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NOTE

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Comment on "The first description of an archaeal hemicellulase: the xylanase from *Thermococcus zilligii* strain AN1": evidence that the unique N-terminal sequence proposed comes from a maltodextrin phosphorylase

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Abstract Uhl and Daniel reported in this journal in 1999 (Extremophiles 3:263–267) the characterization of the first archaeal hemicellulase with a unique N-terminal sequence showing no homology with any xylanase or other protein from the databases. A genomic library of the chromosomal DNA of *Thermococcus zilligii* strain AN1 was screened by using a degenerate probe deduced from the N-terminal sequence. A positive clone was identified, and an amino acid sequence analysis revealed that the N-terminal sequence from this protein and the N-terminal sequence from the putative xylanase of *T. zilligii* were identical. However, the comparison of the amino acid sequence of the protein with sequences in the main protein databases revealed significant similarities with maltodextrin phosphorylases. In conclusion, it is likely that the N-terminal sequence proposed by Uhl and Daniel is not that of the *T. zilligii* xylanase, but corresponds to an archaeal *T. zilligii* maltodextrin phosphorylase.

Key words Xylanase · Archaea · Maltodextrin phosphorylase · *Thermococcus zilligii*

The complete hydrolysis of xylan, the second most abundant polysaccharide in nature, into assimilable sugars requires a set of enzymes including endo-β-1,4-xylanase (EC 3.2.1.8). Xylanases are responsible for the random cleavage of the xylan backbone and have industrial uses in the pulp and paper bleaching, animal feed, and baking sectors. More than 80 sequences of genes coding for xylanases have been reported (Bergquist et al. 2001) from bacteria, plants, or fungi but none from archaea. In 1999, Uhl and Daniel reported, for the *Thermococcus zilligii* strain AN1, the first characterization of an archaeal xylanase with a unique N-terminal sequence showing no significant homology with any xylanase or other protein from the databases. On the basis of their results, we undertook to clone and express the corresponding gene.

Thermococcus zilligii strain AN1 (DSM 2270, Ronimus et al. 1997) was grown anaerobically on 2216S medium at 80°C, pH 7.5, with a NaCl concentration of 0.3 g/l. A genomic library of *T. zilligii* chromosomal DNA was prepared using pBluescript II SK(+) (Stratagene) as vector and *E. coli* DH5α as host cells using standard procedures (Sambrook et al. 1989). Southern hybridization was performed at 45°C with a degenerate 17-mer oligonucleotide probe 5′-CAYACNGTNGARAAYYT-3′ based on the N-terminal amino acid sequence of the *T. zilligii* xylanase proposed by Uhl and Daniel (Fig. 1). A positive recombinant clone containing an insert of 4 kb was obtained, and the insert revealed an open reading frame (ORF) of 2.3 kb (the nucleotide sequence has been submitted to GenBank under accession number AJ318499). The translated sequence revealed that the N-terminal amino acid sequence of the 2.3 kb ORF and the N-terminal sequence of the putative xylanase of *T. zilligii* described by Uhl and Daniel (1999) were identical (Fig. 1).

However, comparison of the amino acid sequence of the protein with sequences from the databases showed significant sequence isology with certain phosphorylases (Table 1). The amino acid sequence alignment of the sequence of the 2.3 kb ORF from *T. zilligii* AN1 with *Thermococcus litoralis* maltodextrin phosphorylase (Xavier et al. 1999) (based on the first 730 amino acids) revealed a sequence identity of 78%. Moreover, analysis of the sequence revealed the presence of a phosphorylase pyridoxal-phosphate attachment site (Fig. 2) and a highly conserved amino acid sequence around this site, which is

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Txyl    --XXANVSHTVENLIRAKLPYPLEN
Tmalp   MVAIANVSHTVENLIRAKLPYPLEN
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Fig. 1. Alignment of the N-terminal amino acid sequence of *T. zilligii* xylanase published by Uhl and Daniel (1999) (Txyl) and the N-terminal amino acid sequence of the protein obtained from the *T. zilligii* chromosomal DNA library screening (Tmalp)

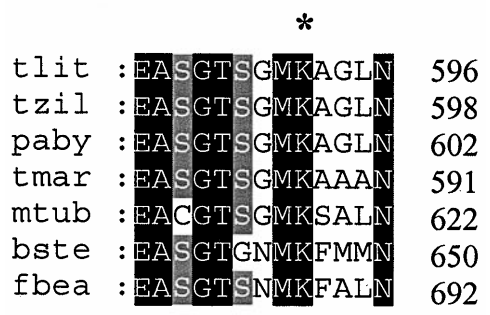

Fig. 2. Sequence alignment of the *T. zilligii* protein (tzil) obtained using the published N-terminal sequence of *T. zilligii* xylanase (Uhl and Daniel 1999) with phosphorylases showing the phosphorylase pyridoxal-phosphate binding site. The pyridoxal-phosphate attachment lysine residue is marked by an *asterisk*. tlit, *Thermococcus litoralis*; tzil, *Thermococcus zilligii*; paby, *Pyrococcus abyssi*; tmar, *Thermotoga maritima*; mtub, *Mycobacterium tuberculosis*; bste, *Bacillus stearothermophilus*; fbea, fava bean

used as a signature pattern of EC 2.4.1.1 phosphorylases (Fukui et al. 1982) (Fig. 2). These phosphorylases are key enzymes in carbohydrate metabolism. They catalyze the formation of glucose 1-phosphate from a polyglucose such as glycogen, starch, or maltodextrin (Fukui et al. 1982), and none of them are linked to xylan degradation. The phosphorylase of *T. litoralis* (MalP), which displays 78% identity with the *T. zilligii* ORF, has been characterized as a maltodextrin phosphorylase with higher activity on short maltodextrins (3–7 glucose units) (Xavier et al. 1999). In addition, it is unlikely that maltodextrin phosphorylases in general, and maltodextrin phosphorylase from *Thermococcus* (*litoralis* and *zilligii*) in particular, possess xylanolytic activity. In support of this point, Uhl and Daniel (1999) did not find any xylanase activity in *Thermococcus litoralis*. An explanation for the misidentification of the N-terminal sequence proposed for the *T. zilligii* xylanase might come from the N-terminal sequencing of an impure protein, the size of the purified AN1 xylanase (95 kDa) and the expected size of the maltodextrin phosphorylase (96.2 kDa, deduced from the *T. litoralis* maltodextrin phosphorylase amino acid sequence) being quite close. We conclude from our results that the N-terminal sequence proposed by Uhl and Daniel (1999) is that of the *T. zilligii* maltodextrin phosphorylase (EC 2.4.1.1) and not a xylanase as reported. Clearly this sequence cannot be used as a probe in a genomic approach for archaeal xylanase gene cloning. Moreover, discussions

Table 1. Amino acid sequence comparison of the *T. zilligii* protein obtained using the published N-terminal sequence of *T. zilligii* xylanase (Uhl and Daniel 1999) with archaeal and bacterial maltodextrin phosphorylases

Organism	Amino acid identity (%)[a]					
	tzil	tlit	paby	tmar	mtub	bste
tzil	100	78	71	67	39	20
tlit		100	73	66	39	20
paby			100	64	39	19
tmar				100	39	19
mtub					100	17
bste						100

tzil, *Thermococcus zilligii*; tlit, *Thermococcus litoralis*; paby, *Pyrococcus abyssi*; tmar, *Thermotoga maritima*; mtub, *Mycobacterium tuberculosis*; bste, *Bacillus stearothermophilus*

[a] Identity values were calculated using the Old Distances program (GCG Wisconsin package) from multiple alignments

of a novel family of xylanase (different from families 10 and 11) based on the N-terminal sequence proposed by Uhl and Daniel seem preliminary, even though we have shown that PCR screening against archaeal genomes (*Thermococcus zilligii*, *T. hydrothermalis*, *T. fumicolans*, *Pyrococcus furiosus*, *P. abyssi*, *P. glycovorans* and *Methanococcus jannaschii*) with DNA primers based on consensus sequences of the active site of families 10 and 11 xylanases yielded no positive results. Thus, purification of the xylanase activity of *T. zilligii* is needed to enable the gene encoding the first archaeal xylanase activity to be cloned and sequenced, giving interesting insight into structure, function, and evolution within this class of enzymes.

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