The phospholipase pl-S.t gene of Sphingobacterium thalpophilum 2015 was cloned and the gene sequence was submitted to NCBI with Accession Number KX674735.1. The phylogenetic analysis showed that this PL-S.t was clustered to phospholipase D (PLD). As far as we know, the PL-S.t with a molecular mass of 22.5 kDa is the lowest of the currently purified bacterial PLDs, which belongs to a non-HKD PLD enzyme. This PL-S.t was resistant to a wide range of alkali pHs (7.5–9.0) after 1 h incubation, retaining more than 90% of its maximum activity. The PL-S.t activity can be enhanced by Ni^{2+}, Co^{2+} and Mn^{2+}. This PL-S.t has only one cysteine residue and fewer negatively-charged amino acids (AAs). The hydrogen bonds network was found around the cystein108, which may be beneficial to the stability and activity of PL-S.t in Ni^{2+} solution. This study has laid the foundation for further research on the molecular mechanism of the catalytic characteristics of low molecular weight alkalic PLD from S. thalpophilum 2015.

Key Words: cloning; enzymatic characteristics; expression; phospholipase D; Sphingobacterium thalpophilum

Abbreviations: pl-S.t, Phospholipase gene from Sphingobacterium thalpophilum 2015; PL-S.t, Phospholipase protein from Sphingobacterium thalpophilum 2015

Introduction

Phospholipases are widely distributed in mammals, plants and microbials (Jenkins and Frohman, 2005). Phospholipases have been developed for a few phospholipid-transforming reactions in laboratory and on industrial scale (Ramrakhiani and Chand, 2011). PLDs attract attention because of their special roles in hydrolysis and transphosphatidylation, which are widely used in the pharmaceutical and food industries, e.g. the conversion of phosphatidylcholine (PC) to phosphatidylserine (PS), which are active in promoting the function of nerve cells. Although a wide range of the molecular weight of PLD enzymes ranging from 16 kDa (from Streptomyces hachijoensis) (Okawa and Yamaguchi, 1975) to 195 kDa (from Homo sapiens), have been reported so far, only a few of them were obtained in the pure state (Simkhada et al., 2010). In addition, the primary structures of PLDs have been elucidated from approximately 40 plant, 18 microbials, and 10 mammalian, PLDs (Jonansen et al., 1996; Kato et al., 1984; Leiros et al., 2000; Lerchner et al., 2005; Simkhada et al., 2010; Ulbrich-Hofmann et al., 2005; Waite, 1999; Zambonelli et al., 2003a). In the case of microbials, only the tertiary structures of PLDs from Streptomyces sp. PMF (PDB entry: 1F01) (Zambonelli et al., 2003b), Streptomyces antibiotics (PDB entry 2ZE9) and Salmonella Typhimurium (PDB entry: 1BYR) have been reported so far (Iwasaki et al., 1994; Lerchner et al., 2005). Therefore, there is a need to study bacterial PLD enzymatic properties and their molecular structure. Some types of PLD, especially from a bacterial origin, have proved to be excellent biocatalysts in the phospholipid industry (Mustranta et al., 1995; Ulbrich-Hofmann et al., 2005). An ability to adapt to harsh conditions will be of benefit to PLD biocatalytic applications (Simkhada et al., 2010).

An organic form of phosphorus always exists in nucleic acids, phytochemicals and phospholipid in the soil. Phospholipid compounds can be further decomposed by microorganisms into inorganic phosphoric acid, glycerol, and...
fatty acids, choline, etc. The starting point of the present work was a phospholipase-producing bacterium, Sphingobacterium thalpophilum 2015, which was isolated from an oil-contaminated medium. Here, we report on the purification and characterization of an alkali resistant extracellular PLD produced by this strain. The gene cloning, enzyme purification and the biochemical characterization of the purified enzyme were major goals of this study, which will lay the foundation for research on the molecular mechanism of the catalytic action.

Materials and Methods

Bacterial strains and culture conditions. E. coli BL21 (DE3) was used as the expression host, and pET-32a (+) (Novagan Company, Darmstadt, Germany) was used as the expression vector. E. coli cells harboring the recombinant plasmid were grown in a Luria-Bertani (LB) medium with 50 µg/mL of ampicillin added. The culture temperature was 30°C for S. thalpophilum 2015 strains and at 37°C for E. coli strains. Phosphatidylcholine (P 5638) (ε99% purity, from soybeans) was purchased from the Sigma-Aldrich Corporation (Shanghai, China).

Screening, isolation and identification. Soil samples were collected from restaurant grease (rich in soy-oil) contaminated land on the Chengdu University of Technology campus (30°39’S 1.2° N, 104°08’14.9° E). The modified culture medium containing 2.0% soybean phosphatidylcholine, 1.0% CaCl2·2H2O, 0.2% K2HPO4, 0.5% (NH4)2SO4, 0.05% MgSO4·7H2O and 2.0% agar was used to screen for phospholipase producing S. thalpophilum 2015 (Yang et al., 2006). S. thalpophilum 2015 was isolated depending on the cloudy zones formed. The purification process was carried out using Ni2+-NTA affinity chromatography (Harwood, 1994) in BioLogic LP protein purification system (Bio-Rad Inc., Shanghai, China). The protein concentration was determined with the Bradford method (Walker, 2002). The expression recombinant was detected by SDS-PAGE electrophoresis and MALDI-TOF/MS analysis.

Mass determination of PL-S.t protein by MALDI-TOF/MS analysis. MALDI-TOF/MS analysis of the PL-S.t was conducted as follows. The recombinant PL-S.t band from 12% SDS-PAGE was cut out of the gel to be 1 mm × 1 mm square. Trypsin digestion reaction was performed in a enzymatic analyzer under 37°C for 15 h. A 40 µL of peptide extraction solution (50% ACN, 0.1% TFA) was added after the digestion reaction, and incubated under 37°C for 30 min. After lyophilization, dissolving with 0.1% formic acid solution and drying on a 5800 target plate, the plate was sent for analysis by the MALDI-TOF/TOF 5800 mass spectrometer (Applied Biosystems Corporation, CA, USA).

Phospholipase assay. The enzyme assay in this study was presented by hydrolytic activity, which was determined using phosphatidylcholine as a substrate coupled with oxidase peroxidase spectrophotometric assay (Imamura and Horiii, 1978) and modified by Simkhada et al. (2010). The 100 µL reaction mixture contained 2.12 mmol/L phosphatidylcholine, 2 mmol/L CaCl2, 1.5% TritonX-100 (v/v), 30 mmol/L NaCO3-NaHCO3 buffer (pH 9.6; 9.2 mmol/L Na2CO3, 20.8 mmol/L NaHCO3) and enzyme solution. After allowing the reaction to proceed for 20 min at 30°C, 60 µL of EDTA solution (50 mmol/L EDTA; 1mol/L Tris-HCl, pH 8.0) was added and the mixture was boiled for 5 min to stop the reaction. One hundred and fifty microliters of colorimetric reagent (containing 100 U peroxidase and 50 U choline oxidase) was added to the reaction and incubated at 37°C for 1 h. After that, the reaction sample was measured at 500 nm using a UV-5200PC spectrophotometer (MetaSh Corporation, Shanghai, China). One unit (U) was defined as the amount of enzyme required for the release of 1 µmol of choline/min from phosphatidylcholine.

Optimum temperature and pH. To estimate the optimum pH of the activity of PL-S.t, the enzyme solution was in-
Sphingobacterium thalpophilum 2015 phospholipase D

Cubated at 30°C with pH values ranging from 3.5 to 9.5 (0.05 mol/L citric acid-sodium citrate buffer for pH 3.0–5.0, 0.05 mol/L K₂HPO₄–KH₂PO₄ for pH 5.5–8.0 and 0.05 mol/L Tris-HCl for pH 8.5–9.5). The effect of temperature on the enzyme activity was determined by incubating the purified PL-S.t enzyme in 0.05 mol/L K₂HPO₄–KH₂PO₄ (pH 8.0) for 20 min at a temperature between 20 and 70°C (5°C interval). The enzyme activity was assayed using the same method as described above.

Thermal stability and pH stability. The thermostability of PL-S.t was studied by incubating the enzyme samples in K₂HPO₄–KH₂PO₄ (pH 8.0) buffer for 1 h at a temperature between 20–70°C (5°C interval) before adding phosphatidylcholine as a substrate. The enzyme activity was then measured at 30°C. To determine the pH stability of PL-S.t, the PL-S.t was initially incubated in 0.05 mol/L buffers with various values of pH (pH 3.5–9.5, 0.5 intervals) for 1 h at 4°C in the fridge. The remaining PL-S.t activity was measured at 30°C under the method mentioned above.

Effect of metal ions. The effects on the enzyme activity were evaluated by adding different metal ions, including Ca²⁺, Cu²⁺, Ni²⁺, Mn²⁺, Mg²⁺, and Zn²⁺ separately at a concentration of 10 mmol/L to the PL-S.t enzyme solution.

Effect of organic solvents. The effect of organic solvents (10% ethanol, 10% isopropyl alcohol, 10% TritonX-100, 10% hexane, 10% Tween 80 and 10 mmol/L Na₂-EDTA) on the PL-S.t activity was measured.

In all enzymatic characteristics experiments, each sample was tested in triplicate. The results were expressed as the mean ± SD (standard deviation).

Phylogenetic and structural analysis of PL-S.t. A PL-S.t protein phylogenetic tree was constructed with MEGA-X software and the confidence of the tree branches was checked by bootstrap generated from 500 replicates. The characteristics of PL-S.t, including isoelectric point (BioEdit version 7.0.5.3), hydrophobic analysis (ProtScale program on the ExPASy server) and the secondary structure of PL-S.t (PBIL LYON-GERLAND) were analyzed. Homology modeling was constructed with the SWISS-MODEL and the rationalization of the protein structure was assessed using Ramachandran analysis. The hydrogen bond network was analyzed using a Swiss PDB Viewer (SPDBV, version 4.1.0).

Results

Cloning of the pl-S.t gene from S. thalpophilum 2015

The sequencing results of the amplified 621 bp pl-S.t gene DNA fragment (Supplementary Fig. S1a) showed a high homology with Sphingobacterium thalpophilum strain NCTC11429 PL genome assembly, phospholipase gene (LR590484.1). The pl-S.t gene sequence was submitted to NCBI (Accession Number: KX674735.1).

Purification of PL-S.t

After sonication on ice, the enzyme was purified by affinity chromatography. The His-tagged PL-S.t protein was purified with a molecular mass of 22.5 kDa (Supplementary Fig. S1b). The concentration of the purified protein
was 2.69 mg/L and the purified PL-S.t hydrolysis activity was 14.46 U/mg.

**Mass determination of PL-S.t protein by MALDI-TOF/MS analyses**

The peptide mass data showed a unique MS/MS fragmentation of GGTATEILHLSTYLK in recombinant PL-S.t. By MALDI-TOF/MS analysis, the protein was digested into peptides and subjected to mass spectrometry (MS) analysis to obtain the mass information (Supplementary Fig. S2). The PL-S.t peptide was blast compared in NCBI and was identified to be a phospholipase of *S. thalpophilum*.

**Protein homology and structure analysis**

The phospholipase protein sequences were analyzed with the other phospholipase family members, including phospholipase A, B, C, D. The Neighbor-Joining phylogenetic tree was constructed (Supplementary Fig. S3). The phospholipase of *S. thalpophilum* 2015 (ANZ9103.1) was clustered to phospholipase D.

The isoelectric point (PI) of PL-S.t from *S. thalpophilum* 2015 was 5.52. TMHMM prediction implied that all regions of the protein were located outside the membrane without a transmembrane region. The secondary structure of the PL-S.t was composed of 6 spirals and 7 folds, and the stereoscopic conformation was presented in the tertiary structure (Fig. 1A). The Ramachandran Plots showed that the dark area (grey) represents the core area and the preferred conformation area of the residue (>90%). It showed that 96.04% of the scattered points fell into these areas, which implies that these residues have a reasonable conformational space (Fig. 1B).

**Effect of temperature and pH on the PL-S.t activity and stability**

The optimum pH for PL-S.t activity was 8.0. PL-S.t activity was stable over a wide range of pH values (7.5–9.0) after 1 h incubation, retaining more than 90% of its maximum activity. When incubating at a pH lower than 5.0, its relative activity significantly decreased to below 40% (Fig. 2). The optimum temperature for PL-S.t activity was 30°C and the thermostability assays showed that PLD-S.t was stable at 37°C after incubating for 1 h. The changes in temperature caused a significant decrease of activity (Fig. 3).

**Table 1.** Effects of metal ions on phospholipase activity.

| Metal ions (10 mmol/L) | Relative activity (%) |
|-----------------------|----------------------|
| No ions               | 100                  |
| Ca²⁺                  | 161.9 ± 0.03         |
| Ni²⁺                  | 219.8 ± 0.11         |
| Mn²⁺                  | 364.2 ± 0.4          |
| Co²⁺                  | 235.3 ± 0.18         |
| K⁺                    | 105.4 ± 0.06         |
| Cu²⁺                  | 76.9 ± 0.14          |
| Mg²⁺                  | 61.6 ± 0.03          |
| Zn²⁺                  | 39.2 ± 0.17          |

**Table 2.** Effects of organic solvents on phospholipase activity.

| Organic solvents          | Relative activity (%) |
|---------------------------|----------------------|
| No organic solvents       | 100                  |
| Ethanol (10%, v/v)        | 35.9 ± 0.9           |
| Isopropyl alcohol (10%, v/v) | 112 ± 0.4           |
| TritonX-100 (10%, v/v)    | 240.6 ± 1.2          |
| Hexane (10%, v/v)         | 50.6 ± 0.15          |
| Tween80(10%, v/v)         | 148.7 ± 0.3          |
| Na₂-EDTA(10mmol/L)        | 36.8 ± 1.8           |
| Microbial type                  | Molecular weight | Optimum pH or temperature | Activated organic solvents | Inhibitory organic solvents | Activated ion | Inhibitory ion | References                                      |
|--------------------------------|-----------------|---------------------------|---------------------------|-----------------------------|---------------|---------------|------------------------------------------------|
| *Streptomyces PMF*             | 54 kDa          | pH 8.0 and 60°C           | —                         | —                           | —             | —             | Zambonelli et al. (2003b)                       |
| *Streptomyces sp. YU100*       | 57 kDa          | pH 7.0 and 60°C           | Triton X-100              | —                           | —             | —             | Kyu et al. (2002)                              |
| *Streptomyces. chromofuscus*   | 55–58 kDa       | pH 7.5 and 30°C           | —                         | —                           | —             | —             | Li et al. (2008)                               |
| *Streptomyces sp. CS628*       | 50 kDa          | pH 9.6 and 30°C           | —                         | EDTA                        | Ca<sup>2+</sup> | Mg<sup>2+</sup>, Mn<sup>2+</sup>, Co<sup>2+</sup>, Zn<sup>2+</sup>, Cu<sup>2+</sup>, Fe<sup>3+</sup> | Simkhada et al. (2010) |
| *Streptomyces. antibiotics*    | 57 kDa          | —                         | —                         | —                           | —             | —             | Iwasaki et al. (1994)                          |
| *Streptomyces. antibiotics*    | 64 kDa          | pH 5.5 and 60°C           | —                         | —                           | —             | —             | Shimbo et al. (1993)                           |
| *Streptomyces olivochromogenes*| 60 kDa          | pH 8.0 and 75°C           | —                         | —                           | —             | —             | Shimbo et al. (1990)                           |
| *Streptomyces lydus*           | 56 kDa          | pH 6.0 and 55°C           | —                         | —                           | —             | —             | Ogino et al. (1999)                            |
| *Streptomyces. antibiotics 103*| 54 kDa          | pH 6.0 and 50°C           | EDTA                      | —                           | —             | —             | Nakazawa et al. (2010)                         |
| *Streptomyces tendae*          | 55 kDa          | pH 7.5 and 50°C           | —                         | —                           | —             | —             | Mander et al. (2009)                           |
| *Corynebacterium pseudotuberculosis* | 31.7 kDa | pH 8.5–9.8 | — | — | — | — | Egen et al. (1989) |
| *Bacillus licheniformis*       | 44 kDa          | pH 7.0 and 40–45°C        | Triton X-100              | Tween 20                    | Ca<sup>2+</sup>, Mg<sup>2+</sup>, Mn<sup>2+</sup>, Co<sup>2+</sup>, Zn<sup>2+</sup>, Cu<sup>2+</sup>, Fe<sup>3+</sup> | Kang et al. (2011) |
| *Ochrobactrum sp. ASAG-PL1*    | 37 kDa          | pH 7.0 and 40°C           | —                         | —                           | —             | —             | Hu et al. (2013)                               |
| *Psychrophile, Shewanella sp.* | 64 kDa          | pH 7.8 and 40°C           | —                         | —                           | —             | —             | Tsuruta et al. (2007)                          |
| *Acinetobacter radioresistens* | 31.5 kDa        | pH 7.0 and 40°C           | —                         | —                           | —             | —             | Cao et al. (2018)                              |
| *Arcanobacterium haemolyticum* | 31.5 kDa        | pH 7.0 and 40°C           | —                         | —                           | —             | —             | Cuevast and Songer (1993)                      |
| *Sphingobacterium thalpophilum*| 22.5 kDa        | pH 8.0 and 30°C           | Triton X-100, Tween 80, isopropyl alcohol | EDTA, hexane, ethanol | Ca<sup>2+</sup>, Ni<sup>2+</sup>, Mn<sup>2+</sup>, Co<sup>2+</sup>, K<sup>+</sup> | Cu<sup>2+</sup>, Mg<sup>2+</sup>, Zn<sup>2+</sup> | This report
Effect of metal ions and organic solvents on PL-S.t activity

The PL-S.t activity was influenced by various organic agents and metal ions under the controlled conditions (Table 1). Triton X-100, Tween 80 and isopropyl alcohol enhanced the activity, of which Triton X-100 had the best effect, while Na₂-EDTA, hexane and ethanol inhibited the PL-S.t activity. For metal ions, some ions enhanced the activity, such as Ca²⁺, K⁺, Ni²⁺, Mn²⁺, Co²⁺, especially Ca²⁺, while Cu²⁺, Mg²⁺, Zn²⁺ inhibited the PL-S.t activity (Table 2).

Discussion

PLD-producing microbial and PL-S.t

Most microbes which have PLD activity are isolated from soils. But few PLDs from different microorganisms were purified and their structure and characteristics were investigated (Table 3). To this purified microbial PLD list we can add Sphingobacterium thalpophilum 2015, which belongs to Sphingobacteria (Bacteroidetes). This PLD-S.t has a molecular weight of 22.5 kDa, which is the lowest of the purified bacterial PLD reported so far.

For HKD PLDs, they share the conserved HKD motif (histidyl, lysyl, and aspartyl) (Sandoval, 2018). It has been reported that Saccharomyces cerevisiae, Streptomyces sp. PMF and Streptomyces antibioticus contain the HKD motif HXXK₄D (X being any AA residue) (Rudge et al., 2002; Sandoval, 2018). For non-HKD PLDs, they lack the conserved HKD motif, but exhibit PLD activity, and are no less physiologically relevant than members of the PLD superfamily (Selvy et al., 2011). PLD from Streptomyces chromofuscus (scPLD), the sequence of which has been included in NCBI GenBank (Accession No. AAM 77916; Yang and Roberts, 2002) contains no HKD motif, but can hydrolyze a range of phospholipids and possibly perform phosphatase activities (Zambonelli et al., 2003a). The highest homology of the phospholipase sequences recorded in NCBI to PL-S.t is the phospholipase of Sphingobacterium detergens (WP_120258128.1), with a homology of 71.7%. Comparing this with phospholipase A, B, C, and D protein sequences, we found that the PL-S.t has a percent identity of 50.0% to Methylobacterium indicum PLD. From the phylogenetic analysis and phosphatidylcholine hydrolysis activity results, the PL-S.t should belong to the PLD family. The multiple sequence alignment of some classified and unclassified phospholipases are shown in Supplementary Fig. S4.

PL-S.t characterization from S. thalpophilum 2015

For organic agents, Triton X-100, Tween 80 and isopropyl alcohol enhanced the PL-S.t activity, in which Triton X-100 increased the activity of PL-S.t by as much as 2.4 times. The present study showed that TritonX-100 can enhance the hydrolysis activity of PLD from Streptomyces sp. YU100 and Bacillus licheniformis (Table 3). Tween 80 exhibited inhibition to PLD activity of Bacillus licheniformis (Table 3), while in our experiment, Tween 80 stimulated the PL-S.t activity. Triton X-100 and Tween 80 are both moderate non-ionic detergents and soluble in a lipid medium. These detergents have been employed in hydrolysis and transphosphatidylation reactions as emulsifiers (Imamura and Horuti, 1979). PL-S.t demonstrated a good adaptability to detergents which may help its hydrolysis action in different phospholipid substrates. Another organic solvent, EDTA, exhibited an obvious inhibition to PL-S.t activity, which is consistent with the results of PLDs from Streptomyces chromofuscus and Streptomyces tendae (Table 3). We also found that EDTA can increase the activity of PLD from Streptoverticillium cinnamoneum (Table 3). EDTA has a chelate function in the presence of many metal ions (such as Ca²⁺). Some enzymes need to play roles in the presence of metal ions, so that EDTA can be used as an inhibitor to these enzymes, such as some nuclease (Barra et al., 2015), protease (Wei and Bobek, 2005), and phospholipase (Mander et al., 2009). On the other hand, EDTA also has the ability to chelate toxic heavy metals from enzyme (Shahid et al., 2014). In the future, the comprehensive effect of EDTA and ions on PL-S.t will be studied. Ethanol inhibited PLD-S.t activity, while isopropanol alcohol promote it, and these results are consistent with the characteristics of Staphylococcus saprophyticus M36 organic solvent tolerant lipase (Tang et al., 2009). Hexane has been reported to have the ability to inhibit microbial growth and lipase activity, but no report has yet been published about its influence on phospholipase activity.

Most PLD sequences contain potential glycosylation and phosphorylation and phosphoinositide binding motifs, which are known to mediate in enzyme activation, and this ability seems relative with Ca²⁺ activation (Zheng et al., 2000). The effect of Ca²⁺ on PL-S.t activity was consistent with the study of microbial PLD (from Streptomyces sp. CS628, Streptomyces olivochromogenes, Streptomyces tendae, Bacillus licheniformis and Psychrophile Shewanella sp.) (Table 3). Very few microbial PLDs exhibit inhibition by Ca²⁺ (Ogino et al., 1999). The activation effects of Co²⁺ and Mn²⁺ were consistent with PLDs from Streptomyces sp. YU100 and Bacillus licheniformis (Table 3). The inhibition effects of Cu²⁺ have been reported in pure microbial PLD, and also in our experiments. The inhibition effects of Mg²⁺ are consistent with PLDs from Streptoverticillium cinnamoneum, Streptomyces tendae, Streptomyces sp. CS628 and Psychrophile Shewanella sp. (Table 3). The inhibition effects of Zn²⁺ on PLDs were found from Bacillus licheniformis, Psychrophile Shewanella sp. and Streptomyces tendae (Table 3). The
positive effect of K+ on thermolabile alkaline PLD of Streptomyces sp. CS628 was also found in PL-S.t from S. thalpophilum 2015. Especially in this study, the activity of PL-S.t was enhanced by Ni2+ without inhibition. Studies have indicated that Ni2+ could improve the phosphatase activity (Dotaniya and Pipalde, 2018), and decrease urease activity (Kanso et al., 2018). Normally, Ni2+ can bind to cysteine or negatively-charged residues in active sites of enzymes (Paulo et al., 2017). PL-S.t has only one cysteine residue and fewer negatively-charged AAs compared with neutral AAs, and the hydrogen bonds network was found around Cys108 (Supplementary Fig. S5). It was found that hydrogen bonds have the ability to stabilize protein (Chen et al., 1993; Pace et al., 2014). Nevertheless the mechanism of PL-S.t stability in an Ni2+ solution does require further study.

From the optimum pH and stability experiments, we found the PL-S.t from S. thalpophilum 2015 is an alkaline PLD and is alkali tolerant. Simkhada et al. (2010) found that alkali tolerant characteristics also existed in Streptomyces sp. CS628. The molecular mechanism of phospholipase alkaline stability is still not clear at the present time. Through research on the alkali adaptability of M-protease from alkaliophilic Bacillus sp. KSM-K16 and alkali cellulase K from Bacillus sp. KSM-635, the alkali resistance mechanism was found to be related to the decreasing amount of lysine, negatively-charged AAs and an increasing amount of arginine and neutral AAs (histidine, asparagine and glutamine) (Suplatov et al., 2014). By analyzing the PL-S.t sequence, the negatively-charged AA residues and lysine together occupied 9.2% of total AAs, which is lower than arginine and neutral AAs (histidine, asparagine and glutamine), with an occupation of 13.5%. Whether the low content of negatively-charged AAs and lysine of PL-S.t has an effect on the alkali tolerance of PL-S.t still needs to be further studied.

The optimum growth temperature of Sphingobacterium thalpophilum was around 30°C (Fu et al., 2017; Zhou et al., 2005). Interestingly, the optimum temperature of PL-S.t was also found to be 30°C and the activity decreased above or below 30°C, indicating that PL-S.t is temperature sensitive. Extremophile was found to produce enzymes that catalyze best when the conditions are favourable for extremophile growth (Burg, 2003). There are a lack of studies on the relationship between the optimum temperature of mesophile enzyme and mesophile growth.

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Supplementary Materials

Supplementary figures are available in our J-STAGE site (http://www.jstage.jst.go.jp/browse/jgam).

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