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ORIGINAL PAPER

Characterization of the smallest dimeric bile salt hydrolase from a thermophile *Brevibacillus* sp.

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Abstract A thermophilic microorganism producing bile salt hydrolase was isolated from hot water springs, Pali, Maharashtra, India. This microorganism was identified as *Brevibacillus* sp. by 16S rDNA sequencing. Bile salt hydrolase (BSH) was purified to homogeneity from this thermophilic source using Q-sepharose chromatography and its enzymatic properties were characterized. The subunit molecular mass of the purified enzyme was estimated to be 28 kDa by SDS-PAGE and, 28.2 kDa by MALDI-TOF analysis. The native molecular mass was estimated to be 56 kDa by gel filtration chromatography, indicating the protein to be a homodimer. The pH and temperature optimum for the enzyme catalysis were 9.0 and 60°C, respectively. Even though BSH from *Brevibacillus* sp. hydrolyzed all of the six major human bile salts, the enzyme preferred glycine conjugated substrates with apparent K_M and k_{cat} values of 3.08 μM and 6.32 × 10^2 s^{-1}, respectively, for glycodeoxycholic acid. The NH_2-terminal sequence of the purified enzyme was determined and it did not show any homology with other bacterial bile salt hydrolases. To our knowledge, this is the first report describing the purification of BSH to homogeneity from a thermophilic source.

Keywords Purification · Bile salt hydrolase · *Brevibacillus* sp. · Dimeric intracellular enzyme · Thermophile

Abbreviation
BSH Bile salt hydrolase

Introduction

Elevated levels of cholesterol are a risk factor for cardiovascular diseases. The reduction in serum cholesterol could be an important health benefit, as 1% reduction in serum cholesterol is associated with an estimated reduction of 2–3% in the risk of coronary artery diseases (Manson et al. 1992). Exploring new methods to reduce serum cholesterol is of great importance at present. Cholesterol excretion is mediated by bile acids, the steroid molecules synthesized de novo in the liver from cholesterol. The solubility of the hydrophobic steroid nucleus is increased by conjugation as an *N*-acyl amidate with either glycine or taurine prior to secretion in gall bladder. Intake of food stimulates the release of bile salts into duodenum which result in the emulsification of dietary lipids. Conjugated bile acids are poorly absorbed by passive diffusion in the small and large intestines and mainly absorbed at the distal ileum by an active transport system, which is called as ileum bile acid transporter (IBAT) and the members of the ATP binding cassette (ABC) family of transporters. (Lack and Weiner 1966). After absorption, the mixture of bile salts is partly returned to the liver by hepatic portal circulation in the

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process known as enterohepatic circulation (Hofmann 1999).

Enzymatic deconjugation of bile salts has been related to a reduction of serum cholesterol levels in mammals (Klaver and van der Meer 1993; Pereira et al. 2003). Deconjugation is catalyzed by bile salt hydrolases (BSH; E.C.3.5.1.24), the enzymes which hydrolyze the amide bond and liberate the glycine/taurine moiety from the steroid core of bile salts. The resulting acids are termed as free bile acids or deconjugated bile acids, which are excreted via feces. Bacteria with BSH activity may effectively reduce serum cholesterol by enhancing the excretion of free bile acids, with a consequent increase in the synthesis of bile salts from serum cholesterol or by decreasing the solubility of cholesterol, and thus reducing its uptake from the gut.

Many reports are available for the production of bile salt hydrolases from mesophilic source. A large number of bacterial strains possessing deconjugation activity have been isolated from intestine and feces of human and other mammals and also from fermented milk products (Kim and Lee 2005). BSH activity is observed in bacteria such as *Lactobacillus* sp. (Lundeen and Savage 1990), *Bifidobacterium longum* (Grill et al. 1995), *Clostridium perfringens* (Gopal-Srivastava and Hylemon 1988), *Enterococcus* (Knarreborg et al. 2002), *Bacteroides vulgatus* (Kawamoto et al. 1989), *Bacteroides fragilis* (Stellwag and Hylemon 1976). In addition to these intestinal microorganisms, BSH activity was also reported from an enteropathogenic strain of *Listeria monocytogenes* (Dussurget et al. 2002) and bile adapted strain of *Xanthomonas maltophilia* (Dean et al. 2002).

Inspite of this wide distribution and high activity of BSH among mesophilic microorganisms, there are no reports so far regarding the isolation and purification of BSH from the thermophilic source with an optimum growth temperature range 40–65°C. Screening for BSH producing thermophiles in our laboratory led to the identification of thermophilic bacteria isolated from hot water springs in Pali, Maharashtra, India. This thermophile was identified as *Brevibacillus* sp. based on 16S rDNA analysis and is a source of intracellular BSH (Sridevi and Prabhune 2008). In the present study we are reporting for the first time purification and characterization of BSH from a novel thermophilic source, the *Brevibacillus* sp. with an optimal growth temperature of 55°C.

Materials and methods

Q-sepharose, sephacryl S-200, glycodeoxycholic acid, glycocholic acid, glycochenocholic acid, taurodeoxycholic acid, taurocholic acid, taurochenocholic acid, 2% ninhydrin reagent solution, trichloro acetic acid, native molecular weight marker kit were purchased from Sigma. Low molecular weight calibration kit for SDS electrophoresis was obtained from Amersham Biosciences. All other chemicals used were of analytic grade.

Isolation and growth conditions of *Brevibacillus* sp.

Brevibacillus sp. was grown under optimal conditions for production of BSH (Sridevi and Prabhune 2008). Briefly a series of 500-ml Erlenmeyer flasks containing 125 ml of sodium glutamate medium (sodium glutamate 0.5%, yeast extract 0.5%, peptone 1.0% and sodium chloride 0.2%) were seeded with 10% inoculum and incubated for 24 h at 55°C and 180 rpm. Cells in a log phase culture were harvested by centrifugation at 7,000 × *g* for 30 min at 4°C. The harvested cells were used for homogeneous preparation of purified enzyme.

Bile salt hydrolase assay and protein assay

Bile salt hydrolase activity was determined by measuring the amount of amino acids resulting from the hydrolysis of amide bond of bile salts using ninhydrin assay (Suresh Kumar et al. 2006). Briefly, the enzyme sample was incubated in a reaction mixture containing 1-mM sodium glycodeoxycholate, 100-mM potassium phosphate buffer, pH 6.5 at 40°C for 10 min. An aliquot of 25 µl was removed and mixed immediately with 25 µl of 15% (w/v) trichloroacetic acid. The sample was spun at 15,000 × *g* for 2 min and the supernatant was mixed with an equal volume of 2% ninhydrin reagent solution before boiling for 15 min. The absorption was recorded at 570 nm and the amount of product formed was estimated from a calibration curve. One unit of BSH activity is defined as the amount of enzyme that liberates 1 µmol of the amino acid from substrate per min. Protein concentration was determined in accordance with the method of Lowry et al. (1951) with BSA as a standard.

Purification of bile salt hydrolase

Preparation of cell-free extract

Harvested cells from batch fermentation were washed twice in 20-mM Tris buffer pH 8.0, resuspended in the same buffer and disrupted by sonication in ice bath for 1 min at 80 amplitude with 0.5-s pulse on and 0.8-s pulse off using Branson Sonifier. Cell debris was removed by centrifugation at 15,000 × *g* for 30 min and the supernatant was stored at −20°C.

Ion exchange chromatography

The cell-free extract was subjected to anion exchange chromatography using Q-sepharose column (2 × 6 cm) pre-equilibrated with 20-mM Tris buffer pH 8.0. The column was washed with equilibration buffer and the unbound protein was collected as fractions (2 ml). Flow rate was 10 ml h^{-1}. Fractions with BSH activity were pooled, concentrated by ultrafiltration using speed vac (LABCONCO). Concentrated sample was then dialyzed against 20-mM Tris buffer, pH 8.0 and rechromatographed as above, on another freshly packed, equilibrated Q-sepharose column (1.5 × 5 cm) and eluted with 20-mM phosphate buffer, pH 8.0. Enzyme was concentrated and stored in aliquots at −20°C.

SDS-PAGE

Denaturing polyacrylamide gel electrophoresis (SDS-PAGE, 12%) was carried out by the method of Laemmli (1970) to check the purity at each step of purification and to determine the molecular mass of the purified enzyme. Silver staining was used to visualize protein bands on the gels (Morrissey 1981) and the molecular weight was estimated based on concurrently electrophoresed marker proteins (Amersham Biosciences). The Molecular weight markers used for the SDS-PAGE were phosphorylase b (97 kDa), bovine serum albumin (66 kDa), ovalbumin (45.0 kDa), carbonic anhydrase (30.0 kDa) and α lactalbumin (14.4 kDa). The precise molecular weight of the subunit was determined using a MALDI-TOF mass spectrometer (Applied Biosciences).

Gel filtration chromatography

The molecular weight of native protein was calculated according to Andrews (1964).

Gel filtration was carried out using a sephacryl S-200 column (100 × 1 cm) at a flow rate of 0.1 ml min^{-1} with 50-mM Tris-HCl buffer at pH 8.0, containing 15-mM NaCl. The relative molecular weight (Mr) was determined by using protein standards under similar conditions (Sigma MW-GF-200Kit) which includes β-amylase 200 kDa, alcohol dehydrogenase 150 kDa, bovine serum albumin 66 kDa, carbonic anhydrase 29 kDa and cytochrome *c* 12.4 kDa.

Substrate specificity

Substrate specificity of the *Brevibacillus* BSH was determined with six major bile salts such as glycodeoxycholic acid, glycocholic acid, glychenodeoxycholic acid, taurocholic acid and taurochenodeoxycholic acid. Conjugated bile salt hydrolysis was determined at at 37°C for 10 min in 100-mM phosphate buffer, pH 6.5. The amount of amino acid released due to enzymatic reaction was determined by ninhydrin assay.

Effect of temperature and pH on BSH activity and stability

To ascertain the pH optimum of the enzyme, 50 μg of the enzyme was measured for BSH activity at different pH values under standard assay conditions. The following buffers were used: 100-mM acetate buffer (pH 3.0–6.0), phosphate buffer (pH 6.0–8.0), Tris-HCl (pH 8.0–9.0), carbonate–bicarbonate buffer (pH 9.0–11.0). pH stability of the enzyme was examined in the range of pH 3.0–11.0 by using buffers mentioned above. Enzyme was incubated at 25°C for 1 h and the residual activity was determined under standard assay conditions.

For the determination of the optimum temperature of BSH activity, 50 μg of the enzyme was assayed over the temperature range 30–80°C in 100-mM Tris buffer, pH 9.0 for 10 min. For thermal stability, 50 μg of the enzyme was incubated at different temperatures ranging from 40–80°C for 1 h in 100-mM potassium phosphate buffer, pH 7.0 and residual activity was estimated under standard assay conditions.

Kinetic study

The effect of substrate concentration on the reaction rate was determined using glycodeoxycholic acid by standard enzyme assay. The Michaelis–Menten constant (K_m) and maximum velocity for the reaction (V_{max}) was calculated from Lineweaver–Burk Plot. Catalytic turnover number (k_{cat}) and catalytic efficiency (k_{cat}/K_m) of purified enzyme were calculated using glycodeoxycholic acid.

Effect of various metal ions and amino acid modifying reagents on BSH activity

The effect of different metal ions on BSH activity was assessed. The enzyme was incubated with various metal salts (10 mM) for 1 h at 30°C and then the residual enzyme activity was determined. Resulting enzyme activities were compared to those of the standard enzyme reaction as a control.

Various potential amino acid modifying reagents such as *N*-bromosuccinamide, phenylymethylsulphonyl fluoride, 5, 5′-dithio bis-(2-nitrobenzoic acid), phenyl glyoxal, *N*-acetylinidazole, Woodwards reagent K, citraconic anhydride, trinitrobenzene sulphonic acid were tested for their effect on BSH activity. For this, 50 μg of homogenous preparation of the enzyme was incubated with various

concentrations of above-mentioned reagents at 30°C for 30 min and relative BSH activity was measured. The percent residual enzyme activity was determined by standard enzyme assay with reference to the activity of the enzyme in a reaction without the addition of modifying reagent as supplement.

Determination of the N-terminal amino acid sequence

The purified enzyme from Q-Sepharose chromatography was concentrated and applied to 15% SDS-PAGE. After separation, the proteins were blotted onto a polyvinylidene fluoride membrane and stained with Coomassie brilliant blue R-250. BSH band was cut out and used for N-terminal amino acid sequencing, which was performed using Applied Biosystems Procise 494 protein with standard reagents and methods recommended by manufacturer.

Results and discussion

Purification

The purification procedure is summarized in Table 1. It was observed that protein was purified to homogeneity using ion exchange chromatography on Q-Sepharose. The most common protein purification approach is to let the protein of interest adsorb to the column matrix and allow the contaminants to pass through. However, in some cases the vice versa may be a better approach, i.e., to let the protein of interest flow through and to get the contaminants adsorbed. We have adapted the second condition in this purification step. BSH with positive charge, eluted as it flows through whereas contaminant proteins with negative charge remain bound to column. This step minimized the contamination to maximum extent with 83% yield and a 12-fold increase in specific activity. The pooled active fractions were concentrated using speedvac (LABCONCO). Extra band of non-specific proteins observed in this step was purified further by rechromatography on another freshly packed and pre-equilibrated Q-sepharose column, as described above. BSH active fractions were detected for homogeneity on a 12% SDS-PAGE (Fig. 1a). As a result of purification using this procedure 58% yield was obtained and the protein was purified 13-fold. The enzyme was electrophoretically homogenous and the molecular weight of the enzyme was estimated to be 28 kDa from SDS-PAGE analysis. For a more precise molecular mass determination, a MALDI-TOF analysis was performed. The purified fraction gave a major peak with a molecular mass of 28.2 kDa. The molecular weight (Mr) of the pure native enzyme determined to be approximately 56 ± 1 kDa by gel filtration chromatography (Fig. 1b), suggesting that the enzyme is a dimeric protein.

Bile salt hydrolase with different native molecular weights and structures have been reported previously. The native enzymes are octamers in *B. fragilis* (Stellwag and Hylemon 1976), hexamers in *B. longum* BB536 (Grill et al. 1995), tetramers in *C. perfringens* (Srivastava and Hylemon 1988), *B. longum* SBT2928 (Tanaka et al. 2000), or trimers in *L. johnsonii* (Lundeen and Savage 1990). Dean et al. (2002) reported a dimeric bile salt hydrolase from a bile adapted strain *X. maltophila* with a native and subunit molecular weight 100 and 52 kDa, respectively. We are reporting here another dimeric enzyme with smallest functional unit (28 kDa), not documented so far.

Substrate specificity

Brevibacillus BSH showed a broad substrate range for six major bile salts. The enzyme was active towards glycine and taurine conjugated substrates. The highest levels of activity were observed with glycodeoxycholic acid (defined as 100% activity) and with glycine conjugated bile acids (Fig. 2). Substrate specificity of *Brevibacillus* BSH is similar to the other bile salt hydrolases (Tanaka et al. 2000; Kim et al. 2004a, b). The enzyme showed relatively less activity (50%) with taurine conjugated substrates compared to glycine conjugated substrates.

Effect of pH and temperature on BSH activity/stability

Studies on the effect of pH on enzyme catalysis revealed that the enzyme was active in broad range of pH, i.e., pH 3.0–11.0 (Fig. 3a). Maximum activity of the enzyme was observed at pH 9.0, in contrast to the pH optimum reported for the other enteric bile salt hydrolases. Slightly acidic pH optima, in the range of 3.5–7.0 was observed for BSH activity in such bacteria. Only exception is the one purified from *X. maltophila* with a pH optimum range 7.9–8.5

Table 1 Purification summary of bile salt hydrolase from *Brevibacillus* sp.

Steps of purification	Total activity (U)	Total protein (mg)	Specific activity (U mg^{-1})	Fold purification	Yield (%)
Sonicate	158	152	1.03	1.0	100
Q-Sepharose chromatography (I)	132	10.8	12.2	11.8	83
Q-Sepharose chromatography (II)	91	6.6	13.8	13.3	58

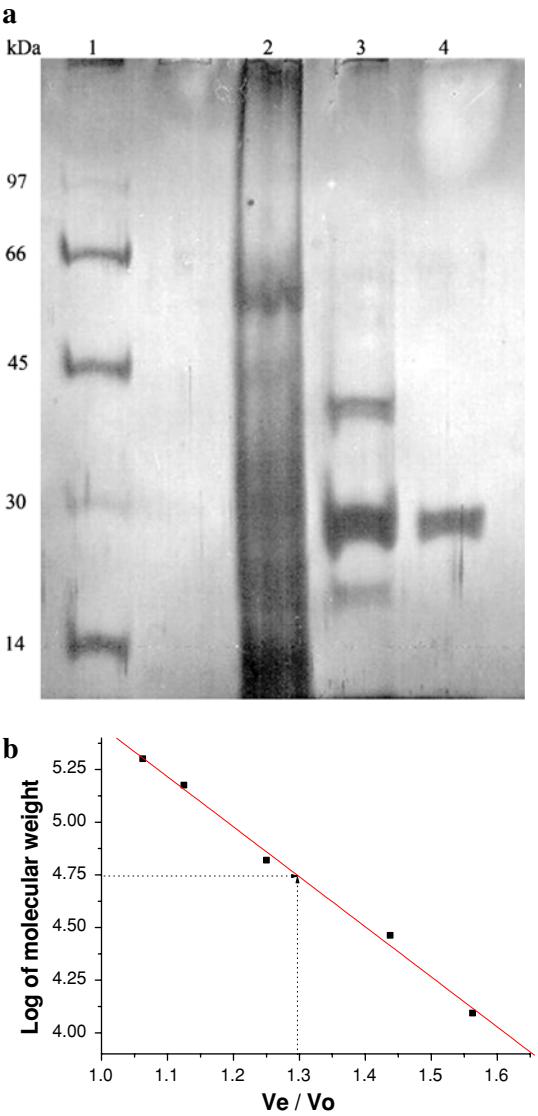

Fig. 1 **a**. Profile of BSH in 12% SDS-PAGE stained with silver nitrate. *Lane 1* molecular weight marker, *lane 2* fraction after sonication, *lane 3* fraction after Q-sepharose I and *lane 4* fraction after Q-sepharose II. **b**. Molecular weight estimation of purified *Brevibacillus* BSH by gel filtration on Sephacryl S-200

(Dean et al. 2002; Kim and Lee 2005). *Brevibacillus* BSH showed pH stability over the range of 3.0–11.0 and the stability optimum was observed at pH 8.0 (Fig. 3b). BSH purified from *B. longum* is reported to be stable in the pH range 4.0–8.0 and rapidly inactivated on either side of this range Tanaka et al. (2000). The purified enzyme from the *Brevibacillus* sp. has potential applications for in vivo reduction of cholesterol levels due to its broad range of pH stability. The enzyme was presumed to withstand pH conditions of the stomach (1.0–2.0) since more than 70% of the activity was still retained at pH 3.0. When the effect of temperature on BSH activity was studied, the enzyme was active in the range of 30–80°C with optimum

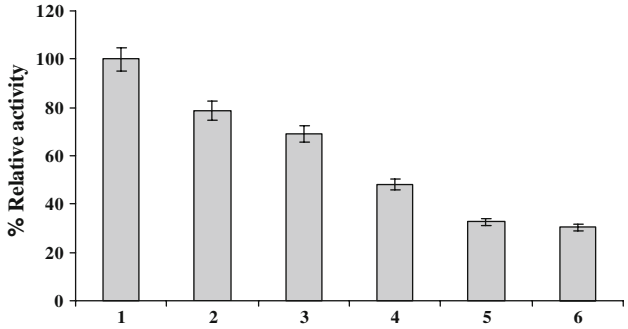

Fig. 2 Substrate specificity of BSH from *Brevibacillus* sp. *1* glycodeoxycholic acid, *2* glycocholic acid, *3* glycochenodeoxycholic acid, *4* taurocholic acid, *5* taurodeoxycholic acid, *6* taurochenodeoxycholic acid

temperature at 60°C (Fig. 3c) and differs significantly from the temperature optima (30–45°C) described for other bile salt hydrolases (Tanaka et al. 2000). The enzyme is very stable at 60°C and remains active for more than 1 h. It retains 100% activity at 30°C for 12 h. The enzyme looses about 65% activity at 70°C after 1 h (Fig. 3d). Temperature stability studies conclude that the enzyme is stable over a broad temperature range.

Kinetic studies

The substrate saturation curve of *Brevibacillus* BSH against glycodeoxycholic acid followed Michaelis–Menten kinetics. The apparent values of K_M and k_{cat} were calculated from a Lineweaver–Burk plot, and were approximately 3.08 μM and 6.32×10^2 s^{-1}, respectively. The catalytic efficiency (k_{cat}/K_M) was calculated to be 0.2×10^3 M^{-1} s^{-1}. K_M value of the purified enzyme against glycodeoxycholic acid is significantly less in comparison with BSH purified from *B. longum* with an optimal growth temperature 37°C (Tanaka et al. 2000, Suresh Kumar et al. 2006) indicating an high substrate affinity of BSH from *Brevibacillus* sp.

Effect of metal ions and amino acid modifying reagents on BSH activity

The effects of metal ions and enzyme inhibitors are summarized in Table 2. BSH activity was strongly inhibited by Cu^{++} and Hg^{++}. Complete inhibition was observed with Cu^{++}. Complete inactivation of BSH activity by Cu^{++} was also observed in BSH from *X. maltophila* and *B. longum* (Dean et al. 2002; Tanaka et al. 2000). Moderate inhibition was observed with Ba^{++}, Ag^{++}, Zn^{++}, little inhibition (19%) was observed with metal ion chelator (EDTA). The enzyme activity was enhanced by 14.7% with Fe^{++}.

Effect of specific amino acid modifying agents on BSH activity was studied and the results are summarized in the

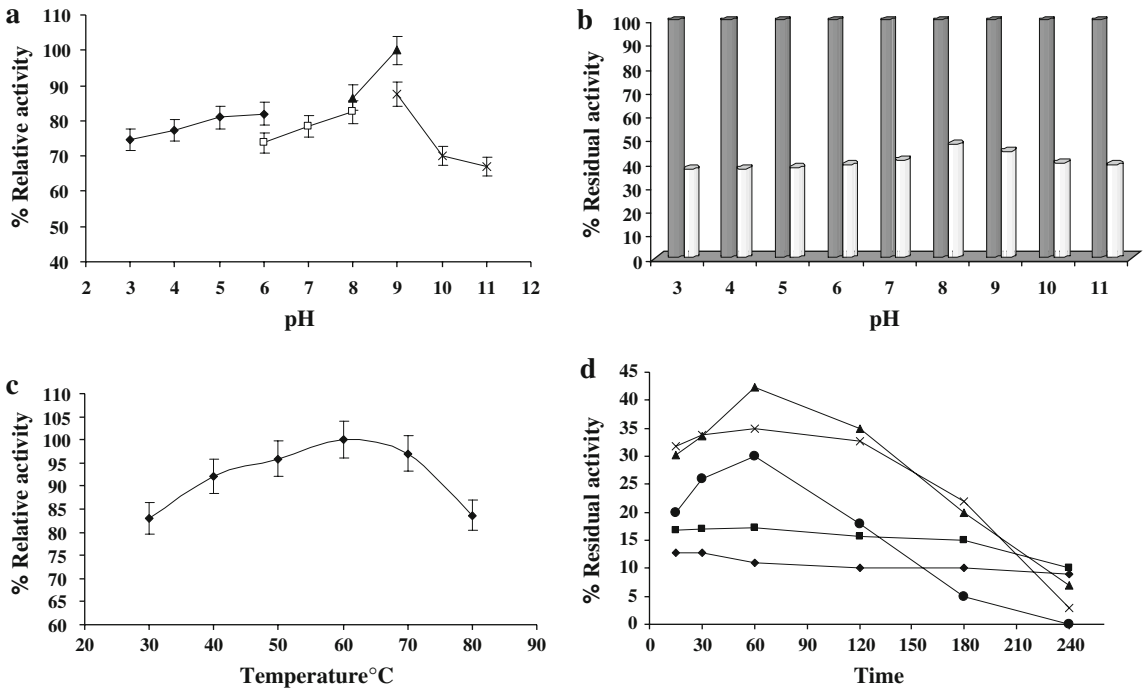

Fig. 3 **a** Effect of pH on BSH activity of *Brevibacillus* sp. The effect of the pH on the enzyme activity was examined in the pH range of 3–11 with the following 100-mM buffers under the standard assay conditions: *filled diamond* acetate buffer (pH 3–6), *open square* potassium phosphate buffer (pH 6–8), *filled triangle* tris buffer (pH 8–9), *cross* carbonate-bicarbonate buffer (pH 9–11). Relative activity is expressed as percentage of the maximum activity. **b** pH stability of *Brevibacillus* BSH. Fifty microgram of the enzyme was pre-incubated in buffers at different pH values at 25°C for 1 h and the residual activity was determined under standard assay conditions. Symbols: *filled cylinder* bile salt hydrolase activity at zero hour of incubation, *open cylinder* bile salt hydrolase activity after one hour of incubation. **c** Effect of temperature on BSH activity of *Brevibacillus* sp. The activity was determined at different temperatures at pH 9.0 in 100-mM Tris buffer. **d** Thermostability of *Brevibacillus* BSH. Purified enzyme was incubated at different temperatures (40–80°C) for a period of 1 h and the residual enzyme activity was assessed under standard conditions. Symbols: *filled diamond* 40°C, *filled square* 50°C, *filled triangle* 60°C, *cross* 70°C, *filled circle,* 80°C

Table 2 Influence of metal ions on the BSH activity

Metal ions	% Residual activity
$CuSO_4$	0
$BaCl_2$	78.4
$AgNO_3$	77.3
$HgCl_2$	43.2
$CoCl_2$	90.9
$NiSO_4$	92.0
$MnSO_4$	95.5
$ZnSO_4$	76.1
$MgSO_4$	87.5
$FeSO_4$	114.7
EDTA	80.69

Activity without metal ion was considered as 100%

Table 3. Trinitrobenzene sulphonic acid and citraconic anhydride strongly inhibited the enzyme activity at a concentration of 25 mM, with residual BSH activity of 30 and 33%, respectively. Inhibition of BSH activity with trinitrobenzene sulphonic acid and citraconic anhydride can be predicted as involvement of lysine in the catalytic activity of enzyme. There was no inhibition observed with modifying agents such as *N*-acetylimidazole and phenylmethylsulfonyl fluoride. About 50% inhibition was observed with Woodward's reagent K and phenylglyoxal. Suresh Kumar et al. (2006) observed that chemical modification of BSH from *B. longum* with cysteine modifier 5,5′-dithio bis-(2-nitrobenzoic acid) lowered the activity by 78%. Interestingly, in our study it was observed that *Brevibacillus* BSH was inhibited only by 35% with 5,5′-dithio bis-(2-nitrobenzoic acid) under identical conditions.

N-terminal amino acid sequencing

N-terminal amino acid sequencing of the purified protein resulted in the following sequence: Lys-Asn-Thr-Asp-Leu-Lys-Gln-Glu-Asn-Lys-Lys-Phe-Glu-Ser-Arg-Leu. A protein homology comparison revealed that this sequence exhibited no homology to the N-terminal amino acid sequences of the BSH of various lactobacilli and other gastrointestinal microorganisms. Bile salt hydrolases belongs to N-terminal nucleophilic (Ntn) hydrolase super

Table 3 Effect of amino acid modifying reagents on BSH activity

Amino acid modifier	Possible residue modified	Concentration	% Residual activity
Phenyl methyl sulphonyl fluoride	Serine	1 mM	100
5,5′-dithio bis-2-nitrobenzoic acid	Cysteine	1 mM	65
Woodwards reagent k	Aspartic acid, glutamic acid	2 mM	53.6
N-Bromosuccinamide	Tryptophan	50 μM	63.6
N-Acetyl imidazole	Histidine, tyrosine	10 mM	100
Citraconic anhydride	Lysine	25 mM	33.3
Trinitrobenzene sulphonic acid	Lysine	25 mM	30.9
Phenyl glyoxal	Arginine	3 mM	57.4

Activity without inhibitor was considered as 100%

family. Members of this family are synthesized as preproteins and go through post-translational processes, which lead to an autocatalytically activated enzyme. This process is thought to generate a new N-terminal residue, which is designed to act as a nucleophile. The N-terminal nucleophile, cysteine is highly conserved in all BSH enzymes (Kim et al. 2004a, b; Coleman and Hudson 1995; Elkins and Savage 1998; Christiaens et al. 1992). The crystal structure of BSH from *C. perfringes* and *B. longum* reveals that the catalytic site possesses a nucleophilic residue cysteine, which is thought to be crucial for catalysis and is central to the mechanism of catalysis/substrate hydrolysis (Rossocha et al. 2005; Suresh Kumar et al. 2006). The importance of the -SH group was confirmed by the fact that replacement of Cys with other potential nucleophilic residues such as Ser or Thr resulted in the loss of BSH activity (Tanaka et al. 2000; Kim et al. 2004a, b). It was observed by Dean et al. (2002) that BSH purified from a bile adapted strain, *X. maltophila* has alanine as N-terminal residue. In our study, on the basis of N-terminal analysis, we observed a lysine residue at the N-terminus of the enzyme instead of cysteine indicates that there has been appreciable divergence in these enzymes. However, the exact location and role of cysteine in enzyme catalysis will be revealed only after the resolution of three dimensional structure of the *Brevibacillus* BSH. Thermozymes adapt different mechanisms for their thermostability property, one of such mechanisms include small structural modifications that are achieved with the exchange of some amino acids (Li et al. 2005). The significant difference in the amino acid sequence may be due to an adaptation mechanism to improve the resistance to irreversible loss of activity. (Iwakura et al. 1995).

In summary, the present study describes the purification and characterization of BSH from a novel thermophilic source, *Brevibacillus* sp. To the best of our knowledge, this is the first report regarding the purification and characterization of BSH from the thermophilic source. The purified enzyme is the smallest dimeric enzyme and the N-terminal sequence of this protein is distinguishably different from the reported BSH from other sources. The characterization of the enzyme reveals that there was appreciable divergence in the properties of the enzyme when compared with mesophilic proteins and also some properties of the enzyme such as high thermostability and substrate affinity have industrial applications.

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