determined. The optimum pH of the immobilized enzyme was slightly higher than that of the free enzyme at pH 10 and 10.5, respectively. In addition, both free and immobilized enzyme retained 94 to 100% of its initial activity over a wide pH range (pH 6.0 to 11.0). The enzymatic activity of both free and immobilized CGTase was highest at temperature 50°C; however, the relative activities of the immobilized CGTase were slightly higher than those of the free enzyme. Furthermore, investigation of thermostability of the enzyme indicated that the immobilization process of CGTase on chitin significantly protected the enzyme against thermo-inactivation. Kinetic parameters, K_m and V_{max}, values for free and immobilized enzymes were estimated and while there was no change in the V_{max} value (83.3 µmol/min. mg) for both free and immobilized CGTase, the K_m of the enzyme increased from 14.28 to 20 mg/ml upon immobilization. The immobilization of the enzyme showed high operational stability by retaining almost 50% of the initial activity after nine uses.

Key words: Cyclodextrin glycosyltransferase, *Bacillus agaradhaerens*, immobilization, chitin, alkaliphiles.

INTRODUCTION

Cyclodextrin glucosyltransferases (CGTases) (EC 2.4.1.19) represent one of the most important group of microbial amylolytic enzymes. CGTase catalyze the formation of cyclodextrins (CDs) from starch and related α(1 → 4) linked glucose polymers via a transglycosylation reaction (Tonkova 1998; Leemhuis et al., 2010). Bacterial CGTase is a multifunctional enzyme that in addition to formation of CDs it also catalyze other three reactions (van der Veen et al., 2000; Alcalde et al., 2001): (a) Coupling, where the CD molecule is opened and combined with a linear oligosaccharide to produce a longer linear carbohydrate; (b) disproportionation, which is the transfer of part of a linear oligosaccharide chain to an acceptor; and (c) saccharifying or hydrolysis of starch. CDs are doughnut-shaped molecules with a hydrophilic outer surface and a relatively hydrophobic cavity (Martin Del Valle, 2009). With such structural features, CDs and their derivatives can encapsulate hydrophobic molecules within their hydrophobic cavity to form inclusion complexes. This property has been used to stabilize and solubilise various substances of interest in numerous applications in the

*Corresponding author. E-mail: ashebl@ksu.edu.sa. Tel: (+966)-0597359415. Fax: (+966)-014675833.

Abbreviations: CGTases, Cyclodextrin glucosyltransferases; **CDs,** cyclodextrins; **PCR,** polymerase chain reaction; **BLAST,** Basic Local Alignment Search Tool; **NCBI,** National Center For Biotechnology Information.

pharmaceutical, cosmetics and food and textile industries (Biwer et al., 2000; Li et al., 2007; Astray et al., 2009). CDs are also used in the separation of enantiomers to extract toxic chemicals from waste streams (Martin Del Valle, 2009) and in soil bioremediation (Fava and Ciccotosto, 2002).

As the separation of different CDs is costly and time-consuming, CGTase that synthesises predominantly one type of CD are of great interest. Bacterial CGTase usually produced mixture of different types of CDs, however most CGTases from alkaliphilic bacteria convert starch into β-CD as the predominant product, however a mixture of other CD forms are still produced in varying ratios (Horikoshi, 1999a,b). There is increasing interest in developing efficient industrial process for production of cyclodextrins and oligosaccharides for addressing applications in different industries. However, possibly, the main bottleneck in the industrial application of enzymes is the price and the stability of the biocatalyst. Enzyme immobilization is one of the most useful approaches to overcome such difficulties (Biwer et al., 2000). Continuous production of CDs and oligosaccharides using immobilized CGTase would offer several advantages including allowing reuse of expensive CGTase, simplifying product purification process and providing opportunities for scaling up (Leemhuis et al., 2010). Different approaches have been applied for the immobilization of CGTases based on adsorption, entrapment or covalent binding (Leemhuis et al., 2010).

Immobilizations by physical adsorption or entrapment were unsuitable because the enzyme readily leaks from the support during the reaction (Biwer et al., 2000). In this work, we have isolated new alkaliphilic CGTase producing bacterium and investigated the immobilization of partially purified CGTase by covalent attachment to chitin using cross-linking reaction. Chitin has been described as a suitable carrier for covalent immobilization of enzymes, using cross linking reaction, for several industrial applications (Zeng and Zheng, 2002; Krajewska, 2004). Chitin, a polysaccharide containing 2-deoxy-2-amino glucose unit linked through β 1,4 linkage, is present in crustaceans, insects and marine crabs etc (Krajewska, 2004). It has been reported that every five amino out of six in chitin molecule are in acetylated form. The presence of the amino groups in chitin molecule provides a binding site for proteins. This can act as a solid support for preparation of the immobilized enzyme. Recently, a number of studies have shown that chitin is a good carrier for enzyme immobilization with cross linking reaction as cheaper and convenient process (Zeng and Zheng, 2002; Krajewska, 2004).

MATERIALS AND METHODS

Isolation of CGTase producing alkaliphilic bacteria

Soil and water samples were collected in sterile containers from Wadi Natrun soda lakes, Egypt. Wadi Natrun extends in a northwest by southeast direction between latitudes 30° 15´ north and longitude 30° 30´ east. Isolation of CGTase-producing alkaliphilic bacteria was carried out using rich alkaline agar medium containing 0.02 % (w/v) phenolphthalein (Park et al., 1989). The alkaline agar medium (pH 10.5) contained soluble starch (10 g/l; Sigma), yeast extract (5 g/l; Difco), casamino acids (5 g/l; Difco), peptone (5 g/l; Difco), NaCl (50 g/l), Na_2CO_3 (15 g/l), agar (15 g/l) and 300 µl trace elements solution. The trace element solution contained: $CaCl_2.2H_2O$ (1.7 g/l), $FeSO_4.7H_2O$ (1.3 g/l), $MnCl_2.4H_2O$ (15.4 g/l), $ZnSO.7H_2O.7H_2O$ (0.25 g/l), H_3BO_3 (2.5 g/l), $CuSO_4.5H_2O$ (0.125 g/l), Na_2MoO_4 (0.125 g/l), $CoNO_3.6H_2O$ (0.23 g/l) and 2.5 ml 95 to 97 H_2SO_4. Na_2CO_3 and trace elements solutions were autoclaved separately before addition to the medium. Soil samples were suspended in a 10% (w/v) NaCl solution prepared in 50 mM glycine-NaOH buffer, pH 10. Aliquots (100 µl) of different dilutions of soil suspensions and water samples were plated on the alkaline agar medium and incubated at different temperatures, for several days. Formation of halo zone around the colonies, resulting from phenolphthalein-cyclodextrin inclusion complex, was an indication of CGTase activity (Park et al., 1989).

Identification of the CGTase producing strain

The selected strain was identified by 16S rRNA gene sequence analysis. The bacterial isolate was grown overnight in 5 ml alkaline broth medium. Total DNA was extracted using DNeasy Blood and Tissue Kits (Qiagen, NY, USA) following the manufacturer's instructions. Eubacterial-specific forward primer 16F27 (5´- AGA GTT TGA TCC TGG CTC AG-3´) and reverse primer 16R1525 (5´- AAG GAG GTG ATC CAG CCG CA-3´) were used to amplify 16S rDNA gene (Lane, 1991). Polymerase Chain Reaction (PCR) amplification was performed in a final reaction volume of 100 µl, and the reaction mixture contained each primer at a concentration of 0.5 µM, each deoxynucleoside triphosphate at a concentration of 200 µM, 50 mM KCl, 10 mM Tris–HCl (pH 8.3), 1.5 mM MgCl , 0.01% (w/v) gelatin and 2.5 U of *Taq* DNA polymerase. The PCR reaction was run for 35 cycles in a DNA thermal cycler. The following thermal profile was used for the PCR (35 cycles): Initial denaturation at 95°C, denaturation at 95°C for 1 min, primers annealing at 52°C for 1 min and extension at 72°C for 1.5 min. The final cycle included extension for 10 min at 72°C to ensure full extension of the products. PCR products were purified using a QIAquick Gel Extraction Kit (Qiagen, NY, USA,) and sequenced using an automated sequencer (Reserach center (King Faisal hospital, Riyadh, Saudi Arabia). The 16S-rDNA gene sequence of the isolate was aligned with reference 16S-rDNA sequences of the European Microbiological Laboratory (EMBL), GenBank (gb, Germany) and the data base of Japan (dbj) using the Basic Local Alignment Search Tool (BLAST) algorithm (Altschul et al., 1997) available in the National Center for Biotechnology Information (NCBI) homage.

CGTase production

The same alkaline medium used for strain isolation, without phenolphthalein, was used for enzyme production by the selected isolate. A loopful of culture from agar plate was inoculated into 50 ml glass tube containing 5 ml of the liquid medium and incubated overnight at 180 rpm and 37°C. This culture was then inoculated into 500 ml capacity Erlenmeyer flask containing 95 ml of the same medium and incubated at 37°C for 48 h. Cells and insoluble material were removed by centrifugation at 6 000 g for 15 min at 4°C and the cell-free supernatant was used as the source of the enzyme.

Partial purification of CGTase

Cell free-supernatant was used as a crude CGTase enzyme preparation and was partially purified using corn starch adsorption method (Ferrarotti et al., 1997). Corn starch and ammonium sulphate were added to 1000 ml of cell-free supernatant to a concentration of 5% (w/v) and 1 M, respectively and was kept at 4°C with constant moderate mixing for 1 h to allow CGTase adsorption to corn starch. The mixture was then centrifuged at 4 000 rpm for 10 min. The pellet was washed twice with 400 ml cold 1 M ammonium sulphate solution to remove unbound proteins. Adsorbed CGTase was eluted from the corn starch pellet with 200 ml of 50 mM Tris-HCl buffer, pH 8.0, containing 1 mM β-CD. The pellet was incubated in elution buffer for 30 min at 37°C in a shaking water bath followed by centrifugation for 10 min at 10 8 000 rpm. The elution was repeated once with 80 ml of elution buffer. The eluate (280 ml) was concentrated using an Amicon ultra-filtration membrane kit (10 kDa cut-off membrane) and then dialyzed against 50 mM Tris-HCl buffer overnight at 4°C and stored at -20°C till use.

Immobilization of partially purified CGTase

Chitin was prepared for immobilization as previously reported by Gill et al. (2006) by adding 150 ml of 0.5 N HCl to 5 g of chitin. The contents were stirred for 2 h at room temperature, washed thoroughly with distilled water till the pH of the filtrates was around 7.0, followed by drying at 50°C for overnight. Two methods were used for chitin crossing linking with glutaraldehyde: (A) Refined chitin (0.2 g) was treated with 5 ml of glutaraldehyde solution at definite concentration in the presence of 0.5 ml acetic acid as a catalyst and stirred at room temperature for 24 h (Zeng and Zheng, 2002): (B) refined chitin was treated with 6% acetic acid at 50°C for 30 min, washed thoroughly with distilled water till the pH was around 7.0. Then the treated chitin was treated with 5 ml of glutaraldehyde solution at definite concentration and stirred at room temperature for 24 h (Vaillant et al., 2000). The chitin-glutaral-dehyde complex (from both methods) was washed several times with distilled water until the washing was free of glutaraldehyde. Partially purified CGTase was added to the chitin-glutaraldehyde complex and allowed to stand for 24 h at 4°C with occasional stirring. The supernatant was removed by centrifugation at 2000 g and the chitin-CGTase complex was washed with distilled water until protein in the washing could not be detected. The immobilized CGTase was then collected and stored at 4°C. CGTase activity of the immobilized enzyme and in the washout was measured under the standard assay conditions. Protein content was determined using Bradford (1976).

Immobilization yield (%)

Immobilization yield was calculated from the following equation (Woodward 1985):

Immobilization yield = Bound enzyme (U/g carrier) / [Added enzyme (U/g carrier) - Unbound enzyme (U/g carrier)] %

Scanning electron microscope

The immobilized CGTase and free carrier were subjected to scanning electron microscope examination (SEM) according to the method previously reported (Wojtasa et al., 2008) at the Electron microscopy Unit, Research Centre, Faculty of Science, King Saud University.

Enzyme assay

CGTase activity was measured as β-CD forming activity (Mäkelä, 1988). 750 µl of a 5% (w/v) maltodextrin solution prepared in 50 mM glycine-NaOH buffer, pH 9 was pre-incubated at 50°C for 5 min. 25 µl of enzyme sample was added and after incubating for 20 min at 50°C, the reaction was quenched by adding 375 µl of 0.15 M NaOH. Subsequently, 100 µl of 0.02% (w/v) phenolphthalein prepared in 5 mM Na_2CO_3 was added and after standing at room temperature for 15 min, the colour intensity was measured at 550 nm. One unit of CGTase activity was defined as the amount of enzyme releasing 1 µmol of β-CD per min under the defined assay conditions. A calibration curve was made using 0.0055 to 0.22 µmol of β-CD solution in 50 mM glycine-NaOH buffer, pH 9, as a standard. Protein concentration was determined according to Bradford (1976) using bovine serum albumin as standard protein.

Immobilized enzyme assay

About 0.2 g (wet) of immobilized CGTase and 1.5 of 5% maltodextrin glycine-NaOH buffer (pH 9) was incubated at 50°C for 20 min and 750 µl of reaction mixture was taken for enzyme assayed as above.

Determination of optimum pH and stability measurement of CGTase

The influence of pH on the enzyme activity of free and immobilized CGTase was determined by measuring the enzyme activity at varying pH values ranging from 5 to 13 at 50°C using different suitable buffers, 50 mM sodium acetate (pH 5.0 and 6.0), 50 mM sodium phosphate (pH 7.0, 7.5 and 8.0), 50 mM glycine-NaOH buffer (pH 8.5, 9.0, 9.5 and 10) and 50 mM carbonate-bicarbonate buffer (pH 10.5 to 13.0), respectively. The pH stability of the free and immobilized enzyme was examined by incubating the enzyme for 1 h at 25°C in pH range 4 to 13 prior to determination of the residual activity; under the standard assay conditions.

Determination of optimum temperature and thermal stability of CGTase

The optimum temperature of the free and immobilized enzyme activity was determined by performing the enzymatic reaction in temperature range of 25 to 65°C (with 5°C increments) under the standard reaction conditions. Thermal stability were investigated for free and immobilized enzyme by measuring the enzyme residual activities after being incubated for 0.5, 1, 2 and 3 h in the temperature range of 25 to 65°C. The residual enzyme activity was assayed under standard assay conditions. The initial value of enzyme activity in each set was assigned the value of 100% activity.

Determination of kinetic parameters

Kinetic constants, K_m and V_{max} values, were determined for free and immobilized CGTase by measuring initial rates of the reaction at different concentrations of soluble starch (2 to 20 mg/ml) as a substrate in glycine buffer solution (50 mM, pH 10) at 50°C. The kinetic constants K_m and V_{max} were calculated by Lineweaver–Burk plot (Mathews and Van Holde, 1990).

Operational stability of the immobilized CGTase

It was performed with 0.2 g of immobilized CGTase (wet weight)

containing about 35 U of the enzyme. The immobilized CGTase was incubated with 1.5 ml of maltodextrins substrate in glycine-NaOH buffer (50 mM, pH 5.2) at 40°C for 20 min. At the end of the reaction period, it was collected by centrifugation at 2000 g for 10 min, washed with distilled water and re-suspended in 1.5 ml of freshly prepared substrate to start a new run. The reaction was repeated up to 10 cycles (Martin et al., 2003). All results are means of at least three experiments.

RESULTS AND DISCUSSION

Isolation of aerobic CGTase producing alkaliphilic bacteria

Isolation of CGTase producing alkaliphilic bacteria using alkaline agar medium containing 0.02 % (w/v) phenolphthalein resulted in the isolation of 10 isolates showing halo zones around their margins. The positive isolates (n = 10) were propagated in liquid medium and CGTase activity was measured. One of these isolates, designated as KSU-A11, showing the highest CGTase activity (2.1 U/ml) in alkaline liquid medium, was selected for further study. Comparative sequence analysis of the 16S rDNA gene of strain KSU-A11 and other 16S rDNA sequences available in data base indicated that KSU-A11 had the highest similarity (99%) with *Bacillus agaradhaerens*. *B. agaradhearens* was firstly described as a new alkaliphilic *Bacillus* species by Nielsen et al. (1995) and variety of polysaccharides-degrading enzymes from *B. agaradhaerens* have been studied for different applications (Schulein et al., 1999; Xu, 2000; Martins et al., 2000).

Immobilization of partially purified CGTase on chitin

CGTase was partially purified from the filtered cell free supernatant using adsorption to corn starch followed by enzyme elution with 50 mM Tris-HCl buffer containing 1 mM β-CD. The specific activity of the enzyme increased from 5.2 to 69.5 Umg^{-1} for the crude and the partially purified enzyme, respectively. For immobilization of the partially purified CGTase, it was found that method B including treatment of the refined chitin separately with acetic acid at 50°C for 30 min followed by activation with glutaraldehyde as a cross liker resulted in higher immobilization yield than cross linking with glutaraldehyde in the presence of acetic acid with immobilization yield of 55.5 and 35.2%, respectively. Therefore, this method was used throughout all the immobilization procedures.

Effect of glutaraldehyde concentrations on CGTase immobilization

Refined chitin was treated with different concentrations of glutaraldehyde ranging from 0.5 to 8% and the chitin-glutaraldehyde complex was then treated with the partially purified enzyme and the immobilization yield was determined as described above. As shown in Figure 1, the immobilization yield increased by increasing glutaraldehyde concentration, reaching maximum value (72.5%) at 3%. Further increase of the glutaraldehyde concentration had no significant effect on the immobilization yield; however, it was significantly decreased at 8%. As bifunctional agent, glutaraldehyde is capable of reacting with the amino groups present both in supports and in the enzyme, thus forming inter and intra-molecular bonds in the enzyme and between the enzyme and the support (Spagna et al., 1998). The optimum concentration of the cross linker varied (1 to 6%) according to the structure and source of enzyme and support (Spagna et al., 1998; Zeng and Zheng, 2002; Gill et al., 2006).

Effect of amount of added enzyme on CGTase immobilization

Different amount of partially purified CGTase, ranging from 0.1 to 6 mg (protein)/gm carrier (wet), was added to chitin-glutaraldehyde complex. The results presented in Figure 2 indicated that the immobilization yield increased by increasing the enzyme amount with maximum value (83.5%) at 2.5 mg (protein)/g carrier. This result is relatively similar to that reported by for immobilization of chitosanase on chitin by cross-linking reaction where the optimum amount of added enzyme was 2.0 mg/g carrier (Zeng and Zheng, 2002).

SEM of the immobilized CGTase

The immobilized CGTase and free carrier were subjected to SEM, (Electron microscopy Unit, Research Centre, Faculty of Science, King Saud University). As shown in Figure 3, the CGTase was successfully immobilized on the carrier by a cross linking reaction.

Characterization of the immobilized CGTase

Effect of pH on the CGTase activity and stability

The influence of pH on the activity and stability of the free and immobilized enzyme have been studied in the pH range 4 to 13. The results indicated that the optimum pH of the immobilized enzyme was slightly higher than thatof the free enzyme, pH 10 and 10.5, respectively (Figure 4). Similar result has been reported by Marten et al. (2003) where the pH/activity profile of immobilized CGTase was shifted toward alkaline value. This obser-vation basically agree with the general observations that charged supports shifted the pH/activity towards higher pHs when the concentrations of the hydroxyl ions in the immediate vicinity of the support surfaces is lower than the bulk

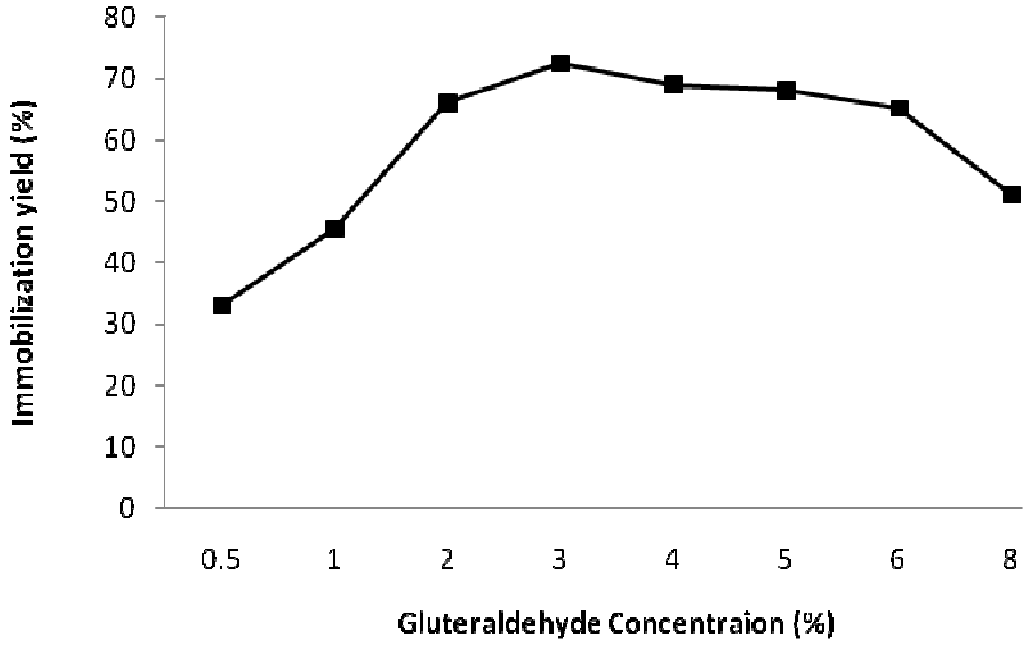

Figure 1. Effect of concentration of glutaraldehyde on CGTase immobilization. The results are means of three experiments.

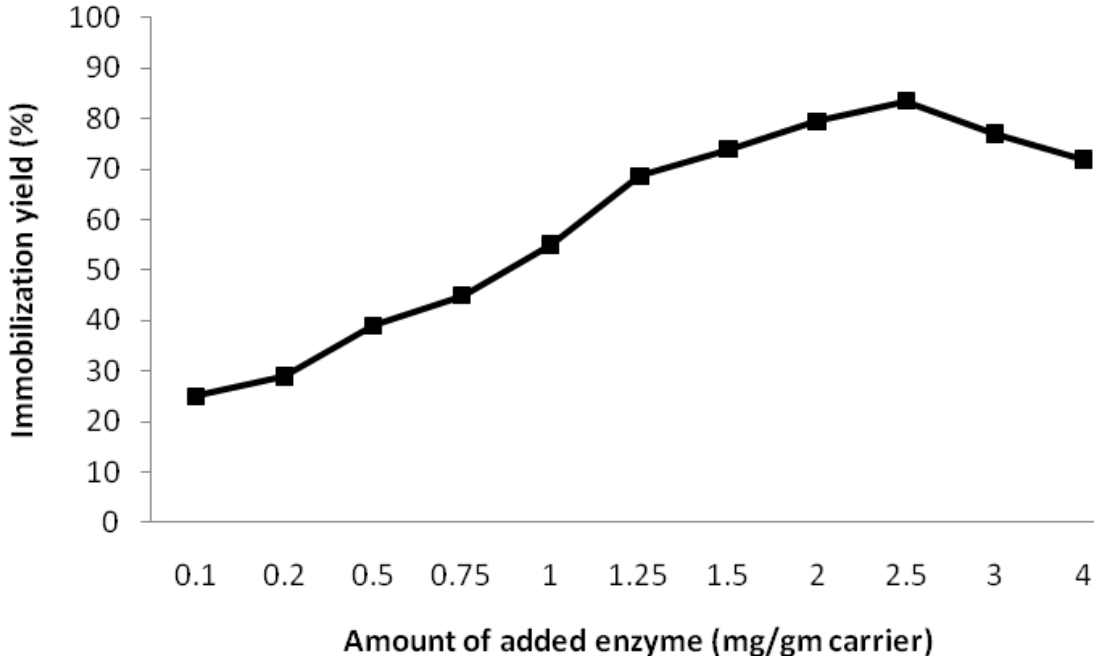

Figure 2. Effect of amount of added enzyme on CGTase immobilization. The results are means of three experiments

solution or towards higher pH values when the contrary occurs (Vaillant et al., 2000). However, chito-sanase immobilized on chitin showed optimum pH for the immobilized enzyme activity which was shifted toward acidic value (Zeng and Zheng, 2002). The pH stability of *B. agaradhaerens* KSU-A11 CGTase was studied by incubating the free and immobilized enzyme for 1 h at 25°C under varying pH values prior to determination of the residual activity under standard assay conditions. There was no significant difference in the residual activity

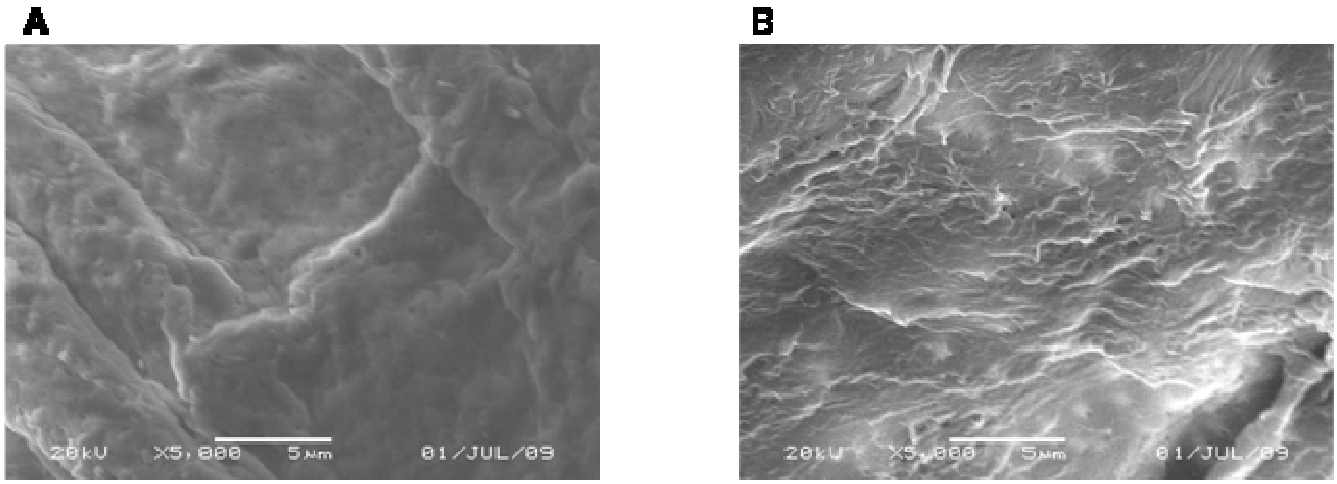

Figure 3. (A) SEM micrograph of free carrier (B) Immobilized enzyme.

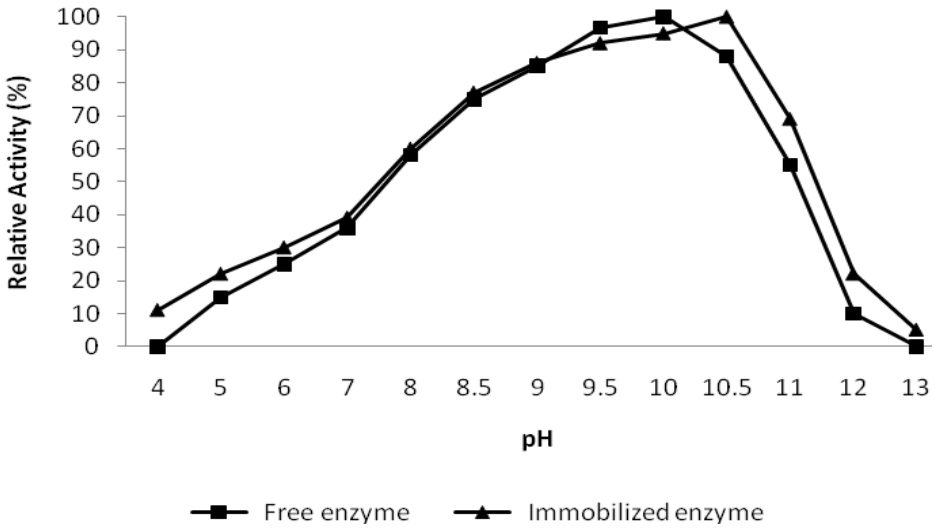

Figure 4. Effect of pH values on the activity of free and immobilized CGTase. The results are means of three experiments.

of both free and immobilized enzyme.The enzymes retained 94 to 100% of its initial activity over a wide pH range (pH 6.0 to 11.0). At pH 5.0 the residual activity was 83.5% while at pH 12.0, only 16.2% of initial activity was retained (Figure 5).

Effect of temperature on the activity and stability of CGTase

The activity of free and immobilized CGTase was measured at different temperatures ranging from 30 to 65°C. The temperature activity profile of both free and immobilized enzyme showed increasing enzyme activity up to 50°C and the activity decreased thereafter (Figure 6). The optimal temperature for both free and immobilized CGTase was 50°C; however, the relative activities of the immobilized CGTase were slightly higher than those of the free enzyme. The thermal stability of free and immobilized CGTase was investigated in the temperature range between 35 to 65°C at the optimum pH for the catalytic activity (pH 10 and 10.5 for free and immobilized enzyme, respectively). The results indicated that the immobilization process of CGTase on chitin protected the enzyme against thermo-inactivation (Figure 7). Similar results of enhancing thermostability of CGTase upon covalent binding immobilization have been reported (Martin et al., 2000; Ferrarotti et al., 2006). The formation of intermolecular covalent bonds between the enzyme molecules and between these and those of the support confer rigidity on the structure of the enzyme molecule, so that the enzyme is less affected by the denaturing

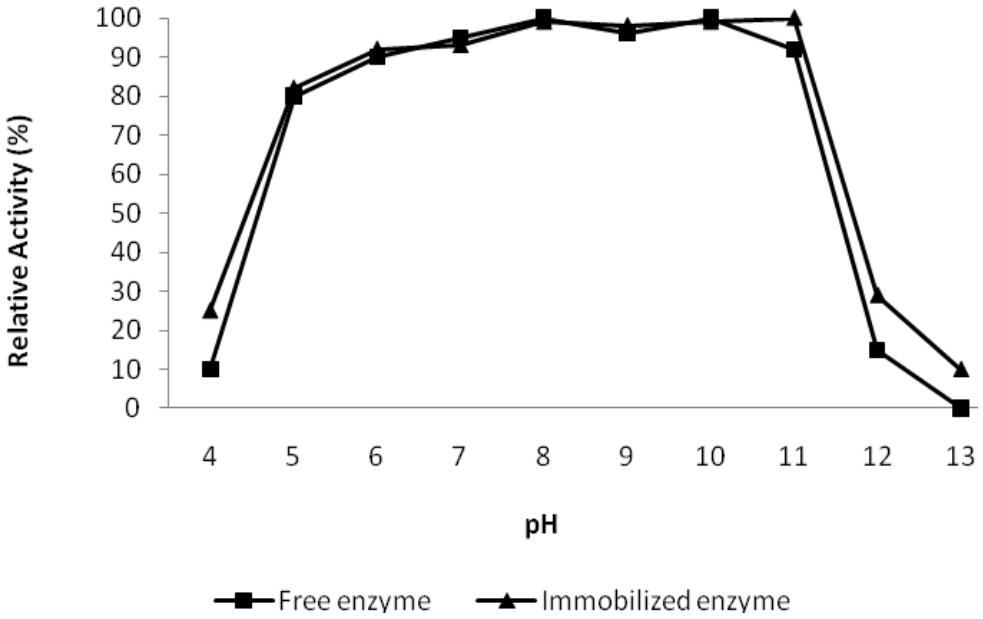

Figure 5. Effect of pH values on the stability of free and immobilized CGTase. The results are means of three experiments.

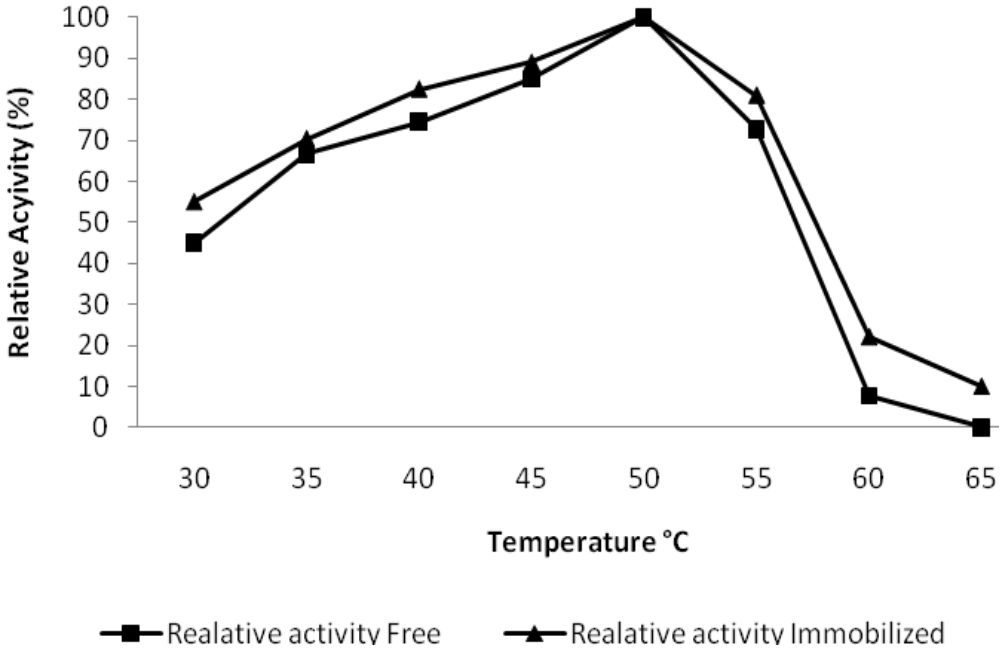

Figure 6. Effect of temperature values on the activity of free and immobilized CGTase.

effect of temperature (Martin et al., 2000; Ferrarotti et al., 2006).

Kinetic parameters

Kinetic parameters including Km and Vmax values for free and immobilized enzymes were estimated using Lineweaver-Burk plots for free and immobilized enzymes (Figure 8). While there was no change in the V_{max} value (83.3 µmol/min. mg) for both free and immobilized CGTase, the K_m of the enzyme increased from 14.28 to 20 mg/ml upon immobilization, which is true for most of the immobilization system (Arya and Srivastava, 2006).

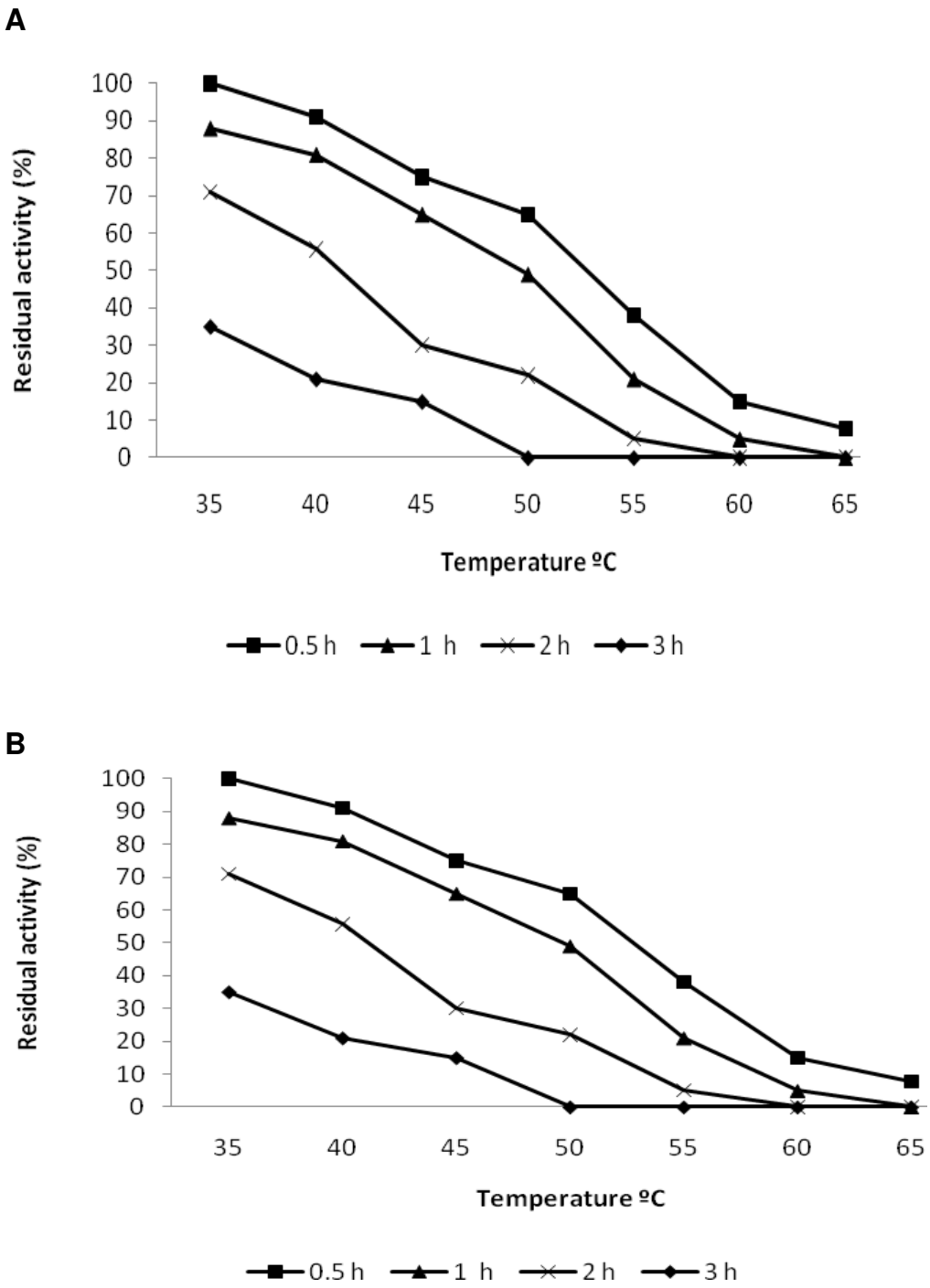

Figure 7. (A) Thermal stability of free and (B) immobilized CGTase at different temperatures.

Higher K_m value of the immobilized enzyme means that the affinity between the enzyme and the substrate decreased after immobilization. This may be due to the fact that the local concentration of the substrate near and within the carrier microenvironment was lower than that in the bulk solution because of a possible electrostatic repulsion between the substrate and the carrier (Kweona et al., 2005; Tardioli et al, 2006).

Operational stability

The reuse stability of immobilized enzymes is one of the most important factors affecting the utilization of an immobilized enzyme system. The immobilized CGTase was recycled and assayed ten times to check its activity; the immobilized enzyme was washed thoroughly with assay buffer until there was no trace of starch solution.

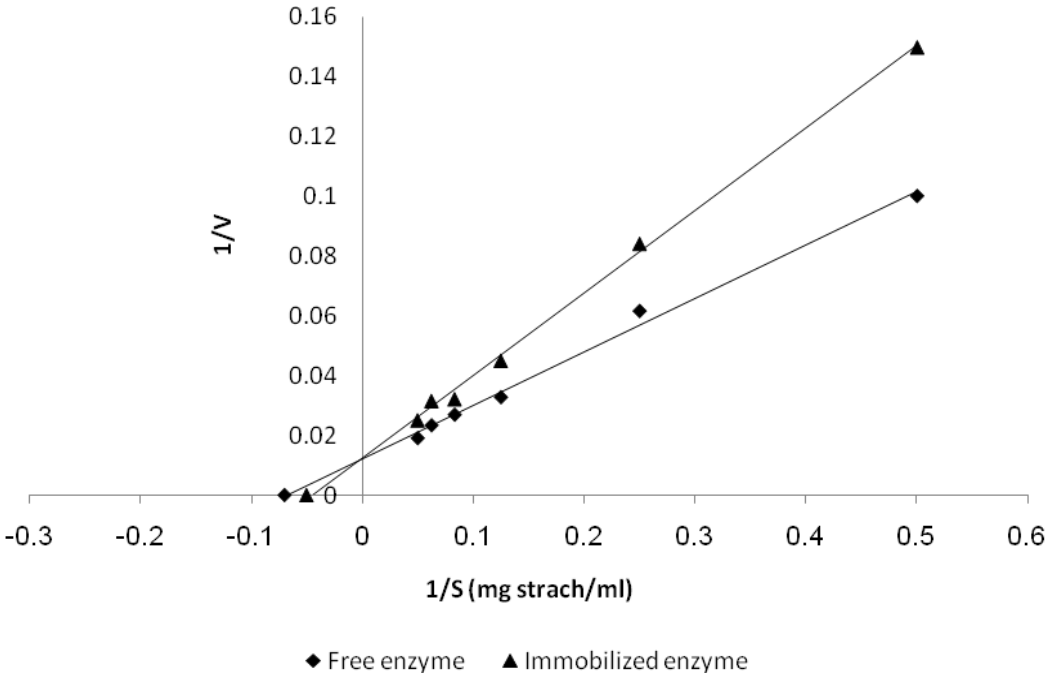

Figure 8. Lineweaver-Burk plot for estimation of Km and Vmax.

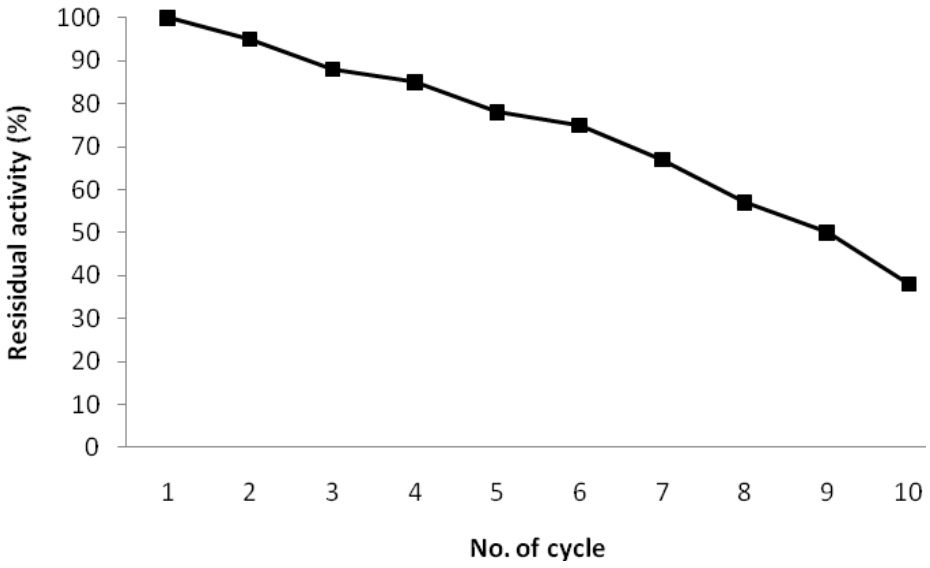

Figure 9. Operational stability of immobilized CGTase.

The results indicated that on repeated use of immobilized CGTase, 50% of the initial activity was retained for up to nine cycles (Figure 9). After ten cycles, there was significant enzyme denaturing due to physical loss of enzyme.

Conclusion

Screening of alkaliphilic bacteria, isolated from Egyptian soda lakes, for cyclodextrin glycosyltransferase-producing alkaliphilic bacteria resulted in isolation of 10 positive strains. Strain KSU-A11 was selected as the best producer of CGTase which was identified as *B. agaradhaerens* using 16S rDNA sequence analysis. The partially purified CGTase was immobilized on chitin by covalent binding technique using cross linking reaction with high immobilization yield. The properties of the free and immobilized CGTase were determined. It was shown that the immobilization process significantly improved the

enzyme properties indicating a possible application of the immobilized CGTase on chitin, using cross linking reaction, in different industrial appliaction of CGTase.

ACKNOWLEDGMENT

This work was financially supported by Research Center, College of Science, King Saud University (Research Project No Bot/2010/19).

REFERENCES

Alcalde M, Plou FJ, Teresa MM, Valdes I, Mendez E, Ballesteros A (2001). Succinylation of cyclodextrin glycosyltransferase from *Thermoanaerobacter* sp. 501 enhances its transferase activity using starch as donor. J. Biotechnol. 86: 71-80.

Altschul SF, Madden TL, Schaffer AA, Zhang J, Zhang Z, Miller W, Lipman DJ (1997). Gapped Blast and PSI-Blast: a new generation of protein database search programs. Nucleic acid program Res. 25: 3389-3402.

Arya SK, Srivastava SK (2006). Kinetics of immobilized cyclodextrin gluconotransferase produced by *Bacillus macerans* ATCC 8244. Enzyme Microbial Technol. 39: 507-510.

Astray G, Gonzalez-Barreiro C, Mejuto J, Rial-Otero R, Simal-Gandara J (2009). A review on the use of cyclodextrins in foods. Food Hydrocoll. 23: p. 1631.

Biwer A, Antranikian G, Heinzle E (2000). Enzymatic production of cyclodextrins. Appl. Microbiol. Biotechnol. 59: 609-617.

Bradford MM (1976). A rapid and sensitive method for quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal. Biochem. 72: 248-254.

Fava F, Ciccotosto VF (2002). Effects of randomly methylatedbeta-cyclodextrins (RAMEB) on the bioavailability and aerobic biodegradation of polychlorinated biphenyls in three pristine soils spiked with a transformer oil. Appl. Microbiol. Biotechnol. 58: 393-399.

Ferrarotti SA, Bolivar JM, Mateo C, Wilson L, Guisan JM, Fernandez-Lafuente R, (2006). Immobilization and stabilization of a cyclodextrin glycosyltransferase by covalent attachment on highly activated glyoxyl-agarose supports. Biotechnol. Prog. 22: 1140-1145.

Ferrarotti SA, Rosso AM, Maréchal MA, Krymkieweiz N, Maréchal LR (1996). Isolation of two strains (S_R type) of *Bacillus circulans* and purification of a cyclodextrin-glucanotransferease. Cell Mol. Biol. 42: 653-657.

Gill PK, Manhas RK, Singh P (2006). Hydrolysis of inulin by immobilized thermostable extracellular exoinulinase from *Aspergillus fumigates.* J. Food Eng. 76: 369-375.

Horikoshi K (1999a). Extracellular enzymes, In: Alkaliphiles 1999, p. 147 Harwood academic publisher Neatherlands.

Horikoshi K (1999b). Alkaliphilies: Some Applications of their products for biotechnology. Microb. Mol. Biol. Rev. 63: 735-750.

Krajewska B (2004). Application of chitin- and chitosan-based materials for enzyme immobilizations: a review. Enzyme Microbial Technol. 35: 126-139.

Kweona D, Kim S, Lee N, Chung K, Seo J (2005). Immobilization of *Bacillus macerans* cyclodextrin glycosyltransferase fused with poly-lysine using cation exchanger. Enzyme Microbial Technol. 36: 571-578.

Lane DJ (1991). 16S/23S rRNA sequencing. In: Nucleic acid techniques in bacterial systematic. Stackebrandt E, Goodfellow M (eds). John Whiley, Sons, New York, pp. 115-175.

Leemhuis H, Kelly R, Dijkhuizen L (2010). Engineering of cyclodextrin glucanotransferases and the impact for biotechnological applications. Appl. Microbiol. Biotechnol. 85: 823-835.

Li Z, Wang M, Wang F, Gu Z, Du G, Wu J, Chen J (2007). γ-Cyclodextrin: a review on enzymatic production and applications. Appl. Microbiol. Biotechnol. 77: 245-255.

Mäkelä MJ, Korpela TK, Puisto J, Laakso SV (1988). Non-chromatographic cyclodextrin assays: evaluation of sensitivity, specificity and conversion mixture applications. J Agri. Food Chem. 36: 83-88.

Martin Del Valle EM (2009). Cyclodextrins and their uses: a review. Proc. Biochem. 39: 1033-1046.

Martin MT, Alcalde M, Plou FJ, Dijkhuizen L, Ballesteros A (2000). Synthesis of malto-oligosaccharides via the acceptor reactioncatalyzed by cyclodextrin glycosyltransferases. Biocatal. Biotrans.19: 21-35.

Martin MT, Plou FJ, Alcalde MI, Ballesteros A (2003). Immobilization on Eupergit C of cyclodextrin glucosyltransferase (CGTase) and properties of the immobilized biocatalyst. J. Mol. Catal. Enzyme 21 299-308.

Martins RF, Davids W, Abu Al- Soud W, Levander F, Radström P, Hatti-Kaul R (2000). Starch-hydrolyzing bacteria from Ethiopian soda lakes. Extremophiles. 5: 135-144.

Mathews CK, Van Holde KE (1990). Dynamic of life: Catalysis and control of biochemical reactions. In Biochemistry. The Benjamin/Cummings publishing company, Inc. USA.

Nielsen P, Fritz D, Priest FG (1995). Phenitic diversity of alkaliphilic *Bacillus* strains: proposal for nine new species. Microbiology, 141: 1745-1761.

Park CS, Park KH, Kim SH (1989). A rapid screening method for alkaline β cyclodextrin-methylorange containing solid medium. Agric. Biol. Chem. 53: 1167-1169.

Schulein M, Outtrup H, Jorgensen PL, Bjornvad ME (1999). Alkaline xyloglucanases from *Bacillus* suitable for fabric detergents. PCT Int. Appl. WO99o2663, Denmark, January 21, 1999.

Spagna G, Andreani F, Salatelli E, Romagnoli D, Pifferi PG (1998). Immobilization of Ø-l-arabinofuranosidase on chitin and chitosan. Process Biochem. 33: 57-62.

Tardioli PW, Zanin GM, Moraes FF (2006). Characterization of *Thermoanaerobacter* cyclomaltodextringlucanotransferase immobilized on glyoxyl-agarose. Enzyme Microbial. Technol. 39: 1270-1278.

Tonkova A (1998). Bacterial cyclodextrin gluconotransferase. Enzyme Microb Technol. 22: 678-686.

Vaillant F, Millan A, Millan P, Dornier M, Decloux M, Reynes M (2000). Co immobilized pectinlyase and endocellulase on chitin and Nylon supports. Process Biochem. 35: 989-996.

Van der Veen BA, Uitdehaag JC, Dijkstra BW, Dijkhuizen L (2000). The role of arginine 47 in the cyclization and coupling reactions of cyclodextrin glycosyltransferase from *Bacillus circulans* strain 251 implications for product inhibition and product specificity. Eur. J. Biochem. 267: 3432–3441.

Woodward J (1985). Immobilized Cells and Enzymes: a Practical Approach, p. 44, Oxford, IRL, Press Limited, England.

Wojtasa AP, Hansen LT, Paulson AT (2008). Microstructural studies of probiotic bacteria-loaded alginate microcapsules using standard electron microscopy techniques and anhydrous fixation. LWT 41: 101-108.

Xu F (2000). Methods for using pectate lyases in baking. PCT Int. Appl. WO0015038, United States, March 23, 2000.

Zeng J, Zheng L (2002). Studies on *Penicillium* sp. ZDZ1 chitosanase immobilized on chitin by cross-linking reaction. Process Biochem. 38: 531-535.