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Production of β-xylanase and β-xylosidase by the extremely halophilic archaeon *Halorhabdus utahensis*

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Abstract The extremely halophilic archaeon, *Halorhabdus utahensis*, isolated from the Great Salt Lake, Utah, produced β-xylanase and β-xylosidase activities. Both enzymes were active over a broad NaCl range from near zero to 30% NaCl when tested with culture broth. A broad NaCl optimum was observed for β-xylanase activity between 5% and 15% NaCl, while β-xylosidase activity was highest at 5% NaCl. Almost half of the maximum activities remained at 27%–30% NaCl for both enzyme activities. When dialyzed culture supernatant and culture broth were employed for determination of β-xylanase and β-xylosidase stabilities, approximately 55% and 83% of the initial β-xylanase and β-xylosidase activities, respectively, remained after 24 h incubation at 20% NaCl. The enzymes were also shown to be slightly thermophilic; β-xylanase activity exhibiting two optima at 55° and 70°C, while β-xylosidase activity was optimal at 65°C. SDS-PAGE and zymogram techniques revealed the presence of two xylan-degrading proteins of approximately 45 and 67 kDa in culture supernatants. To our knowledge, this paper is the first report on hemicellulose-degrading enzymes produced by an extremely halophilic archaeon.

Keywords *Archaea* · β-xylanase · β-xylosidase · Halophilic · *Halorhabdus utahensis* · Halostable

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Introduction

Next to cellulose, hemicellulose is the second most abundant renewable polysaccharide in nature, produced at a rate of 10^{10} tons per year (Biely 1985; Wilkie 1983). Xylan, being the most important of the hemicelluloses, is usually a heteropolymer, composed of a backbone of 1,4-linked β-D-xylopyranose residues and branches of L-arabinofuranose, D-glucuronic acid, or 4-*O*-methyl-D-glucuronic acid. The degree of branching depends on the source of plant material (Biely 1985; Puls et al. 1988), and the xylans are often acetylated (Biely 1985).

Hydrolysis of the xylose backbone of xylan involves endo-β-xylanases (1,4-β-D-xylan xylanohydrolase: EC 3.2.1.8) and β-xylosidases (1,4-β-D-xylan xylohydrolase: EC 3.2.1.37) (Wong et al. 1988). The degradation of xylan is further enhanced by the action of side-group cleaving enzymes such as α-L-arabinofuranosidases, acetyl esterases, and α-glucuronidases (Puls et al. 1988).

Xylanases have been reported in bacteria, marine algae, fungi, invertebrates, and plants (Dekker and Richards 1976). Although most of the extracellular xylanases studied derive from mesophilic bacteria and fungi, psychrophilic fungi (Bradner et al. 1999) as well as thermophilic (e.g., Lüthi et al. 1990; Winterhalter and Liebl 1995) and alkalophilic (e.g., Honda et al. 1985) bacteria producing xylanases have also been described. Furthermore, production of xylanolytic enzymes by the hyperthermophilic archaeon, *Pyrodictium abyssi*, was briefly reported by Andrade and co-workers (1996). More recently, a small survey of archaeal organisms revealed the production of xylanolytic activities by species within the euryachaeal genera *Thermococcus* and *Pyrococcus* and provided the first characterization of an archaeal hemicellulase, i.e., the xylanase produced by *Thermococcus zilligii* strain AN1 (Uhl and Daniel 1999). So far, no reports seem to exist on the isolation and characterization of hemicellulases from extremely halophilic archaea, although degradation of cellulose by bacteria adapted to hypersaline environments has been

reported previously (e.g., Elazari-Volcani 1943; Simankova et al. 1993; Vreeland et al. 1998).

In this paper we, for the first time, report on the characterization of xylanase and xylosidase activities produced by the extremely halophilic euryarchaeon, *Halorhabdus utahensis*, recently isolated from sediments of the Great Salt Lake, Utah, USA (Wainø et al. 2000).

Materials and methods

Organism and culture conditions

For the production of enzymes, cells of *Halorhabdus utahensis* strain AX-2 (DSM 12940^T) were aerobically cultured at 30°C in a Tris-based medium (TRIS 10 medium) containing (g/l): NaCl, 270 g; $MgSO_4 \cdot 7H_2O$, 20 g; KCl, 5 g; NH_4Cl, 2 g; NaBr, 0.1 g; yeast extract (Difco), 1 g; TRIS-HCl, 12 g; birchwood xylan (Roth, Karlsruhe, Germany), 2 g; trace metal solution (TMS 3) (Ingvorsen and Jørgensen 1984), 2 ml. The pH was adjusted to 7.8. After sterilization and cooling of the medium to 5°C, 2.5 ml of a sterile phosphate solution (KH_2PO_4, 50 g/l), 0.5 ml of a sterile $CaCl_2$ solution ($CaCl_2 \cdot 2H_2O$, 100 g/l), and 0.25 ml of a sterile $FeCl_2$/$MnCl_2$ solution ($FeCl_2 \cdot 4H_2O$, 20 g/l + $MnCl_2 \cdot 4H_2O$, 20 g/l) were added. The final pH of the medium was approximately 7.6.

Preparation of enzyme sources

Enzyme solutions for enzyme assays comprised culture broth, cell-free supernatant, dialysate, or crude purified enzyme. Supernatant was obtained by centrifugation of culture broth for 3 min at 11,000 *g*, while dialysate was prepared by dialyzing the supernatant overnight against 10 mM sodium phosphate buffer (pH 7.0) at 4°C. The final salinity of the dialysate was 0.5% (w/v) NaCl. For separation of proteins by gel electrophoresis, the dialysate was concentrated about 40-fold by air-drying in a covered, sterile Petri-dish left overnight in a flow-bench. The concentrated dialysate was subsequently redialyzed before being subjected to sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS-PAGE).

A crude purification procedure for xylanase was performed by incubation of the concentrated dialysate with about 6% (w/v) beechwood xylan (Lenzing AG, Lenzing, Austria) and 10% (w/v) NaCl at 30°C for 60 min. Subsequently, the sample was centrifuged for 5 min at 11,000 *g*. The supernatant, containing the non-adsorbed enzyme activity (fraction A), was removed, whereas the pellet was resuspended in an equal volume of 10 mM sodium phosphate buffer without NaCl (pH 7.0) and incubated at 30°C for 30 min. Following centrifugation (5 min at 11,000 *g*) the resulting supernatant containing the desorbed xylanase activity (fraction B) was removed. Finally, the pellet containing the non-desorbable xylanase fraction (fraction C) was resuspended in an equal volume of 10 mM sodium phosphate buffer (pH 7.0). Fraction B was further concentrated by centrifugation at 4°C through a Centricon-10 (Amicon, Beverly, USA) concentrator for 60 min at 5,000 *g*, thereby achieving a roughly 500-fold concentrated enzyme solution. This solution was also subjected to SDS-PAGE.

For localization of enzymatic activities in *Halorhabdus utahensis*, culture broth was treated as follows: the supernatant obtained by centrifugation (15 min at 11,000 *g*) of culture broth was dialysed against a 10 mM sodium phosphate buffer containing 20% NaCl and 1% $MgSO_4 \cdot 7H_2O$, pH 7.0 (hereafter referred to as NaMP buffer), and served as the extracellular fraction. The cell pellet was resuspended in 10 mM sodium phosphate buffer containing 2% NaCl to induce cell lysis and incubated at 30°C with shaking (the progress of cell lysis was followed by microscopy). After 30 min the lysate was centrifuged at 16,000 *g* for 30 min. The supernatant containing the cytosolic/periplasmatic fraction was dialyzed against NaMP buffer. The pellet containing the cell wall/membrane fractions was resuspended in an equal amount of NaMP buffer.

Enzyme assays

The reaction mixture for determination of β-xylanase activity contained 10% (v/v) of enzyme solution incubated in a total of 1.0 ml NaMP buffer containing 0.1% (w/v) AZCl-xylan (Megazyme, Wicklow, Ireland). The reaction mixture was incubated at 30°C in an Eppendorf thermomixer for a period of 15–240 min (enzyme activity was found to be constant within this time period, $R > 0.99$). After incubation, reaction mixtures were centrifuged (11,000 *g* for 3 min) and the dye-release from AZCl-xylan was measured spectrophotometrically at 595 nm. One unit of β-xylanase activity was defined as the amount of enzyme which releases 1 μmol of reducing sugar as equivalent to D-xylose per minute under the above conditions. To inter-convert xylanase activities, a calibration curve was made correlating dye-release from AZCl-xylan to reducing-sugar equivalents produced from beechwood xylan. Reducing-sugar equivalents were measured by the method of Miller (1959). The effect of pH on β-xylanase activity was tested at 12% (w/v) NaCl by replacing the NaMP buffer of the standard assay with Britton–Robinson (I) buffer. In short, buffer solutions of different pH values were obtained by adding increasing amounts of a 0.2 M NaOH solution to a stock solution of 40 mM each of H_3PO_4, CH_3COOH, and H_3BO_3 (Rauen 1964).

β-xylosidase activity was determined using a reaction mixture containing 2% (v/v) culture broth in a total volume of 1 ml 0.5 mM p-nitrophenyl-β-D-xylopyranoside dissolved in NaMP buffer. The reaction mixture was incubated for a suitable period at 30°C (hydrolysis was found to be linear within incubation periods applied, $R > 0.99$). The amount of *p*-nitrophenol released was determined spectrophotometrically at 405 nm in supernatant samples immediately upon centrifugation (11,000 *g* for 3 min). One unit of β-xylosidase activity was defined as the amount of enzyme, which liberates 1 μmol of *p*-nitrophenol per minute under the above-mentioned assay conditions. The effect of pH was determined at 10% (w/v) NaCl using a reaction mixture containing 2% (v/v) culture broth in 1 ml 0.5 mM *p*-nitrophenyl-β-D-xylopyranoside dissolved in 40 mM Britton–Robinson (I) buffer.

All enzyme assays were done in duplicate or triplicate.

Gel electrophoresis and zymogram

Proteins were separated by SDS-PAGE using an 8%–18% polyacrylamide gradient gel (ExcelGel^{TM} SDS, Pharmacia Biotech). The gel was run at 15°C at a constant current of 25 mA for approx. 80 min. After separation, the analytical gel was immediately placed on a substrate gel (TRIS 10 medium, 1.5% agar, 0.2% beechwood xylan, 10% NaCl; pH 7.4) and incubated at 30°C for 60 min. Hydrolysis of xylan in the substrate gel were visualized using the Congo Red technique (Williams 1983), and proteins on the SDS-PAGE gel were silver-stained (Pharmacia Biotech).

Results

Time courses of cell growth, production and cellular location of β-xylanase and β-xylosidase activity

When *Halorhabdus utahensis*, strain AX-2, was grown in TRIS 10 medium containing birchwood xylan as the carbon source, both β-xylanase and β-xylosidase activities were produced (Fig. 1). β-xylanase activity reached a maximum of around 206 mU/ml at the end of the exponential growth phase and decreased throughout the stationary phase. β-xylosidase activity also increased during the growth phase, reaching a maximum level of 18 mU/ml towards the end of the exponential growth phase, but stayed constant during the remaining cultivation period.

The cellular locations of the β-xylanase and β-xylosidase activities were determined with cultures in either exponential growth phase (about 120 h of incubation) or in the stationary phase (about 200 h of incubation). As shown in Table 1, β-xylanase activity was almost evenly distributed between the cell wall/membrane fraction and the extracellular environment during exponential growth, with an increasing proportion (about 73%) being extracellular in the stationary phase. This increase is probably not due to cell lysis, since the extracellular fraction of the β-xylosidase activity was low and constant over time. In contrast, the β-xylosidase activity was mainly associated with the cell wall/membrane fraction of the cells (87%–96% of total activity). It is speculated that the relatively high presence of β-xylosidase activity in the cytosolic/periplasmic fraction during exponential growth but not in the stationary phase is the result of intracellularly produced β-xylosidases, which have not yet become finally incorporated in the cell wall or cell membrane.

The effect of hydrolysis products on β-xylanase activity

The effect of some potential hydrolysis products on β-xylanase activity was tested using the standard assay procedure in the presence of 0–250 mM xylose or glucuronate (data not shown). No inhibition was found at any concentrations of glucuronate tested, whereas β-xylanase activity decreased slightly with increasing concentrations of xylose, resulting in an inhibition of approximately 20% at 250 mM xylose. However, since the concentration of reducing sugars in the culture broth rarely exceeded 1 mM, inhibition of the xylanase activity, and hence growth by hydrolysis products, is most likely negligible during the cultivation of strain AX-2 on xylan.

Effects of NaCl on β-xylanase and β-xylosidase activities and stabilities

The β-xylanase and β-xylosidase enzymes produced by the extremely halophilic *Halorhabdus utahensis* were catalytically active within a very broad salinity range (Fig. 2). Supernatant dialyzed against distilled water (final NaCl concentration about 0.002% w/v) exhibited 33% of the β-xylanase activity measured at 20% NaCl. The activity could not be increased above this level by

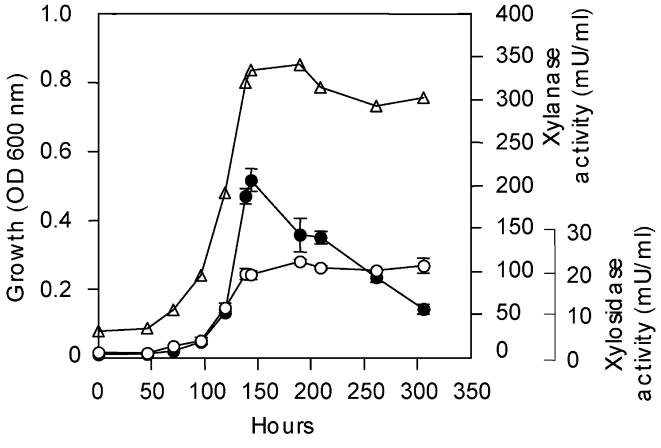

Fig. 1 Time course of growth (△) and production of β-xylanase (●) and β-xylosidase (○) activities. Cells were cultivated in TRIS 10 medium containing 0.2% (w/v) birchwood xylan and 27% (w/v) NaCl at 30°C on a shaker (180 rpm). Enzyme assays were performed with culture broth. *Error bars* indicate SE of two assay replicates

Table 1 Cellular locations of β-xylanase and β-xylosidase activities of *Halorhabdus utahensis*

Locality	β-xylanase	β-xylosidase
Extracellular	47.8 ± 0.8 (73.4 ± 1.0)	1.8 ± 0.0 (3.2 ± 1.0)
Cell wall/membrane	52.2 ± 7.6 (24.9 ± 0.3)	87.0 ± 3.7 (95.9 ± 2.0)
Cytosol/periplasm	0.0 ± 0.0 (1.7 ± 0.3)	11.2 ± 1.0 (0.9 ± 1.5)

Cultures grown for about 120 h or 200 h (figures in parentheses) were tested for distribution of enzymatic activities. Data (with standard errors) are given as percentage of total enzyme activity and represent the means of triplicate samples

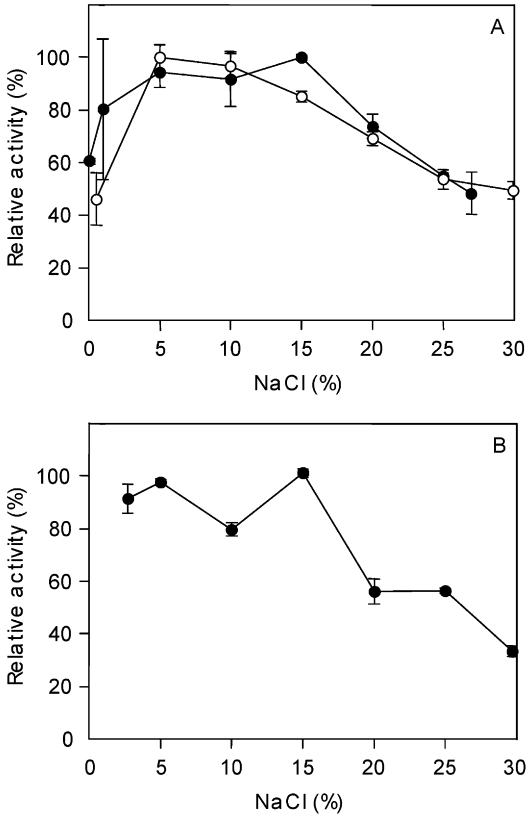

Fig. 2A, B Effects of NaCl on β-xylanase and β-xylosidase activities in presence of 1% (w/v) $MgSO_4 \cdot 7H_2O$. Standard assays were performed after preincubation of enzyme solution with NaCl for 10 min before addition of the substrate solution. **A** β-Xylanase activity in dialysate (●); β-xylosidase activity in culture broth (○). **B** β-Xylanase activity in culture supernatant (●). *Error bars* indicate SE of three assay replicates

re-addition of 20% NaCl. β-xylanase activity was maximal at 15% NaCl (Fig. 2A). When culture supernatant was employed to test the salinity response of β-xylanase activity, two activity optima at 5% and 15% NaCl could be inferred, indicating that isozymes might be produced by *Halorhabdus utahensis* (Fig. 2B). The β-xylanase activity of the dialysate retained at least 49% of the maximum activity at concentrations between 0.05% and 27% NaCl (Fig. 2A). When using supernatant as the enzyme solution, about 32% of the maximum activity remained at 30% NaCl (Fig. 2B). β-Xylosidase activity was also present over a broad salinity range. It displayed optimum activity at 5% NaCl and exhibited more than 45% of maximum activity at all salinities tested (Fig. 2A).

The halostability of β-xylanase and β-xylosidase activities was tested at 30°C during a 24-h assay. β-Xylanase activity was nearly equally stable at 0.05% NaCl and 27% NaCl, whereas β-xylosidase activity was equally stable at 0.5% NaCl and 25% NaCl (data not shown). Thus, about 83% and 50% of the initial β-xylanase and β-xylosidase activities remained after 24 h incubation at these salinities. However, at salinities between 1% and 10% NaCl, β-xylanase stability was higher than β-xylosidase stability, showing no loss of activity within the experimental period. Furthermore, the halostability of β-xylanase at 50°C was considerably higher at salinities above than at those below 10% NaCl, implying a positive correlation between NaCl concentration and thermostability. Other authors, e.g., Kamekura and Seno (1990), have also reported a thermostabilizing effect of NaCl.

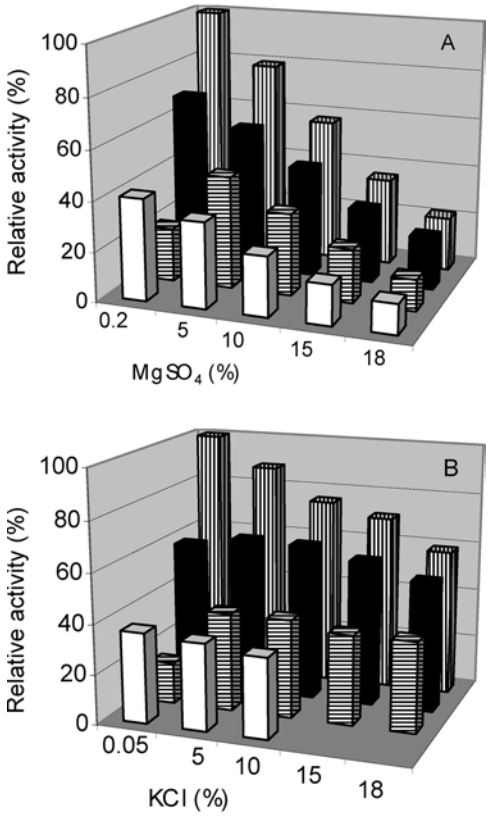

Fig. 3 Effect of five different concentrations of $MgSO_4$ (**A**) and KCl (**B**) on β-xylanase activities in dialysate containing 0.05% NaCl (columns with horizontal lines) or culture supernatant containing 3% NaCl (columns with vertical lines), 7% NaCl (closed columns) or 21% NaCl (open columns). Standard assays were performed in duplicate with the average standard error being approximately 2% of the mean activities

Effects of other salts on β-xylanase activities

The effects of 0%–18% (w/v) $MgSO_4$ or KCl on β-xylanase activity were tested at four different NaCl concentrations. At 0.05% NaCl, $MgSO_4$ stimulated the activity up to a concentration of about 15% with a maximum at 5% $MgSO_4$ (Fig. 3A). At 3%, 7%, and 21% NaCl, β-xylanase decreased linearly with increasing $MgSO_4$ concentrations. At 0.05% NaCl, KCl stimulated the activity at all concentrations tested (Fig. 3B). Increasing KCl concentrations caused increased inhibition of the β-xylanase activity at 3% NaCl, although the inhibition was less pronounced than that of equal amounts of $MgSO_4$. At 7% and 21% NaCl, increasing concentrations of KCl resulted in negligible effects on β-xylanase activity.

Effects of temperature and pH on β-xylanase and β-xylosidase activities

The effect of temperature on β-xylanase and β-xylosidase activities at 20% NaCl and 1% $MgSO_4$ is shown in Fig. 4A. Both enzyme activities could be detected up to 75°C in the standard assay using a 15-min incubation period. β-xylosidase activity showed a sharp optimum at 65°C, while β-xylanase activity exhibited two activity maxima; at 55° and 70°C, respectively, indicating the presence of isozymes. β-xylosidase activity was present over a broad pH range; showing optimum activity at pH 7.6 (Fig. 4B). β-xylanase activity was displaced towards the acidic side as compared to β-xylosidase activity, exhibiting a broad optimum around pH 6.5. No activity was found above pH 9.0, but 35% of maximum activity remained at pH 5.1.

Thermostability of the β-xylanases

The thermostability of β-xylanase activity was investigated by incubation of supernatant in the absence of substrate at 50°, 55°, and 60°C at 20% NaCl and 1% $MgSO_4$ (data not shown). At 50°C the activity remained stable for about 8 h, whereas only 16% and 32% of the initial β-xylanase activity was present after 8 h at 55°C and 0.5 h at 60°C, respectively. β-xylanase activity was absent after 24 and 2 h at 55° and 60°C, respectively, while approx. 75% of initial β-xylanase activity was present after 24 h incubation at 50°C.

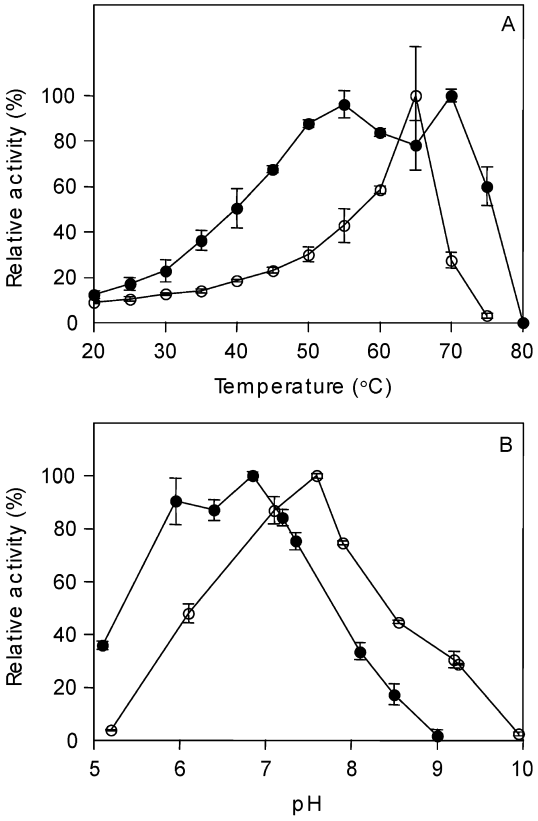

Fig. 4A, B Effects of temperature (**A**) and pH (**B**) on β-xylanase activity in culture supernatant (●) and β-xylosidase activity in culture broth (○). **A** Standard assay with incubation for 15 min. Substrate solutions were preincubated at each temperature for 5 min before the assays were initiated. **B** Determination of β-xylanase and β-xylosidase activities was performed using Britton–Robinson buffer at 12% and 10% (w/v) NaCl, respectively. *Error bars* indicate SE of three (**A**) or two (**B**) assay replicates

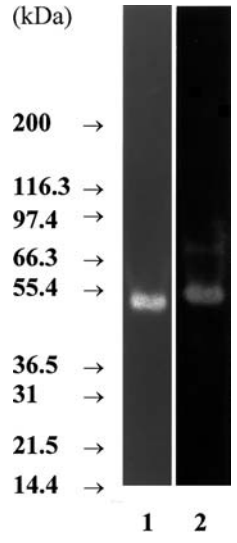

Fig. 5 Xylanase-degrading activity in *Halorhabdus utahensis* resolved by SDS-PAGE of dialysate and activity staining using Congo Red. Cells were cultivated in TRIS 10 medium containing 0.2% (w/v) birchwood xylan and 27% (w/v) NaCl. *Lane 1*, preliminary purified enzyme solution (fraction B); *Lane 2*, untreated 40-fold concentrated dialysate

Preliminary purification, gel electrophoresis and zymogram staining

Preliminary experiments showed that a substantial portion of the total cell-free β-xylanase activity could be adsorbed/desorbed at high and low salinities, respectively. These findings formed the basis of a simple purification procedure, which yielded three fractions: fraction A containing the β-xylanase activity unable to adsorb to xylan at high salinity, fraction B containing the activity which adsorbed to xylan at high salinity but was released at low salinity, and fraction C containing the activity which remained bound to xylan at low salinity. Comparison with the activity of an untreated sample (concentrated dialysate of culture supernatant) under identical standard assay conditions revealed that fractions A, B, and C constituted 38%, 45%, and 5% of total β-xylanase activity, respectively. It should be noted, however, that the activity of fraction C was inherently underestimated, since the enzymes in the assay mixture were already bound to xylan prior to the addition of the dyed test substrate.

SDS-PAGE of fraction B (500-fold concentrated) followed by silver staining revealed a range of very faint protein bands (data not shown). Nevertheless, a xylan-degrading band with an estimated molecular weight of approx. 45 kDa was clearly detected in the zymogram (Fig. 5, lane 1). SDS-PAGE and silver staining of the untreated sample (40-fold concentrated dialysate produced from culture supernatant) did not reveal any protein bands. However, when applying the zymogram technique, two distinct xylan-degrading activities were detected (Fig. 5, lane 2). The molecular masses of the enzymes were estimated at around 45 and 67 kDa.

Discussion

Very few reports exist on the degradation of plant polymers by microorganisms adapted to hypersaline environments, although Elazari-Volcani in 1943 was able to enrich for aerobic cellulose decomposers using inoculum from the Dead Sea (Elazari-Volcani 1943). An extensive screening of 160 eubacterial halophilic strains carried out by Kamekura about 40 years later did not reveal the presence of cellulolytic or hemicellulolytic strains (Kamekura 1986). Nonetheless, Vreeland et al. (1998) reported the isolation of a number of cellulolytic strains from various sources in a salt mine. These so far uncharacterized strains, apparently able to slowly hydrolyze cellulose, were not tested for their ability to degrade hemicellulose. Production of both cellulose- and hemicellulose-degrading enzymes by two strains of the extremely halophilic actinomycete *Actinopolyspora halophila* was, however, described by Johnson et al. (1986). *Actinopolyspora halophila* exhibited optimal production of xylanase activity at 15% (w/v) NaCl. The

xylanase activity was not investigated at different concentrations of NaCl, although it was shown that removal of NaCl by dialysis resulted in lower xylanase activity, which could not be restored by the addition of NaCl. A marine eubacterium, *Thermotoga maritima* MSB8, was shown to produce two xylanases, one having optimal activity in the presence of 0.5 M NaCl (Winterhalter and Liebl 1995). Both enzymes tolerated high NaCl concentrations with 49%–65% of maximum activity remaining at 2.0 M (about 12% w/v) NaCl. Simankova et al. (1993) characterized an anaerobic eubacterium, *Halocella cellulolytica* (now *Hallocella cellulosilytica*, Oren 2000), isolated from a hypersaline lagoon, which was capable of degrading cellulose, but not xylan, at 20% NaCl. Another unidentified organism (strain z-41) degrading cellulose at 25% NaCl was isolated but not further characterized (Simankova and Zavarzin 1993). Except for the recent description of xylanase production by the hyperthermophilic archaeon *Thermococcus zilligii* strain AN1 (Uhl and Daniel 1999), there have been no reports on the production of hemicellulose activities by archaea. Thus, this article for the first time reports the production of β-xylanase and β-xylosidase activities by an extremely halophilic archaeon.

Not surprisingly, the β-xylanase and β-xylosidase activities produced by *Halorhabdus utahensis* exhibited halophilic characteristics, albeit with substantial catalytical activity at low salinity. Extracellular enzymes from microorganisms of the family *Halobacteriaceae* usually irreversibly lose activity at low ionic strength (e.g., Larsen 1967; Ryu et al. 1994). This is, for instance, the case for amylase from *Natronococcus* sp. strain Ah-36 (Kobayashi et al. 1992) and lipases and proteases from different strains of the genus *Halobacterium* (González and Gutierrez 1970; Kamekura and Seno 1990; Ryu et al. 1994). An exception to this is the extracellular amylase produced by *Halobacterium halobium* (now *Halobacterium salinarum*), which regained over 90% of its activity after dialysis against distilled water and subsequent addition of 0.25% NaCl or KCl (Good and Hartman 1970). However, in contrast to most exo-enzymes produced by extremely halophilic archaea, this amylase was halotolerant rather than halophilic, displaying optimal activity at 0.05%–1.0% NaCl. The β-xylanase and β-xylosidase activities of *Halorhabdus utahensis* differ from most extracellular enzymes produced by extremely halophilic archaea by tolerating very low ionic strengths: 0.002% and 0.5% NaCl, respectively. Also their salt responses are different from that of *Halobacterium salinarum* amylase by exhibiting optimum activities at higher salinities and by retaining considerably higher activities at very high NaCl concentrations. Nearly 50% of the maximum β-xylanase activity of *Halorhabdus utahensis* remained at 27%–30% NaCl, while only 33% of the maximum amylase activity of *Halobacterium salinarum* remained at 23% NaCl. Consequently, the β-xylanase and β-xylosidase activities of *Halorhabdus utahensis* may be considered the first truly halophilic enzymatic activities reported from an extremely halophilic archaeon, which also remain active at very low NaCl concentrations.

Xylan-binding domains have been demonstrated in β-xylanases produced by bacteria, e.g., *Thermonospora fusca* (Irwin et al. 1994) and *Cellulomonas fimi* (Black et al. 1995), and affinity binding to xylan has previously been applied as a means of purifying xylanases from *Streptomyces chattanoogensis* (López-Fernández et al. 1998). Interestingly, nearly half of the extracellular β-xylanase activity of *Halorhabdus utahensis* could be adsorbed to xylan. This indicates that extremely halophilic archaea may also produce polymer-binding extracellular enzymes, though it is at present unknown whether the affinity towards xylan is due to specific binding mediated by xylan-binding domains or unspecific binding as a result of ionic interactions. The latter mechanism was found to be the primary reason for xylan adherence of xylanases produced by nonhalophilic fungi (Tenkanen et al. 1995).

Currently, xylanases obtained from nonhalophilic microorganisms are used in the manufacture of coffee (Woodward 1984) and as an ingredient in flour for the bakery industry and in animal feeds (Hilhorst et al. 1999; Veldman and Vahl 1994). Although, to our knowledge, there are no current applications of halotolerant xylanases, investigations into the structure–function relationship of halophilic and halotolerant enzymes will be of general scientific interest because of their unique adaptation to environments of low water potential (e.g., Ventosa and Nieto 1995). Halophilic carbohydrases may have potential applications in wastewater treatment (Biely 1985) and in a variety of industrial processes, such as solvent-based reaction systems, e.g., the production of carbohydrates and hydrolysis of polysaccharides at low water potentials (e.g., Klibanov 1986; Hilhorst et al. 1999).

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