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Beta-Glucosidase From Streptomyces Griseus: Nanoparticle Immobilisation and Alkyl Glucoside Synthesis.

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β-Glucosidase from *Streptomyces griseus*: nanoparticle immobilisation and alkyl glucoside synthesis.

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ABSTRACT

A novel β-glucosidase from *Streptomyces griseus* was cloned and overexpressed in *E. coli*. The purified β-glucosidase (44 kDa) had a K_m of 8.6±0.5 mM and a V_{max} of 217±5.0 μmoles^{-1}min^{-1}mg at 37 °C, pH 7.2 with *p*-nitrophenyl-β-D glucopyranoside as substrate. The enzyme was characterised in terms of pH optimum (pH 6.9), temperature optimum (69 °C) and the influence of solvents and effectors. Purified *S. griseus* β-glucosidase was successfully immobilised, by simple absorption, onto zinc oxide (ZnO) nanoparticles without covalent modification. It remained tightly bound even after extensive washing and could be reused up to ten times without significant loss of activity. The immobilised enzyme had a higher optimum temperature and greater thermostability than the free enzyme. In immobilised form the enzyme readily catalysed the synthesis of alkyl glucosides.

Keywords: β-glucosidase, *Streptomyces griseus,* nanoparticle, alkyl glucoside, immobilisation

List of abbreviations: ORF, Open Reading Frame; GST, Glutathione S Transferase; NCBI, National Centre for Biotechnology Information; GYM, Glucose Yeast Malt medium; *bglsG*, β-glucosidase from *Streptomyces griseus;* pNGP, para nitrophenyl-β-D glucopyranoside.

Introduction

β-glucosidases (*bglsG*, EC 3.2.1.21) are hydrolytic enzymes that cleave β-glycosidic bonds of carbohydrates [1], [2]. They act on a broad range of β-glycosides and play important biological roles in both eukaryotic and prokaryotic organisms. They play a particularly key role in the degradation of celluloses [3]. β-glucosidases have been studied from many different living organisms such as marine invertebrates, bacteria, fungi, plants, and mammals [4]. Commercially, they are used in the food and pharmaceutical industries, chemicals production, textiles and in the biotechnology sector. Indeed, β-glucosidases are amongst the most widely used enzymes for biotechnological applications [5],[6].

Chemical synthesis using β-glucosidases as a biocatalyst is a significant area of research interest. Such chemical transformations may involve transglycosylation or reverse hydrolysis reaction pathways. Reverse hydrolysis involves the direct esterification of a glycosyl donor and an acceptor. Thus, β-glucosidases have been used to synthesise alkyl, flavanoid, stilbenoid and vitamin glycosides among others (see [7] for review). Such transformations are examples of green chemistry that minimize the use of organic solvents.

In many cases β-glucosidases for use in industrial processes are immobilised on a solid support. Immobilisation of an enzyme catalyst allows for its separation from reactants, reusability, and often increases its thermostability [8]. β-glucosidases have been immobilized on different supports, such as alginate [9], silica gel [10], magnetic chitosan microspheres [11], Eupergit C [12], magnetic nanoparticles [13] and, most recently, silicone polymeric thin films [14]. A significant drawback with immobilisation is that it often requires covalent attachment of the enzyme to a support using crosslinking agents such as glutaraldehyde or carbodiimide. The process of immobilization is time consuming, expensive and may lead to loss of enzyme activity (see [15]).

In this study, a putative nucleotide sequence of a β-glucosidase was selected from the *Streptomyces griseus* subsp. *griseus* genome database. Several β-glucosidase Open Reading Frames ranging from 406 to 768 amino acids were identified in the genome

of *Streptomyces griseus subsp. griseus* using the NCBI genomic database. The smallest ORF having a size of 406 amino acids was selected since it was thought that a smaller protein might be more compact in structure and therefore more solvent and temperature tolerant. Overexpression of this β-glucosidase was carried out under the inducible control of a *tac* promoter based *Glutathione-S-Transferase* (GST) fusion protein expression system to avoid the formation of inclusion bodies. A simple method to immobilise this enzyme on zinc nanoparticles was developed and the application of this enzyme in the synthesis of alkyl glucosides was demonstrated.

Materials and Methods

Materials:

Wizard Genomic DNA Purification Kit (Cat. No. A1125), Pure Yield™ Plasmid Midi Prep System (Cat. No. A9281), Gel extraction kit, 6x DNA loading buffer, T4 DNA ligase, and GoTaq® DNA polymerase were obtained from Promega, MSC, Dublin, Ireland. Gene specific primers were obtained from Eurofins MWG Operon. Restriction endonucleases and their corresponding buffers were obtained from New England Biolabs (NEB, UK). Glutathione Sepharose 4B resin and PreScission protease were obtained from VWR International Ltd. Blanchardstown, Dublin, Ireland. All chemicals were obtained from Sigma-Aldrich, Ireland.

Bacterial strains, culture media, and growth conditions:

Streptomyces griseus subsp. *griseus* was inoculated from stock culture into GYM *Streptomyces* medium and incubated for three days at 220rpm and 37°C. Luria-Bertani media was used for the maintenance of *E. coli* strains at 37°C. *Escherichia coli* strains; JM109 (Promega) and BL21 (DE3) cells were used for cloning and protein expression respectively. Phenotypically, protease-plus *E. coli* (JM109) was used for maintenance of the vector pGEX-4T1 and *S. griseus* β-glucosidase (*pGST-bglsG*) clones.

Amplification of β-glucosidase gene:

Amplification of the β-glucosidase (Gene ID: 6212454) from *S. griseus* genomic DNA was performed in a G-Storm GS1 Thermal Cycler (GRI, Promega). Forward, (*bglsG*_for_*Nco*I) 5'CATGCCATGGGGACACACACCCCTGCTTGG3' and reverse (*bglsG*_rev_*Eco*RI) 5'CCGGAATTCTCAGGCTGCCGTGCGCGG3' primers containing *Nco*I and *Eco*RI restriction sites (underlined) respectively were used for the amplification of the β-glucosidase gene. PCR conditions were as follows: denaturation (94 °C for 1 minute), annealing (66 °C for 45 seconds), and extension (72 °C for 1 minute) for 33 cycles with a final extension (72 °C for 5 minutes) and an indefinite hold at 4°C. Amplification was carried out with GoTaq® DNA polymerase. Ligation reactions were carried out using T_4 DNA *Ligase* and the resulting recombinant DNA was used for transformation into JM109 *E. coli* cells.

The β-glucosidase from *Streptomyces griseus* (*bglsG*) was cloned into a *Glutathione-S-Transferase* plasmid expression vector (pGEX-4T1).

Expression of *S. griseus* β-glucosidase:

E. coli transformants were incubated at 37 °C and 220 rpm until an optical density (OD_{600nm}) of 0.4-0.6 was reached. IPTG was added to a final concentration of 0.3 mM. The culture was further incubated at 30°C for 6 hours and then centrifuged at 5,000 x*g* for 10 minutes at 4°C to pellet cells. The pellet was washed in 10 mM Na_2HPO_4, 1.8 mM KH_2PO_4, 140 mM NaCl, 2.7 mM KCl, pH 7.3. The supernatant was discarded and the pellet was resuspended in 100 ml of cell lysis buffer (10 mM Na_2HPO_4, 1.8 mM KH_2PO_4, 140 mM NaCl, 1% (v/v), Triton X-100, 1 mM DTT, 1mM PMSF, pH 7.3). 10 ml of lysozyme (10 mg ml^{-1}) was added to the resuspended cells. Cell lysis was achieved by three cycles of freeze (-196°C, liquid nitrogen) and thaw (at 30°C in a temperature controlled water bath). The lysed cells were centrifuged at 14,000x*g* for 60 minutes at 4°C. The supernatant containing recombinant protein was syringe filtered (pore size of 0.45 μm) and used for protein purification.

Purification of *S. griseus* β-glucosidase:

Lysed and clarified bacterial supernatant was loaded onto a Glutathione Sepharose 4B resin column at a flow rate of 0.5 ml per minute to optimise binding. A specific *PreScission* protease was used for cleavage of fusion protein from the GST tag to yield a highly purified β-glucosidase. This protease cleaved the fusion protein at an rTEV Protease Cleavage Site. The column was washed with 20 ml of cleavage buffer to ensure all cleaved protein was eluted. The eluted enzyme was dialysed against 1.0L of 50 mM potassium phosphate buffer, pH 7.2 at 4°C for 36 hours with three changes and constant gentle mixing. Purified protein was concentrated using Centricon® 10 filters (5000×*g*, 20 minutes, 4°C) with 10 kDa Molecular Weight Cut–Off.

Standard assay of β-glucosidase:

β-glucosidase activity was determined spectrophotometrically at 37°C using the substrate *p*-nitrophenyl-β-D glucopyranoside (pNPG). Briefly, 20 μl of appropriately diluted enzyme (typically 0.5 μg μl^{-1}), was mixed with 120 μl of 50 mM potassium

phosphate buffer, pH 7.2. The assay was initiated with 30 µl of 7.0 mM pNPG (in 50 mM potassium phosphate buffer, pH 7.2) After 20 min the reaction was stopped by adding 30µl of 1M Na_2CO_3 solution The mustard yellow colour, developed due to the liberation of *p*-nitrophenol, was monitored at 405nm in a microplate reader (BioTek PowerWave). For immobilised enzyme the reaction mixture was centrifuged to remove nanoparticles before measurement.

Effect of pH and temperature on *S. griseus* β-glucosidases:

The pH optimum for *S. griseus* β-glucosidases activity was determined in three different buffers: pH 3.5-5.5: 50 mM sodium citrate, pH 6.0-8.0: 50 mM potassium phosphate and pH 8.5-9.0: 50 mM glycine. The temperature optimum for *S. griseus* β-glucosidase was obtained from measurements of activity (at the optimum pH of 6.9) in the temperature range from 20 °C to 100 °C. Thermostability was assessed by incubating an enzyme preparation (free or immobilised) at the appropriate temperature for 3 hours: samples were withdrawn at intervals of 20 minutes and tested using the standard assay. All assays were subject to three intra- and inter-experimental repeats.

Influence of effectors on *S. griseus* β-glucosidase:

All effectors investigated were used at a final concentration of 1 mM. The final concentration of solvent used was 40% (v/v) in all cases. Inhibition of *S. griseus* β-glucosidase by glucose was carried out using different concentrations (ranging 0.056 to 0.336 mM) of glucose.

Kinetics constants for *S. griseus* β-glucosidase:

Kinetic parameters were calculated from an activity *versus* substrate concentration plot based on triplicate independent assay results by regression analysis using the software EnzFitter v2.o.18.0 (Biosoft, Cambridge, UK). Kinetic parameters were obtained using the substrate *p*-nitrophenyl-β-D glucopyranoside (pNPG) using a range of substrate concentrations from 4 to 30 mM.

Nanoparticle immobilisation:

The immobilisation of β-glucosidases onto zinc oxide (ZnO) nanoparticles (NPs) was carried out by incubating 1 ml of enzyme (50 μg ml^{-1}) with 1.0 ml of nanoparticles (1.0 mg/ml) with gentle mixing. The immobilisation was carried out for 90 minutes at 4°C.

Synthesis of glucosides:

Synthesis of hexyl-β-D-glucoside was performed in a total reaction volume of 1 ml. A 160 μl aliquot of a solution containing Glucose (10 mM) and β-glucosidase (0.1-1.0 mg/ml) in 50 mM potassium phosphate buffer pH 6.9 was incubated with 50 μl acetonitrile for 1 hour. An alcohol substrate (790 μl) was then added to initiate the reverse hydrolysis reaction. The reaction was allowed to proceed at 60°C with agitation on a shaking incubator (Innova, 200 rpm) for a further hour. A sample of supernatant containing the solvent phase was withdrawn for TLC analysis.

Thin Layer Chromatography (TLC) of glucosides:

Enzymatically synthesised glucosides were separated on thin layer chromatography (TLC) plates using silica gel 60 F254nm aluminium sheets (dimension: 20 X 20 cm). Briefly, 2.0 μl of each reaction product was applied to a TLC plate by capillary injection with a disposable micropipette. Spotted samples were fixed and activated at 110 °C for 30 minutes. Plates were developed in a mobile phase of 1-propanol/ethyl acetate/water (6:2:2,v/v/v). The plates were dried at room temperature for 5 minutes and sprayed using an atomizer (TLC sprayer) containing 0.3% (w/v) N-(1-naphthyl) ethylenediamine dihydrochloride and 5% (v/v) H_2SO_4 in methanol. The plates were oven dried at 110°C until coloured spots were observed.

Error Estimates

Unless indicated, replicate errors were within the area of the data points drawn on the graphs shown below.

Results and Discussion

Expression and Purification of *S. griseus* β-glucosidase

The molecular weight (44 kDa) of purified *S. griseus* β-glucosidase and its pI (4.87) were established using Expasy tools (http://web.expasy.org/protparam). A highly purified preparation of *S. griseus* β-glucosidase was readily prepared using the protocols described herein (Figure 1).

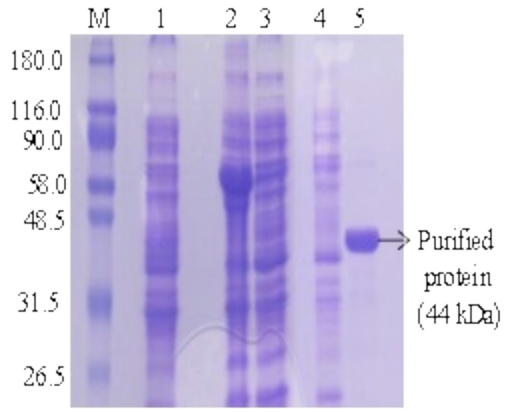


Figure 1: SDS-PAGE of *S. griseus* β-glucosidase purification. SDS gel electrophoresis of purification stages for *S. griseus* β-glucosidase: Lane M is a broad range protein markers (kDa), Lane 1: Non-induced cell lysate, Lane 2: IPTG induced cell lysate, Lane 3: Glutathione Sepharose 4B column flow through, Lane 4: Column washing: Lane 5: Eluted purified β-glucosidase.

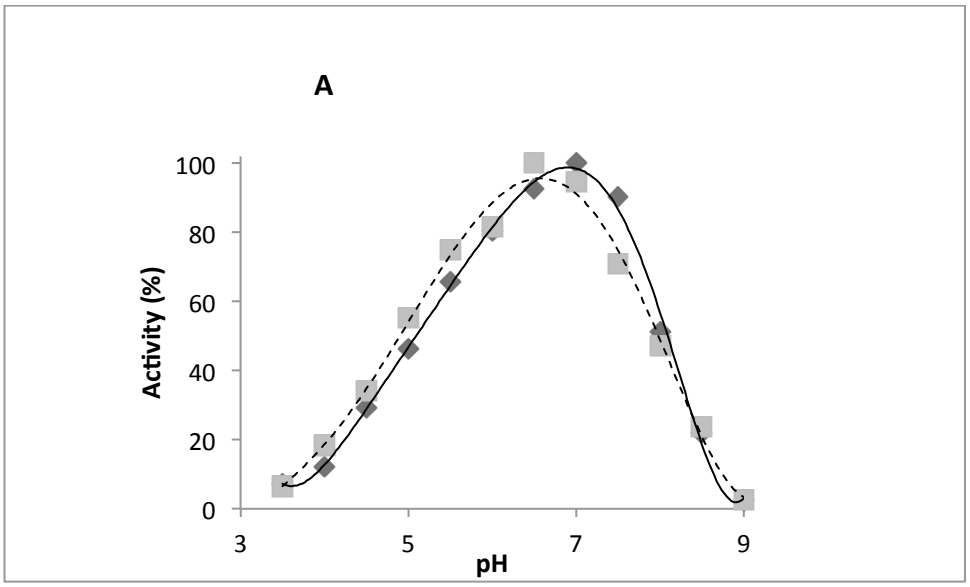
This report is the first on the expression and purification of this novel enzyme. Initially the expression proved problematic due to the formation of insoluble protein. This problem was solved when the *Glutathione-S-Transferase* based expression vector pGEX-4T1 was chosen for cloning and the enzyme was overexpressed as a fusion protein with GST. The GST tag was proteolytically removed before characterisation of the purified enzyme. The specific activity of purified protein (217±5.0 µmol/min/mg) was roughly 100 times higher than the crude extract (2.15 µmol/min/mg) of β-glucosidase. A typical purification starting with 500 ml of culture yielded 3 to 4 mg of purified protein.

Effect of pH and temperature on *S. griseus* β-glucosidase

The effect of pH and temperature on the activity of *S. griseus* β-glucosidase was examined (Figure 2. A,B). The commercially available β-glucosidase from sweet almond was used as a comparator for these studies since it is a well characterised enzyme widely used for a variety of biotechnological applications.

S. griseus β-glucosidase had a pH optimum of 6.9 (Figure 2A), slightly higher than commercial almond β-glucosidase (pH 6.6) under the same assay conditions. β-glucosidases typically have pH optima in the region of 3 to 7 ([15]; [16]; [17]; [18]; [19]) and the *S. griseus* enzyme lies at the upper end of this range. Activity at higher pH values may be useful for specific industrial applications where processing is carried out under more alkaline conditions *e.g*. the paper pulp industry ([20]; [21]).

The effect of temperature on activity was examined in the range 20 °C to 100 °C. The optimum temperature of *S. griseus* β-glucosidase was estimated to be 69 °C. Under the same conditions the commercially available almond β-glucosidase showed a temperature optimum of 59 °C. The profile for *S. griseus* β-glucosidase immobilised on a nanoparticle support is also shown (Figure 2.B): immobilisation increased the enzyme's optimum temperature to 75 °C. This probably reflects an increase in stability at elevated temperatures that is often seen with immobilised enzymes.



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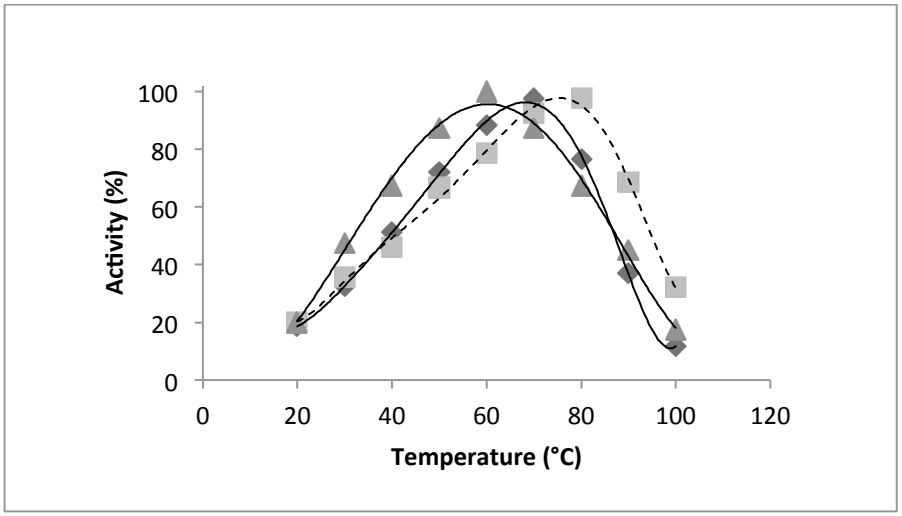
259 **B**



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263 **Figure 2: Effect of pH and temperature on the activity of recombinant *S. griseus***
264 **β-glucosidase – comparison with commercial Almond enzyme.** **A**. Enzyme
265 activity was estimated in the pH range from 3.5 to 9.0. *S. griseus* β-glucosidase (♦);
266 commercial Almond β-glucosidase (■). **B**. Optimum temperature was estimated over
267 the range 20 °C to 100 °C. *S. griseus* β-glucosidase (♦); commercial Almond β-
268 glucosidase (▲) nanoparticle immobilised *S. griseus* β-glucosidase (■).

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A recent review of glucosidases and their industrial applications, [20], listed only one enzyme, the hyperthermostable glucosidase from *Thermus thermophilus* HJ6, with a pH optimum higher than 7.0 and a temperature optimum higher than 70 °C. The *T. thermophilis* enzyme had a pH optimum of 8.5 and a temperature optimum of 90 °C ([22]). *The S. griseus* enzyme, therefore, is close to the upper end of the range for most glucosidases examined to date in terms of both pH optimum and temperature optimum.

Thermostability of the *S.griseus* enzyme was examined by incubating the enzyme at its optimum temperature (69 °C) and monitoring activity over time (Figure 3). This figure also shows how thermostability is increased when the enzyme is immobilised on a nanoparticle support.

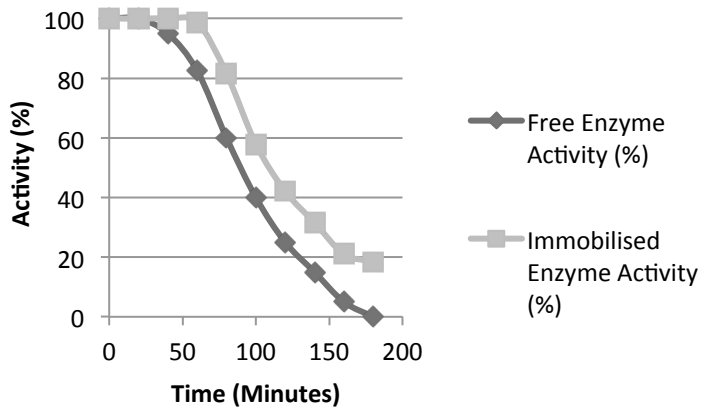


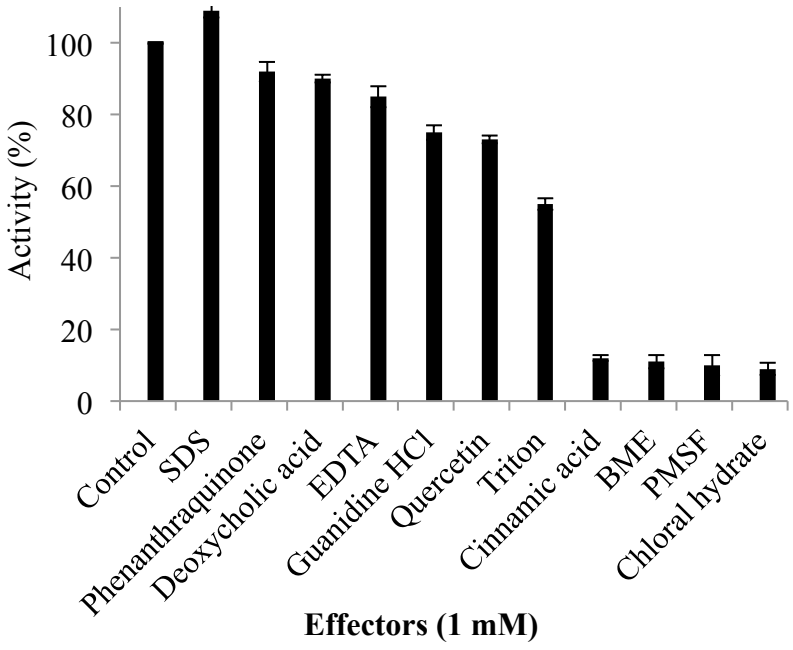
Figure 3: Thermal stability of free and immobilised *S. griseus* β-glucosidase. Immobilisation and thermal stability monitoring as described in Materials and Methods. Reactions were followed for a three hour period using the standard assay. *S. griseus* β-glucosidase (♦); nanoparticle immobilised *S. griseus* β-glucosidase (■).

The free enzyme retained more than 50% of its activity after incubation at 69°C for 1.5 hours while the immobilized form retained more than 50% activity for roughly two hours under the same conditions. After three hours, the free enzyme became inactivated while the immobilised form still retained *ca* 20% activity. In terms of thermostability, the *S. griseus* enzyme is comparable to β-glucosidases commonly used for biocatalytic applications ([17]; [18];[19]). Thus, it is somewhat better than the thermostable β-glucosidase from *Penicillium citrinum* that has an optimum

temperature of 70 °C but becomes inactivated above 60 °C ([23]) or that from *Aspergillus fumigatus Z5* ([17]) which had maximal activity at 60 °C but lost activity rapidly above this temperature. On the other hand, some highly thermostable enzymes have been reported: the glucosidase recently described from *H. orenii* retained more than 90 % of its activity after 3 h of incubation at 65 °C ([24]) while that from *Dictyoglomus thermophilum* reportedly retained a remarkable 70–80% of its initial activity after 7 days of incubation at 70 °C ([25]).

Effectors of *S. griseus* β-glucosidase

The activity of β-glucosidase was examined in presence of a final concentration of 1.0 mM of various effectors: SDS, phenanthraquinone, deoxycholic acid, EDTA, guanidine hydrochloride, quercetin, Triton X-100, cinnamic acid, BME, PMSF, and chloral hydrate (Figure 4A).



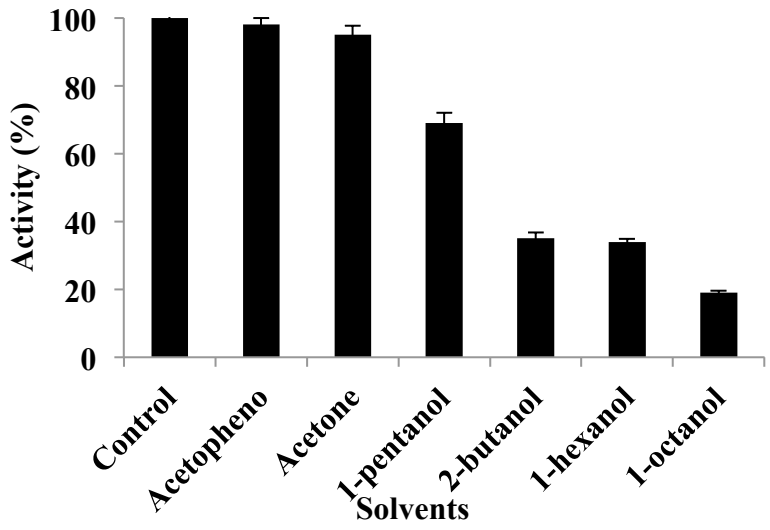


Figure 4: The influence of effectors and solvents on *S. griseus* β-glucosidase activity. Assays were carried out in the absence (control) and presence of 1.0 mM effectors at 37°C. **A** Effectors of *S. griseus* β-glucosidase activity: SDS is sodium dodecyl sulphate; EDTA is Ethylene diaminetetraacetic acid; BME is β-Mercaptoethanol and PMSF is phenyl methane sulfonyl fluoride. Activity was expressed as a percentage of a control reaction without effectors. **B** Effect of solvents on *S. griseus* β-glucosidase activity. Assays were carried out in the absence (control) and presence of 40% (v/v) solvents at 37°C.

Inhibition by the thiol modifying compounds, β-mercaptoethanol and PMSF, suggests the involvement of thiols in the activity of this protein. The enzyme was slightly activated by SDS but inhibited by Triton. The inhibition with Triton might be attributed to its aromatic moiety interacting with a hydrophobic region close to or in the active site. Cinnamic acid has an aromatic ring that may inhibit in a similar manner. Inhibition by chloral hydrate was surprising since it is a rather small molecule. It is a fully hydrated aldehyde which is present in solution as a gem-diol structure and it is possible that this diol is interacting at the glucose binding site.

Solvent tolerance is a highly desirable trait for enzymes that are to be used for industrial chemoenzymatic synthesis [20]: it allows the application of such enzymes in a wider range of solvent environments and by reducing water activity increases the yield of reverse hydrolysis reactions. The stability of *S. griseus* β-glucosidase was

examined in the presence of organic solvents (final concentration of 40% (v/v)). The solvents used were: acetophenone, acetone, 1-pentanol, 2-butanol, 1-hexanol, and 1-octanol. It was found that 1-octanol, 1-hexanol, and 2-butanol inhibited enzyme activity by 19%, 34%, and 35%, respectively (Figure 4B). Thus, the primary alcohol substrates were shown to inhibit the enzyme and this inhibition increased with chain length of the alcohol. Significantly, this inhibition did not impair alkyl glucoside synthesis (see below). The increasing inhibition seen with increasing chain length suggests that alcohols are binding to a hydrophobic region that inhibits substrate turnover but does not inactivate the enzyme.

Inhibition by glucose:

Applications where glucosidases are used to hydrolyse glycosidic bonds require an enzyme that is insensitive to product inhibition. Different concentrations of glucose; ranging from final concentrations of 0.056-0.336 mM were tested (Figure 5). A glucose concentration of 0.336M reduced activity by 50%.

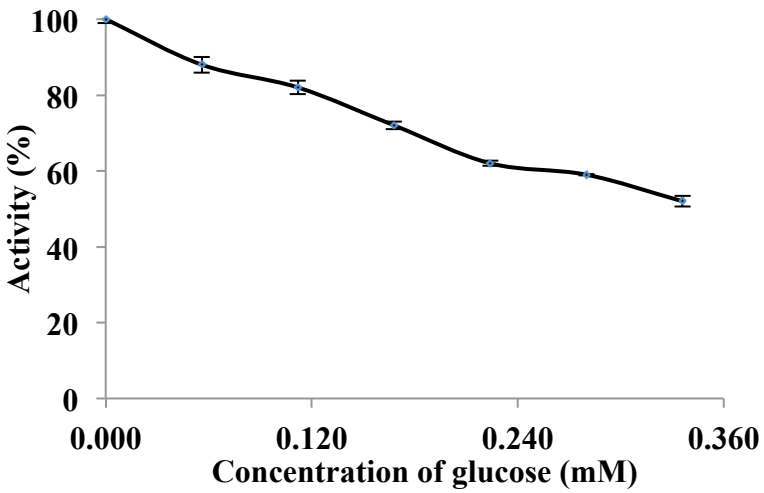


Figure 5: Inhibition of *S. griseus* β-glucosidase by glucose. Inhibition studies were carried out at 37 °C in 50 mM potassium phosphate buffer, pH 6.9 using the standard assay. Different concentrations of glucose were used in the ranges from 0.056-0.336 mM. The percentage activity of control reaction was measured without glucose.

A concentration of 0.336 mM glucose caused 50% loss of activity in the standard assay. In general, most microbial β-glucosidases are inhibited in the presence of glucose ([26]; [27]; [28]). However, a few glucose tolerant enzymes have been reported such as those of *Candida peltata* and *Aspergillus oryzae* ([29]; [30]). On the other hand, the fungal β-glucosidase from *Aspergillus niger* lost 85% activity in the presence of 0.168 mM glucose ([31]). This tight binding of glucose observed in the present work rules out applications where hydrolysis is employed such as bioethanol production. However, tight binding of this substrate is desirable for chemoenzymatic synthetic applications involving reverse hydrolysis.

Kinetic constants

Initial rates of β-glucosidase were determined using different concentrations of *p*-nitrophenyl-β-D glucopyranoside (pNPG) between 4-30 mM. The value K_m and V_{max} were obtained as 8.6±0.5 mM and 217±5.0 μmoles^{-1}min^{-1}mg, respectively (Figure 6).

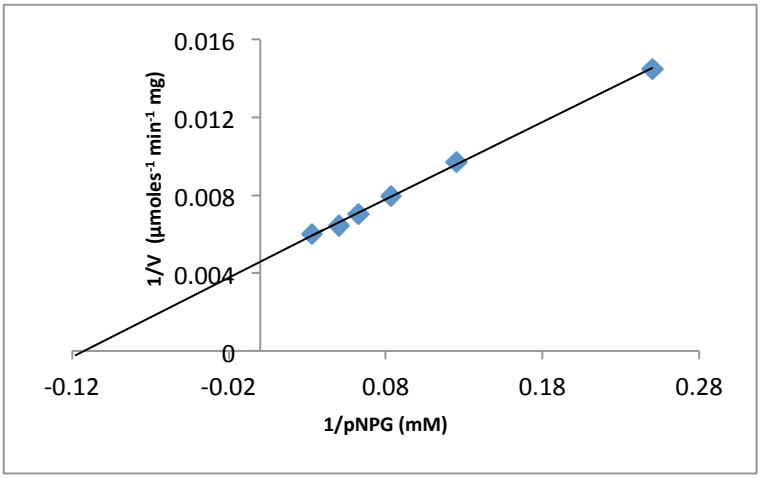
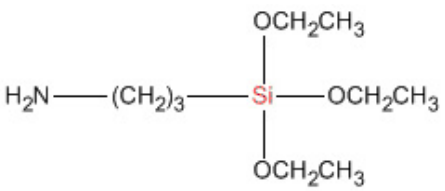


Figure 6. Kinetic constants estimation for hydrolysis of *p*-nitrophenyl-β-D glucopyranoside (pNPG) by *S. griseus* β-glucosidase. Assays were performed at 37 °C using the standard assay while varying the concentration of pNGP.

Nanoparticle immobilisation:

The findings above indicate that immobilisation on a nanoparticle support has a significant effect on thermostability of the *S. griseus* enzyme. Immobilisation typically enhances an enzyme's thermal stability as well as improving its solvent tolerance. In this study, Zinc oxide (ZnO) nanoparticles modified with γ-aminopropyltriethoxysilane (KH550) were chosen based on their cost effectiveness. KH550 is an amino-functional coupling agent which provides hydrophobicity to the surface of ZnO particles (Scheme 1).



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Scheme1: Structure of γ-aminopropyltriethoxysilane (KH550). ZnO nanoparticles are coated with KH550 which provides for ease of dispersion of the nanoparticles.

The *S. griseus* enzyme was found to stick tightly to the Zn nanoparticles used in this study and was not removed even after extensive washing. Nanoparticle immobilised enzyme was washed with buffer solutions containing up to 2.0M KCl without eluting significant enzyme activity. Only 6% of enzyme activity was removed after 10 washes with a 2.0M KCl solution. Similarly, washes in up to 50% (v/v) acetonitrile caused no loss of activity.

The ease of immobilisation is of considerable value since the chemical steps, costs and resulting loss of activity involved in covalent immobilisation are avoided. A similarly facile immobilisation has been reported for a β-galactosidase from *Aspergillus oryzae* [32]. The immobilised enzyme exhibited the same pH optimum as the free enzyme but its optimum temperature was shifted to 75 °C (Figure 2B). Although immobilisation enhanced thermostability, it was not as dramatic an improvement as seen with some other enzymes. This may be due to the method of immobilisation which does not involve multiple covalent points of attachment that are known to stabilise immobilised enzymes. Recently, Verma and coworkers [13] showed a dramatic increase in the stability of *Aspergillus niger* glucosidase following covalent immobilisation on magnetic nanoparticles: the free enzyme lost all activity after 2 h of incubation at 70 °C whereas the immobilized form still retained 67% of its initial activity after 4 h incubation.

Synthesis of Alkyl glucosides:

The application of the immobilised enzyme to the synthesis of hexyl-glucoside was explored. Such glucosides are important biodegradable surfactants with a wide range

of food and pharmaceutical applications [33]. Figure 7 shows a time course of hexyl glucoside synthesis.

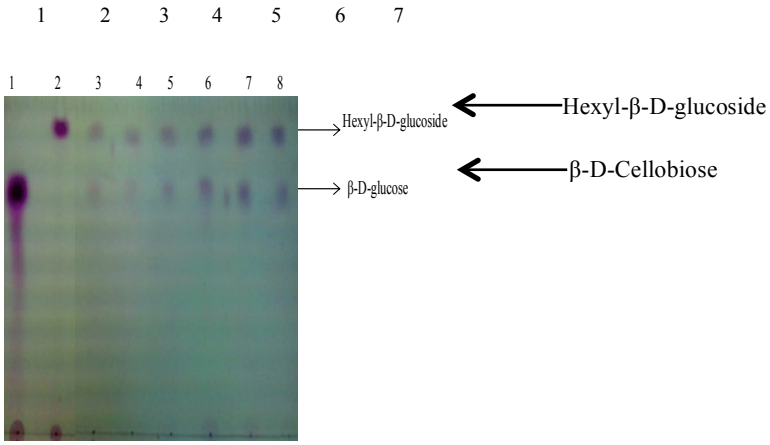

Figure 7: **Synthesis of hexyl glucoside using immobilised *S. griseus* β-glucosidase.** Thin layer chromatography of hexyl-β-D-glucoside synthesis. 20 µl of reaction mixture was withdrawn at different times and 4.0 µl of each sample was analysed by TLC: Lane 1, Standard Hexyl-β-D-glucoside; Lane 2, 2 hours of incubation; Lane 3, 4 hours of incubation; Lane 4, 8 hours of incubation; Lane 5, 16 hours of incubation; Lane 6, 24 hours of incubation and lane 7, 30 hours of incubation.

In addition to hexanol this enzyme was able to utilise a wide range of alcohol substrates as acceptor. Thus, propyl, butyl, pentyl, octyl, benzyl and 2-phenyl-ethyl glucosides were also synthesised by this using the same conditions by simply replacing the alcohol substrate (data not shown). These alcohol substrates were shown to inhibit the glucosidase activity of the enzyme (see above) but clearly do not inhibit the synthesis of alkyl glucosides. This breadth of substrate specificity is a significant advantage for the application of this enzyme. The gycosidation of bioactive compounds, for example, has been pursued to enhance their stability, solubility or receptor binding properties ([7]). Our initial studies establish that this

reaction proceeds readily for this enzyme but further work is clearly required to define the full extent of this synthetic capability.

Conclusions:

The β-glucosidase from *S. griseus* has a number of characteristics that make it suitable as a potential novel biocatalyst for industrial applications: it is optimally active at neutral pH, thermostable up to 69 °C, solvent tolerant and easily immobilised on a nanoparticle support. It has been shown to readily catalyse alkyl glucoside synthesis. The characteristic features of this recombinant *S. griseus* β-glucosidase suggest that it may offer a useful alternative to currently used β-glucosidases for certain green chemistry applications.

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