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FULL ARTICLE

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A novel thermostable xylanase from *Geobacillus vulcani* GS90: Production, biochemical characterization, and its comparative application in fruit juice enrichment

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Abstract

Xylanases have great attention to act as a potential role in agro-industrial processes. In this study, production, characterization, and fruit juice application of novel xylanase from thermophilic *Geobacillus vulcani* GS90 (*Gv*Xyl) were performed. *Gv*Xyl was purified via acetone precipitation and gel-filtration chromatography. The results showed that *Gv*Xyl had 1,671.4 U/mg of specific activity and optimally worked at pH 8 and 55°C. It was also active in a wide pH (3–9) and temperature (30–90°C) ranges. *Gv*Xyl was highly stable at 90°C and relatively stable at pH 3–9. The kinetic parameters of *Gv*Xyl were obtained as K_m, V_{max}, and k_{cat}; 10.2 mg/ml, 4,104 μmol min^{-1} mg^{-1}, and 3,542.6 s^{-1}, respectively. *Gv*Xyl had higher action than commercial xylanase in fruit juice enrichment. These results revealed that *Gv*Xyl might possess a potential influence in fruit juice processing because of its high specific activity and great thermal stability.

Practical applications

Polysaccharides include starch, pectin, and hemicellulose create problems by lowering fruit juice quality in beverages. To overcome this problem, various clarification processes might be applied to natural fruit juices. Even though chemicals are widely used for this purpose, recently enzymes including xylanases are preferred for obtaining high-quality products. In this study, we reported the production and biochemical characterization of novel thermostable xylanase from thermophilic *G. vulcani* GS90 (*Gv*Xyl). Also, apple and orange juice enrichment were performed with the novel xylanase to increase the quality in terms of yield, clarity, and reducing sugar substance. The improved quality features of apple and orange juices with *Gv*Xyl was then compared to commercially available β-1,4-xylanase. The results revealed that *Gv*Xyl might possess a potential influence in fruit juice processing because of its high specific activity and great thermal stability.

KEYWORDS

fruit juice clarification, *Geobacillus vulcani*, thermostability, xylanase

1 | INTRODUCTION

Hemicellulose, the second major polysaccharide in nature after cellulose, is mainly composed of xylan structure. Xylan encompasses a backbone including β-1,4-linked xylopyranose residues and branches that can be β-1,3-linked L-arabinose and α-1,2-linked D-glucopyranose (Wood et al., 1989). Xylan degradation is performed by synergistic action of different xylanolytic enzymes, mainly endo-1,4-β-D-xylanases (E.C. 3.2.1.8), which break up the xylan backbone (Collins et al., 2005). Currently, endo-xylanases have become great attention to act as a potential role in the agro-industrial process in a synergistic manner with the other xylanolytic enzymes. Those processes are mainly clarification of fruit juice, the bleaching of the pulp paper, enhancement of the digestibility of animal feedstock, the increase of bread quality, and bioethanol production (Adigüzel & Tunçer, 2016; Bajpai, 1999; Butt et al., 2008; Collins et al., 2005).

Polysaccharides include starch, pectin, and hemicellulose decrease fruit juice quality in beverages. To overcome this problem, some attempts have been performing to increase the quality of natural fruit juices (Shahrestani et al., 2016). Even though chemicals are widely used for this purpose, enzymes including xylanases are preferred for obtaining the high-quality products. The reasons of enzymes' use are that they have a character of substrate specificity and ordinary reaction conditions, as well as their nontoxic property (Cakmak & Saglam Ertunga, 2016; Shahrestani et al., 2016). Many studies have reported that xylanase is a powerful enzyme for fruit juice extraction and clarification process (Adigüzel & Tunçer, 2016; Bajaj & Manhas, 2012; Cakmak & Saglam Ertunga, 2016; Nagar et al., 2012; Olfa et al., 2007; Sari et al., 2018).

Xylanases are synthesized by a variety of organisms including microorganisms, protozoans, and mollusks (Beg et al., 2001). Mostly, the industrially important xylanases are produced by microorganisms such as bacteria and fungi (Motta et al., 2013). Recently, many studies have been focused on the identification of endo-xylanases from thermophilic microorganisms because thermophilic enzymes can tolerate high temperatures (over 60°C) required for many industrial processes (Morozkina et al., 2010). Although many thermophilic microorganisms have been reported as xylanase sources, xylanase action has been shown in only five species of *Geobacillus* genus; *Geobacillus galactosidasius*, *Geobacillus sp.* TF16, *Geobacillus* sp. *WSUCF1*, *Geobacillus stearothermophilus*, and *Geobacillus thermoleovorans* (Bhalla et al., 2014; Bibi et al., 2014; Cakmak & Saglam Ertunga, 2016; Sari et al., 2018; Verma & Satyanarayana, 2012).

Recently, a thermostable xylanolytic enzyme (α-L-arabinofuranosidase) of *Geobacillus vulcani* GS90, a thermostable α-amylase of *Geobacillus* sp. GS33, and a thermostable protease of *Geobacillus* sp. GS53 from Balçova geothermal region, İzmir, Turkey, have been successfully characterized as a highly active enzyme in a wide range of temperature and pH (Baykara et al., 2021; Burhanoğlu et al., 2020; İlgü et al., 2018); however, a xylanase of *Geobacillus* source from this region of Turkey has not been documented so far. Here, we reported the production and biochemical characterization of a novel thermostable xylanase from thermophilic *G. vulcani* (*Gv*Xyl) for the first time. Also, apple and orange juice enrichment were performed to investigate the influence of novel xylanase on yield, clarity, and reducing sugar substance, compared to the commercially available β-1,4-xylanase.

2 | MATERIALS AND METHODS

2.1 | Materials

All chemicals including beechwood xylan and commercially available β-1,4-xylanase from *Thermomyces lanuginosus* were supplied by Sigma Chemical (St. Louis, MO, USA).

2.2 | Bacterial strains and growth conditions

G. vulcani GS90 obtained from thermal vent of Balçova geothermal region (İzmir, Turkey) and screened for xylanolytic activity (Yavuz et al., 2004) was used as a source of xylanase (*Gv*Xyl). This strain was previously identified by 16S rRNA (Sürmeli et al., 2019). To obtain single colony, Luria–Bertani (LB) agar (1% (w/v) tryptone, 0.5% (w/v) yeast extract, 1% (w/v) sodium chloride, and 1.5% (w/v) agar) was used. For the induction of *Gv*Xyl production, cultivation was performed in a medium containing 1% (w/v) beechwood xylan, 2% (w/v) tryptone, 0.25% (w/v) yeast extract, 0.2% (w/v) NH_4NO_3, 0.2% (w/v) KH_2PO_4, 0.1% (w/v) $MgSO_4.7H_2O$, and 0.005% (w/v) $MnSO_4$ at 55°C and 200 rpm overnight.

2.3 | Protein extraction and purification

The produced *Gv*Xyl was extracted from *G. vulcani* GS90 culture and purified using acetone precipitation and gel-filtration chromatography, respectively. For this purpose, *G. vulcani* GS90 cultures were centrifuged at 4°C and 5,600 *g* for 15 min. The supernatant was kept as an extracellular soluble protein extract. For acetone precipitation, the protein extract was incubated at −20°C for 1 hr, dissolving by 100 ml of cold (−20°C) acetone. After centrifugation at 4°C, 6,000 *g* for 20 min, the pellets were dried, discarding the supernatants. Then, acetone-precipitated soluble protein extract was dissolved in 50 mM of Na-P buffer (pH 7.0) and loaded onto the gel-filtration column (Sephadex G-100, 2.5 × 50 cm, Sigma). The column was equilibrated with 50 mM of Na-P buffer (pH 7.0) and five fractions with the highest xylanase activity were pooled and stored at −20°C. The purified *Gv*Xyl was displayed using 12% SDS-PAGE and zymogram analysis was performed on native PAGE. Protein concentration was quantitatively detected by Bradford method using bovine serum albumin (BSA) standard (Bradford, 1976).

2.4 | Biochemical characterization of the extracellular xylanase

2.4.1 | Standard activity assay

The activity of *Gv*Xyl was determined by 3,5-dinitrosalicylic acid (DNS) method (Miller, 1959). For this purpose, enzyme activity was measured using 1 ml reaction mixture, containing 360 μl of beechwood xylan (1%) in 50 mM of Na-P buffer (pH 7.0) as substrate and 40 μl of the purified enzyme. The reaction was performed at 55°C for 10 min and terminated by 600 μl of DNS reagent. The reaction mixture was boiled for 15 min, cooled down to room temperature, and the released reducing sugars were spectrophotometrically determined at 540 nm. D-xylose was used as the standard. One unit of enzyme activity is defined as the amount of 1 μmol of xylose released by the enzyme per minute under the standard activity assay conditions.

2.4.2 | Effect of pH and temperature

The influence of pH on *Gv*Xyl activity was analyzed using various buffers at different pH, changing from 3 to 9; Na-citrate buffer (pH 3–6), sodium phosphate buffer (pH 7), Tris–HCl buffer (pH 8), and Glycine–NaOH buffer (pH 9). Also, the effect of temperature on enzyme activity was studied at certain temperature points, ranging from 30 to 90°C. All experiments were performed using standard activity assay conditions.

2.4.3 | pH and thermal stability

To investigate pH stability, *Gv*Xyl was pre-incubated at various pH points, ranging from 3 to 9 using the indicated buffers at 55°C for 120 min. For thermal stability, the enzyme was incubated at various temperature points, ranging from 30 to 90°C at pH 8 for 120 min. For both stability assay, the residual enzyme activity was determined using standard activity assay.

2.4.4 | Influence of chemical reagents and metal ions

To study the effect of chemical reagents (10 mM ethylenediaminetetraacetic acid (EDTA), 10 mM sodium dodecyl sulfate (SDS), 10 mM 3-[(3-cholamidopropyl)dimethylammonio]-1-propanesulfonate (CHAPS), 10 mM 1,4-dithiothreitol (DTT), 1% dimethyl sulfoxide (DMSO), 1% Triton X-100, 1% Tween 20, 1% Tween 80, 1% ethanol, 1% methanol, 1% acetone, 1% isopropanol, 1% chloroform, 1% acetonitrile, and 1% β-mercaptoethanol) and 10 mM of metal ions (KCl, NaCl, $CaCl_2$, $MgCl_2$, $MnCl_2$, NaF, $NiCl_2$, $CuSO_4$, $CdSO_4$, $CoCl_2$, and $ZnCl_2$), incubation of *Gv*Xyl was carried out at room temperature for 10 min in the presence of each chemical. Residual enzyme activity was determined under standard activity assay conditions.

2.4.5 | Kinetic studies

Investigation of kinetic parameters for *Gv*Xyl was performed using the Lineweaver–Burk plots originating from a regular Michaelis–Menten kinetics. For this purpose, 360 μl of beechwood xylan in a range of 1–20 mg/ml concentrations and 40 μl of the purified enzyme were used for kinetic reaction at 55°C and pH 7. As a result of standard activity assay conditions, the Lineweaver–Burk graph was plotted.

2.5 | Shelf life determination

Shelf life of *Gv*Xyl was studied at room temperature and 4°C throughout 6 weeks and enzyme activity was checked using standard activity assay once a week.

2.6 | Enzymatic hydrolysis in fruit juice enrichment

*Gv*Xyl was analyzed for the enrichment process of fruit juices to investigate yield, clarity, and reducing sugars, compared to the commercially available β-1,4-xylanase. Fresh fruits (apple and orange), which were purchased from a local market, thoroughly washed, and dried at room temperature. After homogenization, fruit samples were incubated at 50°C for 4 hr by the addition of purified *Gv*Xyl (20 U/ml) and harvested using centrifugation at 5,000 *g* for 10 min. In parallel with this, the same procedure was applied to determine the effect of commercial endo-β-1,4-xylanase (20 U/ml). Thus, supernatants were used to check for yield, clarity, and reducing sugars. The yield of each juice was determined by measuring its volume. Also, determination of the clarity of the juices was performed by measuring the percent transmittance (% T) at 660 nm by UV-Vis spectrophotometer. The percent transmittance was considered as a measure of juice clarity. Beside this, reducing sugars released from fruit juices were determined by DNS method (Miller, 1959; Olfa et al., 2007).

2.7 | Data presentation, statistical analysis, and bioinformatic analysis

Standard errors of data were represented using GraphPad Prism version 6.00 for Windows (GraphPad Software, La Jolla, CA, USA) (www.graphpad.com). All experiments were performed in three repetitions. Also, 16S rRNA gene and amino acid sequences were aligned using Clustal Omega (Madeira et al., 2019) to evaluate the sequence similarity between nucleotide sequences of 16S rRNA genes and amino acid sequences of enzymes.

3 | RESULTS AND DISCUSSION

G. vulcani (former name *Bacillus vulcani* strain 3S-1) is a thermophilic, spore-forming, gram-positive, and motile bacterium

TABLE 1 Purification stages of the extracellular xylanase from *G. vulcani* GS90 (*Gv*Xyl)

Samples	Total activity (U)	Total protein (mg)	Specific activity (U/mg)	Purification fold	Recovery yield (%)
Soluble protein extract	711.4 ± 6.7	1.65	431.1 ± 5.1	1.0 ± 0.005	100 ± 0.546
Acetone precipitation	640.2 ± 5.3	0.98	653.2 ± 8.8	1.52 ± 0.035	90 ± 0.539
Gel filtration	484.1 ± 3.86	0.289	1,671.4 ± 13.3	3.88 ± 0.033	68 ± 0.077

(Caccamo et al., 2000; Nazina et al., 2004). The first report has been on the isolation of *G. vulcani* 3S-1 from sediment of a shallow hydrothermal vent at Vulcano Island (Italy). This report has shown that its growth conditions are in a range of 37–72°C and pH 5.5–9 and it is optimally grown at 60°C and pH 6 (Caccamo et al., 2000). In addition, it has been reported that the strain 3S-1 is phylogenetically the closest strain to the strain GS90 (Sürmeli et al., 2019) used in this study as a xylanase producer source (*Gv*Xyl-producer strain). Another strain *G. vulcani* PSS1 obtained from the gut microbiome of healthy Japanese approximately has 3.4 mb genome (GeneBank ID: JPOI01000001.1) (Nishijima et al., 2016). Some portion of the genome shares a 99.74% identity with 16S rRNA gene sequence of *Gv*Xyl-producer strain GS90 (GeneBank ID: MT611101.1) (Nishijima et al., 2016; Sürmeli et al., 2019) (Supporting Information). Additionally, the strain PSS1 genome encodes endo-1,4-beta-xylanase amino acid sequence of 407 residues and another xylanolytic enzyme alpha-N-arabinofuranosidase of 500 residues, deposited in the NCBI as WP_033842342.1 and WP_202807098.1 accession numbers, respectively (https://www.ncbi.nlm.nih.gov) (Nishijima et al., 2016). The alpha-N-arabinofuranosidase from the strain PSS1 shares 95.80% identity with the alpha-L-arabinofuranosidase from *Gv*Xyl-producer strain GS90 (GeneBank ID: HE653772.2) (İlgü et al., 2018; Nishijima et al., 2016) (Supporting Information). Thus, *Gv*Xyl amino acid sequence from the strain GS90 may be possible to be a highly similar to endo-1,4-beta-xylanase from the strain PSS1.

Xylanases are used in fruit juice enrichment process to increase yield, clarification, and quality properties of juices (İlgü et al., 2018; Long et al., 2018). In this study, we produced, purified, and characterized a novel xylanase from thermophilic *G. vulcani* GS90 (*Gv*Xyl), and then applied the purified enzyme on apple and orange juices. For this purpose, *Gv*Xyl production was induced by a special medium as described in Materials and Methods.

3.1 | Purification of thermostable xylanase from *G. vulcani* GS90 (GvXyl)

Purification of thermostable xylanase from *G. vulcani* GS90 (*Gv*Xyl) was performed using acetone precipitation and gel-filtration chromatography, respectively. The enzyme samples of collected fractions were determined as 68% recovery yield with 3.88-fold purification (Table 1).

*Gv*Xyl purity was checked by SDS-PAGE (Figure 1a) and the xylanolytic activity was confirmed by zymogram analysis, resulting in a clear single band (Figure 1b). The molecular weight of *Gv*Xyl was about 40.1 kDa. Similar to *Gv*Xyl, recombinant thermostable xylanase from *Geobacillus* sp. TF16 exhibited a band of 39.8 kDa (Cakmak & Saglam Ertunga, 2016), whereas another recombinant thermostable endo-xylanase from *Geobacillus sp.* WSUCF1 was 37 kDa 15. Also, endo-1,4-β-xylanase (*Ep*XYN1) from *Eupenicillium parvum* 4–14 has a molecular weight of 40.75 kDa (Long et al., 2018).

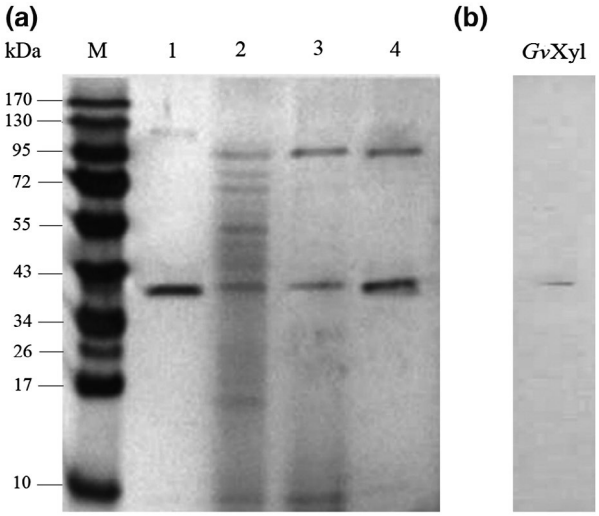

FIGURE 1 SDS-PAGE (a) and zymogram (b) analysis of the extracellular xylanase from *G. vulcani* GS90 (*Gv*Xyl). M. Protein ladder. 1. The purified *Gv*Xyl. 2. Intracellular protein. 3. Soluble protein extract after acetone precipitation. 4. Soluble protein extract

3.2 | Biochemical characterization of GvXyl

3.2.1 | Effect of pH and temperature

Purified *Gv*Xyl from *G. vulcani* GS90 was elaborately characterized, as shown in Figure 2, Tables 2, and 3. *Gv*Xyl was assessed in a broad range of pH (3–9) and temperature (30–90°C). The optimum pH and temperature of the enzyme were determined as 8 and 55°C, respectively (Figure 2a,b). In literature, xylanase from *G. galactosidasius* BS61 had the maximum activity at 60°C and pH 7.0 (Sari et al., 2018). Also, endo-xylanase from *Geobacillus* sp. WSUCF1 possessed maximum activity at pH 6.5 and 70°C (Bhalla et al., 2014). Besides, thermostable recombinant xylanase from *Geobacillus* sp. TC-W7 had optimum working conditions at 75°C and pH 8.2 (Liu et al., 2012).

The *Gv*Xyl stability was determined at temperatures between 30 and 90°C and pH values between 3 and 9. Thermal stability

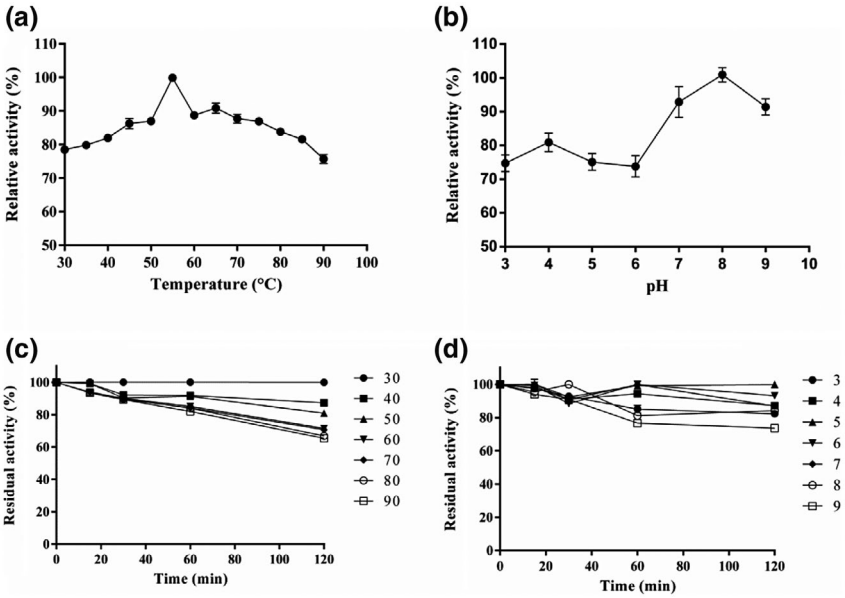

FIGURE 2 Thermal effect (a) and stability (c) of *Gv*Xyl were determined in the range of 30–90°C, the optimal pH (b) and pH stability (d) of *Gv*Xyl were determined in various buffers, such as Na-citrate buffer (pH 3–6), sodium phosphate buffer (pH 7), Tris–HCl buffer (pH 8), and Glycine–NaOH buffer (pH 9)

TABLE 2 Effect of metal ions, chemical reagents, and organic solvents on the enzymatic activity of *Gv*Xyl

Metal ions and chemicals (10 mM)		Organic solvents (1%)	
KCl	89.8 ± 0.04	Hexane	93.4 ± 0.01
NaCl	100.1 ± 0.04	Ethanol	97.4 ± 0.01
$CaCl_2$	177.7 ± 0.05	Methanol	91.9 ± 0.05
$MgCl_2$	81.0 ± 0.04	Acetone	104.7 ± 0.02
$MnCl_2$	184.3 ± 0.08	Isopropanol	86.7 ± 0.04
NaF	105.4 ± 0.01	Chloroform	103.7 ± 0.04
$FeCl_3$	17.0 ± 0.04	Acetonitrile	91.2 ± 0.04
$NiCl_2$	89.4 ± 0.01	β-Mercaptoethanol	65.3 ± 0.06
$CuSO_4$	95.0 ± 0.04	DMSO	91.8 ± 0.02
$CdSO_4$	73.9 ± 0.04	Tween 20	94.1 ± 0.04
$CoCl_2$	126.6 ± 0.08	Tween 80	86.3 ± 0.04
$ZnCl_2$	68.1 ± 0.04		
EDTA	88.2 ± 0.01		
SDS	55.8 ± 0.01		
CHAPS	67.0 ± 0.01		
DTT	55.2 ± 0.05		
Triton X-100	88.8 ± 0.04		

analysis result indicated that the enzyme exhibited a high residual activity profile, above 60%, even at 90°C for 120 min (Figure 2c). Accordingly, xylanase from *Geobacillus galactosidasius* BS61 showed 20% of residual activity at 80°C after 120 min (Sari et al., 2018). Also, thermostable recombinant xylanase from *Geobacillus sp.* TC-W7 reduced its activity below 20% at 80°C after 30 min (Liu et al., 2012). Furthermore, recombinant xylanase from *Myceliophthora heterothallica* had just 30% of residual activity at 70°C after 60 min (de Amo et al., 2019). Moreover, bifunctional endoglucanase/xylanase enzyme from *Chaetomium thermophilum* completely lost its activity at 90°C after 120 min (Hua et al., 2018). Taken together, to the best of our knowledge, there has been no recent report about xylanase enzyme maintaining its activity above 60% at 90°C throughout 120 min (Ariaeenejad et al., 2019; Azouz et al., 2020; Contreras et al., 2021; Ghosh et al., 2019; Hamid & Aftab, 2019; Li et al., 2019; Liew et al., 2019; Long et al., 2018; Xiong et al., 2020; Zhou et al., 2020). pH stability of the enzyme was observed in acidic, neutral, and alkaline conditions. The residual activity of enzyme did not decline below 80% up to 120 min under all the pH conditions (Figure 2d). Similar to our results, it has been reported that thermostable recombinant xylanase from *Geobacillus sp.* TC-W7 exhibited almost full activity at neutral and mildly alkaline pH conditions for 90 min (Liu et al., 2012). Some other studies have shown that xylanases obtained from different organisms showed high stability at mild acidic, neutral, and alkaline pH conditions throughout 24 hr (de Amo et al., 2019; Sari et al., 2018).

3.2.2 | Effect of metal ions and chemical reagents

Thermostable *Gv*Xyl activity was also determined in the presence of different chemicals, organic solvents, and metal ions, using beechwood xylan as the substrate. The enzyme activity, to a varying degree, was enhanced in the presence of 10 mM Na^+ (NaF), Ca^{2+}, Mn^{2+}, Co^{2+} metal ions, and 1% acetone and chloroform among organic solvents. Accordingly, Mn^{2+}, Co^{2+}, and Ca^{2+} considerably stimulated

TABLE 3 Kinetic characteristics of xylanase from *Geobacillus vulcani* GS90 (*Gv*Xyl) and other microorganisms

Microorganisms	Specific activity (U/mg)	V_{max} (μmol min^{-1} mg^{-1})	K_m (mg/ml)	k_{cat} (s^{-1})	References
Geobacillus vulcani GS90	1,671.37 ± 13.31	4,104 ± 18.06	10.2	3,542.60 ± 15.59	This study
Geobacillus galactosidasius BS61	1,203.70 ± 15.96	123	3.18	ND	Sari et al. (2018)
Anoxybacillus kamchatkensis NASTPD13	33	66.64	0.7	ND	Yadav et al. (2018)
Eupenicillium parvum 4–14[a]	384.42 ± 29.42	688.50 ± 32.12	0.85 ± 0.12	608.18 ± 28.37	Long et al. (2018)
Streptomyces sp. B6	763.74	2,894.0	16.1	1,571.4	Liu et al. (2020)

Note: ND refers to "not determined".

[a]kinetic parameters have been calculated according to a reaction time of 5 min.

the enzyme activity, whereas it was slightly increased under acetone and chloroform conditions. Nevertheless, the activity of thermostable *Gv*Xyl was, to a varying degree, inhibited in the presence of the remaining metal ions (especially Fe^{3+} and Cd^{2+}) and chemicals. Some chemicals (especially SDS, CHAPS, and DTT) and organic solvents (especially β-mercaptoethanol) reduced the activity of the enzyme (Table 2). In literature, many studies have been documented that many metal ions including Mn^{2+}, Ca^{2}+, and Co^{2+} and chemicals disrupted the function of xylanase obtained from *Geobacillus* species, as well as the other thermophilic bacteria (de Amo et al., 2019; Hua et al., 2018; Kumar & Shukla, 2018; Liew et al., 2019; Liu et al., 2012; Long et al., 2018; Sari et al., 2018). Different from other studies, bifunctional endoglucanase/xylanase enzyme from *Chaetomium thermophilum*, which optimally works at 55°C and pH 5.0, had a considerable increase in its activity by 5 mM Mn^{2+} and 5 mM Ca^{2+} ions (Hua et al., 2018). Also, some chemicals (Tween 20, β-mercaptoethanol Triton X-100) and some metal ions (Na^{+}, Li^{+}, and K^{+}) increased the recombinant xylanase activity from *Geobacillus sp.* TC-W7 (Liu et al., 2012).

3.2.3 | Enzyme kinetics

The standard activity assay of *Gv*Xyl was applied using beechwood xylan as a substrate at 55°C upon incubation of 10 min. The result showed that specific activity of *Gv*Xyl on beechwood xylan was found as 1,671.37 ± 13.31 U/mg (Table 3). Recently, many studies have reported the specific activity of thermostable xylanases from different microorganisms on beechwood xylan. Accordingly, thermostable xylanase from *Geobacillus galactosidasius* BS61 exhibited 1,203.70 ± 15.96 U/mg of specific activity (Sari et al., 2018). Also, thermostable xylanase from camel rumen metagenome had 1,051 U/mg of specific activity (Ariaeenejad et al., 2019). The specific activity of xylanase from thermophilic fungus *Myceliophthora heterothallica* F.2.1.4 was 101.7 ± 2.2 U/mg (de Amo et al., 2019), whereas xylanase from a halo-thermophilic bacterium *Roseithermus sacchariphilus* strain RA possessed a specific activity of 300 U/mg (Teo et al., 2019). Beside this, xylanase (XynST11) from thermophilic *Streptomyces* sp. B6 had a specific activity of 763.74 U/mg (Liu et al., 2020). However, to the best of our knowledge, there has been no previous report of xylanase that could possess a specific activity, as high as 1,671.37 ± 13.31 U/mg on beechwood xylan so far (Bala & Singh, 2019; Joshi et al., 2020; Long et al., 2018; Zhou et al., 2020).

Kinetic parameter values of thermostable xylanase were obtained by Lineweaver–Burk plot on the certain concentrations of beechwood xylan substrate. This analysis showed that *Gv*Xyl had 10.2 mg/ml of K_m, 4,104 ± 18.06 μmol/min mg^{-1} (U/mg) of V_{max}, and 3,542.60 ± 15.59 s^{-1} of k_{cat} values (Table 3). In the literature, there have been many studies about kinetic parameters of thermostable xylanases from various organisms on beechwood xylan as the substrate. Accordingly, xylanase from *Geobacillus galactosidasius* BS61 had K_m and V_{max} values of 3.18 mg/ml and 123 U/mg, respectively (Sari et al., 2018). Also, extracellular xylanase from *Anoxybacillus kamchatkensis* NASTPD13 possessed K_m and V_{max} values of 0.7 mg/ml and 66.64 μmol min^{-1} mg^{-1}, respectively (Yadav et al., 2018). Beside this, xylanases *Ep*XYN1 and *Ep*XYN3 from *Eupenicillium parvum* 4–14 had 0.85 ± 0.12 and 0.95 ± 0.18 mg/ml of K_m, and 688.50 ± 32.12 and 361.80 ± 26.00 μmol/mg of V_{max}, whereas the k_{cat} values were 608.18 ± 28.37 and 135.06 ± 9.70, respectively, throughout a reaction time of 5 min (Long et al., 2018). Furthermore, xylanases L-LXY and C-LXY from herbivore rumen metagenome had K_m values of 7.16 ± 0.79 and 5.60 ± 0.84, respectively, whereas V_{max} values were 488.04 ± 15.01 and 497.76 ± 28.39, respectively (Zhou et al., 2020). Liu and colleagues (2020) have found that thermostable xylanase XynST11 from *Streptomyces* sp. B6 had 16.1 mg/ml of K_m, 2,894.0 IU/mg of V_{max}, and 1,571.4 s^{-1} of k_{cat} values (Liu et al., 2020). Thus, *Gv*Xyl displayed the highest V_{max}, and k_{cat} values among all known thermostable xylanases in the literature.

To determine the *Gv*Xyl hydrolysis activity on beechwood xylan, the products of the substrate were analyzed via thin-layer chromatography (TLC) (data not shown). TLC analysis indicated that xylobiose was the main product of beechwood xylan as a result of the hydrolysis action of the enzyme. Moreover, xylotriose and xylose detection were observed as faint spots considered side products of the substrate. Non-treated beechwood xylan with *Gv*Xyl did not give any product spots detected by TLC as expected.

The shelf life of *Gv*Xyl was determined along 6 weeks at 4°C and room temperature, determining the residual activity of the enzyme.

TABLE 4 Effect of *Gv*Xyl and commercial β-1,4-xylanase on apple and orange juice clarification, reducing sugar content, and yield

Fruit juices	Tested features	Control	B-1,4-xylanase	*Gv*Xyl
Apple	Yield (ml)	69 ± 1.2	82 ± 0.3	84 ± 0.1
	Reducing sugar (μg/ml)	932.43 ± 2.3	990.11 ± 3.9	1,007.10 ± 1.5
	Clarity (%T)	52.61 ± 2.6	65.65 ± 1.7	71.78 ± 0.6
Orange	Yield (ml)	66 ± 0.8	71 ± 0.7	74 ± 0.3
	Reducing sugar (μg/ml)	369.10 ± 3.1	393.77 ± 1.1	397.00 ± 2.9
	Clarity (%T)	77.04 ± 0.9	81.88 ± 3.1	90.71 ± 4.3

This analysis result showed that the enzyme was highly stable in both conditions throughout the time (data not shown).

3.3 | Fruit juice enrichment application of GvXyl

Recently, xylan-degrading enzymes have been used in fruit juice extraction and enrichment to increase yield, clarification, and quality properties of juices (İlgü et al., 2018; Sharma et al., 2017). So far, many studies have reported the effect of various xylanases on fruit juice extraction and clarification process (Adigüzel & Tunçer, 2016; Bajaj & Manhas, 2012; Cakmak & Saglam Ertunga, 2016; Nagar et al., 2012; Olfa et al., 2007; Sari et al., 2018; Shahrestani et al., 2016). In the present study, the action of thermostable xylanase from *G. vulcani* GS90 (*Gv*Xyl) was researched on fruit juice enrichment of apple and orange pulps at 50°C upon 4 hr of incubation, compared to a commercially available β-1,4-xylanase.

Treatment with purified *GvXyl* resulted in a yield increase of 21.74% and 12.12% of apple and orange juices, respectively, compared to untreated juice (control). However, commercial xylanase increased apple juice and orange juice yield as much as 18.84% and 7.58%, respectively. Apparently, *GvXyl* action caused higher increase in yield than that in β-1,4-xylanase for both juices (Table 4). An increase of juice yield may be correlated with the degradation of all polysaccharides including starches and hemicelluloses in the pulp, resulting in better fruit juice enrichment processing (Sharma et al., 2017).

Juice clarity, measured with % transmittance (%T) at 660 nm, was resulted in an increase of 36.44%T and 17.74%T in apple and orange juices, respectively, treated with *Gv*Xyl. The efficiency of β-1,4-xylanase on the clarity of both treated fruit juices was significantly lower (24.79% for apple and 6.28% for orange) than that of *Gv*Xyl (Table 4). Adigüzel and Tunçer (2016) have shown that xylanase from *Streptomyces* sp. AOA40 led to an increase of about 15% in clarity of apple juice at 50°C after 120 min (Adigüzel & Tunçer, 2016). Also, Nagar et al. (2012) have shown that purified xylanase from *Bacillus pumilus* SV-85S enhanced apple juice clarity by 22.20% in optimized conditions (at 40°C for an incubation time of 30 min) (Nagar et al., 2012). Another study has indicated that *B. licheniformis* P11(C) xylanase decreased the turbidity of apple juice by 30%–40% at the interval of 3–6 hr (Bajaj & Manhas, 2012). A recent study has found an increase in clarity of orange juice as 17.79%, 27.05%, and 48.3% for three different xylanase enzymes (2 U/ml) from *Fusarium* sp. 21 at 45°C for 1 hr (Li et al., 2020). da Silva et al. (2019) have shown that the purified xylanase from *Aspergillus japonicus* increased orange juice clarity by only 2.66% at 55°C upon incubation of 4 hr (da Silva et al., 2019).

Reducing sugar releases with *GvXyl* treatment increased in apple juice by 8.01% and orange juice by 7.56% compared to untreated juices. Moreover, commercial xylanase increased the reducing sugar contents by 6.68% and 6.19% of apple and orange juices, respectively, at relative lower level than that of *Gv*Xyl (Table 4). In the literature, similar studies have reported the released reducing sugar levels by xylanase enzymes from different sources for the application of fruit juices (Adigüzel & Tunçer, 2016; Cakmak & Saglam Ertunga, 2016; Olfa et al., 2007). Accordingly, Cakmak and Saglam Ertunga (2016) has shown that xylanase from *Geobacillus sp.* TF16 increased the reducing sugar content of apple and orange juices by 1.03-fold and 2.85-fold, respectively (Cakmak & Saglam Ertunga, 2016). Also, a xylanase from *Streptomyces* sp. enhanced the reducing sugar content in apple and orange juices by 17.21% and 19.57%, respectively (Adigüzel & Tunçer, 2016). Beside this, xylanase from *Sclerotinia sclerotiorum* S2 fungus resulted in an increase of reducing sugar release in apple and orange juices by 5-fold and 10-fold, respectively, after 24 hr of treatment (Olfa et al., 2007). Li et al. (2020) have reported that three different xylanases of *Fusarium* sp. 21 increased the reducing sugar release in orange juice by 17%, 24.8%, and 42.6% at 45°C upon incubation of 1 hr (Li et al., 2020). Taken together, *Gv*Xyl led to the comparable results in quality improvement of apple and orange juices with other xylanases from different sources.

4 | CONCLUSIONS

In this study, novel thermostable xylanase from *G. vulcani* GS90 (*Gv*Xyl) was produced, purified, and characterized for the first time. *Gv*Xyl can be emerged as an impressive candidate for the use in various industries because it had high specific activity and thermal stability. In line with this, *Gv*Xyl had high thermal stability, retaining its residual activity above 60% at 90°C throughout 120 min. Additionally, kinetic analysis results showed that *Gv*Xyl possessed high specific activity (about 1,671 U/mg), V_{max} (4,104 U/mg), and k_{cat} (about 3,542 s^{-1}) values, to the best of our knowledge, that have not been reported in any previous study for beechwood xylan so far.

Fruit juice enrichment analysis results showed that *Gv*Xyl was more effective than the commercial endo-β-1,4-xylanase, resulting in higher yield, clarity, and reducing sugars on apple and orange juices. Also, it had comparable results with previously reported xylanases from different sources upon fruit juice applications. Thus, *Gv*Xyl could be favorable in facilitating fruit juice enrichment processes.

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AUTHOR CONTRIBUTION

Müge Algan: Data curation; Formal analysis; Methodology. **Yusuf Sürmeli:** Data curation; Formal analysis; Methodology; Visualization; Writing-review & editing. **Gülşah Şanlı Mohamed:** Conceptualization; Investigation; Project administration; Resources; Supervision; Writing-review & editing.

DATA AVAILABILITY STATEMENT

Data available on request from the authors.

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SUPPORTING INFORMATION

Additional Supporting Information may be found online in the Supporting Information section.

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