

# New Glycosyl Hydrolases from *Acidothermus cellulolyticus*

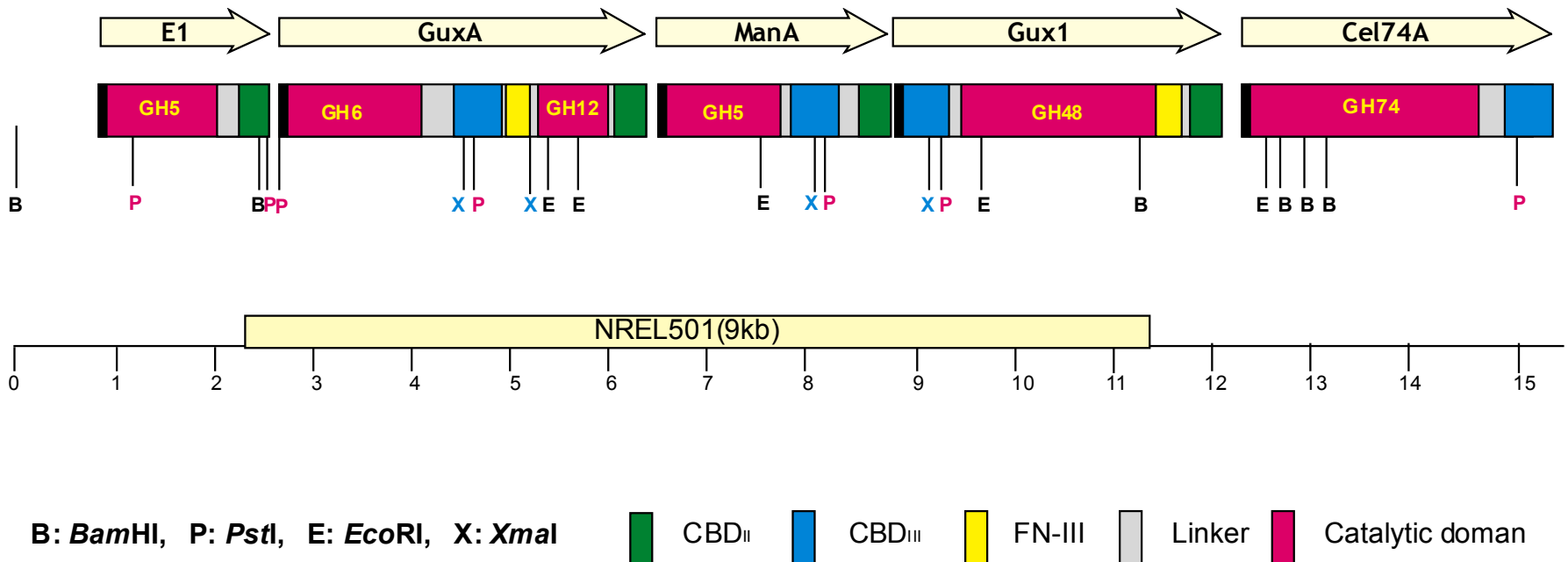
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# Abstract

*Acidothermus cellulolyticus* was isolated from 55-60°C acidic water and mud samples collected in Yellowstone National Park in the early 1980s (Mohagheghi et al. *Int. J. System. Bacteriol.* 1986). Biochemical studies have shown that its hydrolytic enzymes are thermotolerant with maximal activities at temperatures of 75-83°C. One of them, endoglucanase Cel5A, has been cloned and expressed in *E. coli* and other hosts. A lambda clone isolated from a genomic library of *A. cellulolyticus* grown on biomass was selected for DNA sequencing. A 9-kb *Bam*HI fragment from this clone was subcloned into pDR540 and sequenced by primer walking. An inverse PCR technique was then applied to continue the sequencing of the genomic DNA and the primer walking method was used to sequence the large PCR products. Sequence analysis has revealed four additional ORFs downstream of the Cel5A gene. These genes all indicate glycosyl hydrolases with multidomain structure. Domains from GH families 5, 6, 12, 48, and 74 have been identified through sequence homology. Three of these enzymes have been cloned and expressed in *E. coli*. Some characteristics of the purified catalytic domains of the Cel 5a, Cel 12a and Cel 74 a are presented here.

# A Large Gene Cluster From the *A. cellulolyticus* Genome



# Related Glycosyl Hydrolases

Glycoside Hydrolase Families

5(Retention)

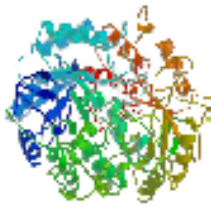
6(Inversion)

12(Retention)

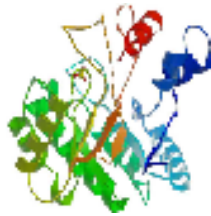
48(Inversion)

74(Retention)

Structure example



*Acidothermus cellulolyticus* endoglucanase E1cd  
(β/α)<sub>8</sub>



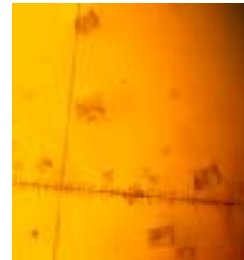
*Thermobifida fusca* endoglucanase E2cd



*Streptomyces lividans* endoglucanase  
β-jelly roll



*Clostridium cellulolyticum* cdf  
(α/α)<sub>6</sub>-helix barrel



*Aspergillus niger*

endo glucanase **GH5**

endoglucanase **GH12**

*Trichoderma reesei*

endoglucanase EGII **GH5**  
mannanase **GH5**

cellobiohydrolase CBHII **GH6**

endoglucanase EGII **GH12**

*Clostridium thermocellum*

endoglucanase CelE **GH5**  
cellulobiohydrolase CelO **GH5**  
endoglucanase CelC **GH5**  
endoglucanase CelB **GH5**  
endoglucanase CelG **GH5**  
endoglucanase CelG **GH26** **GH5**

endoglucanase CelS **GH48**

*Thermobifida fusca*

mannanase **GH5**  
endoglucanase E5 **GH5**

endoglucanase E2 **GH6**  
cellobiohydrolase E3 **GH6**

exoglucanase E6 **GH48**

cellulase **GH74**

*Cellulomonas fimi*

endoglucanase Cel5A **GH5**

cellobiohydrolase CBHA **GH6**  
endoglucanase CenA **GH6**

exocellobiohydrolase GuxB **GH48**

*Acidothermus cellulolyticus*

endoglucanase E1 **GH5**  
mannanase ManA **GH5**

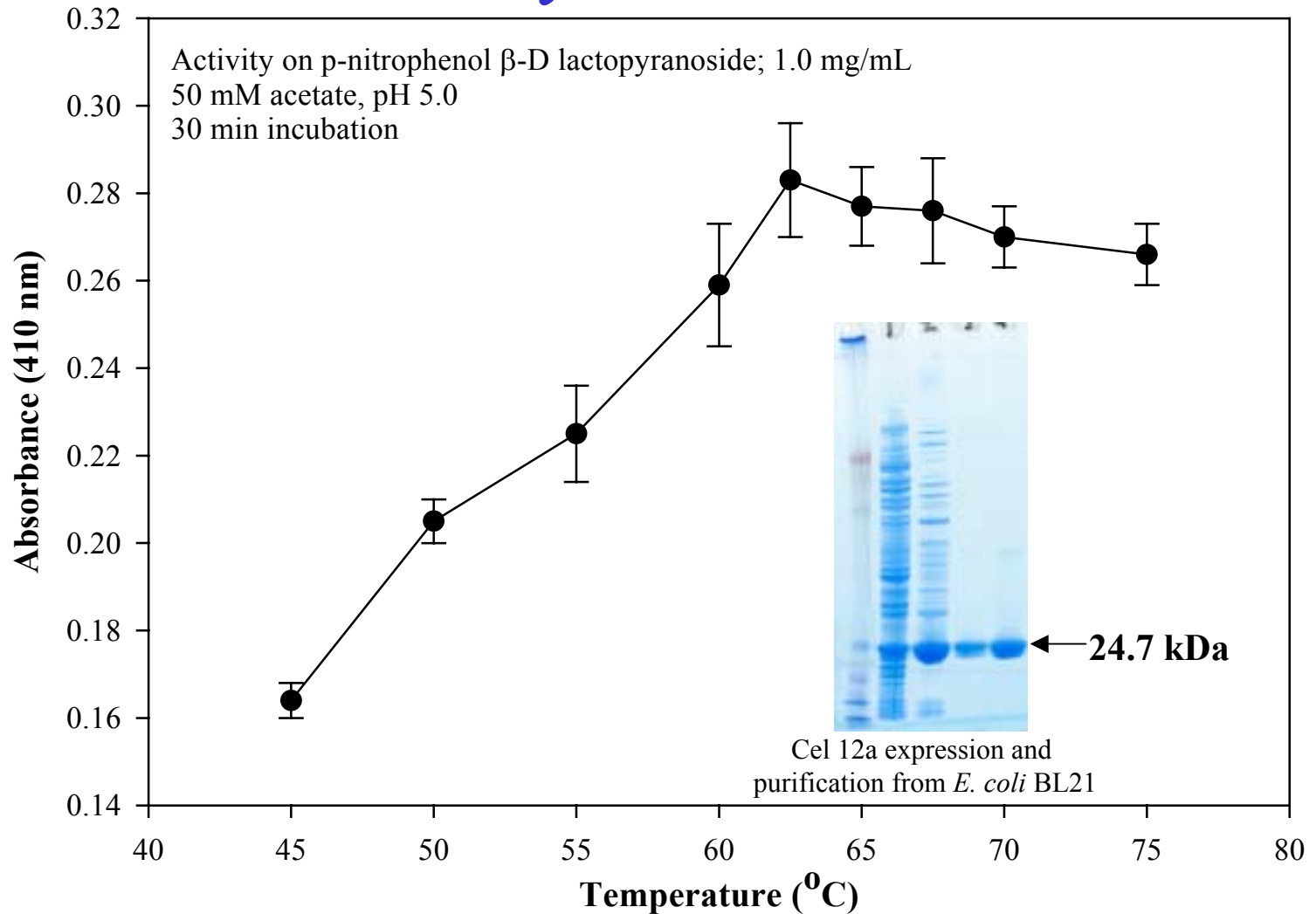
cellobiohydrolase GuxA **GH6** **GH12**

cellulase GuxA **GH6** **GH12**

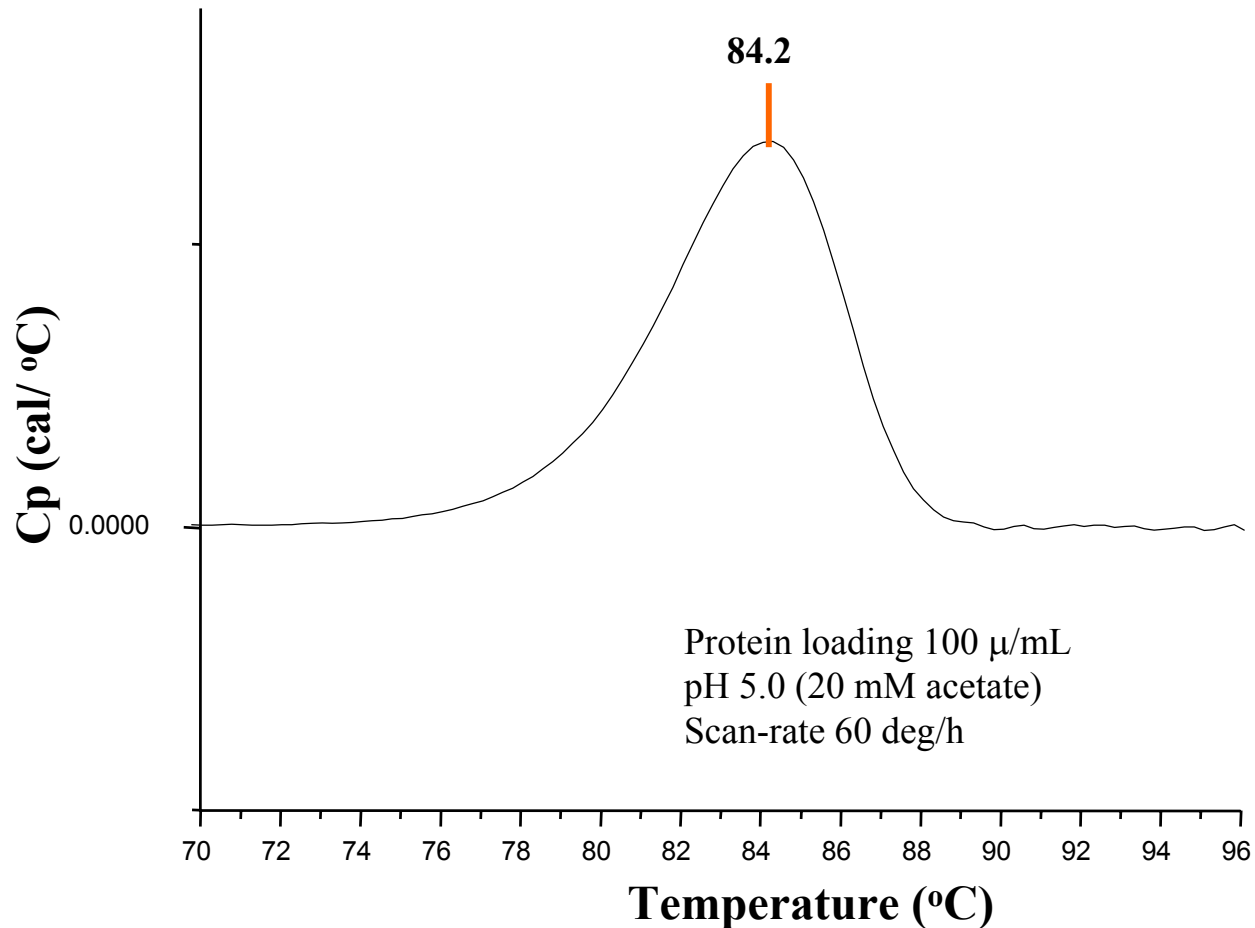
cellulase Gux1 **GH48**

cellulase AviII **GH74**

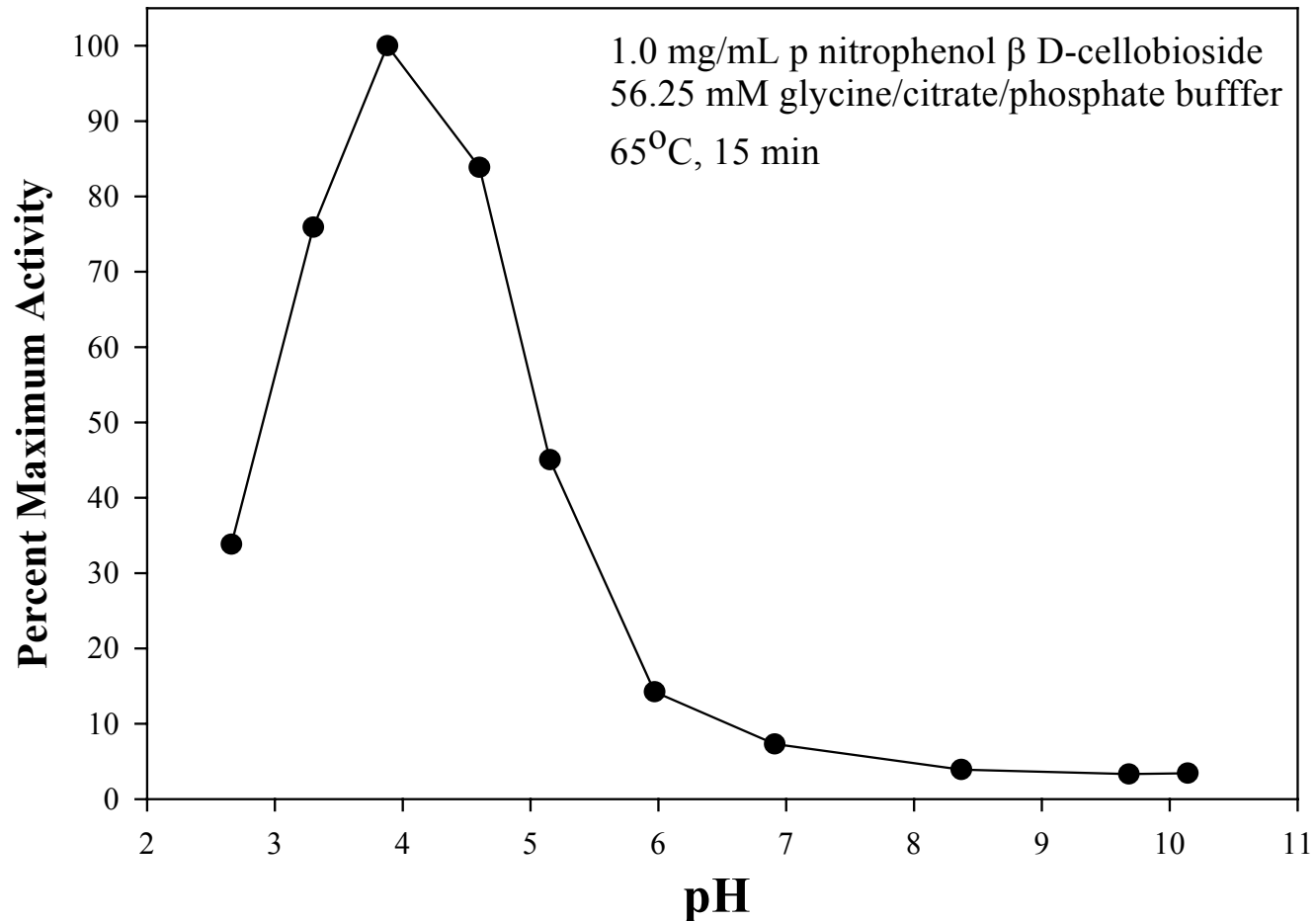
# Temperature Optimum of *A. cellulolyticus* Cel 12A cd



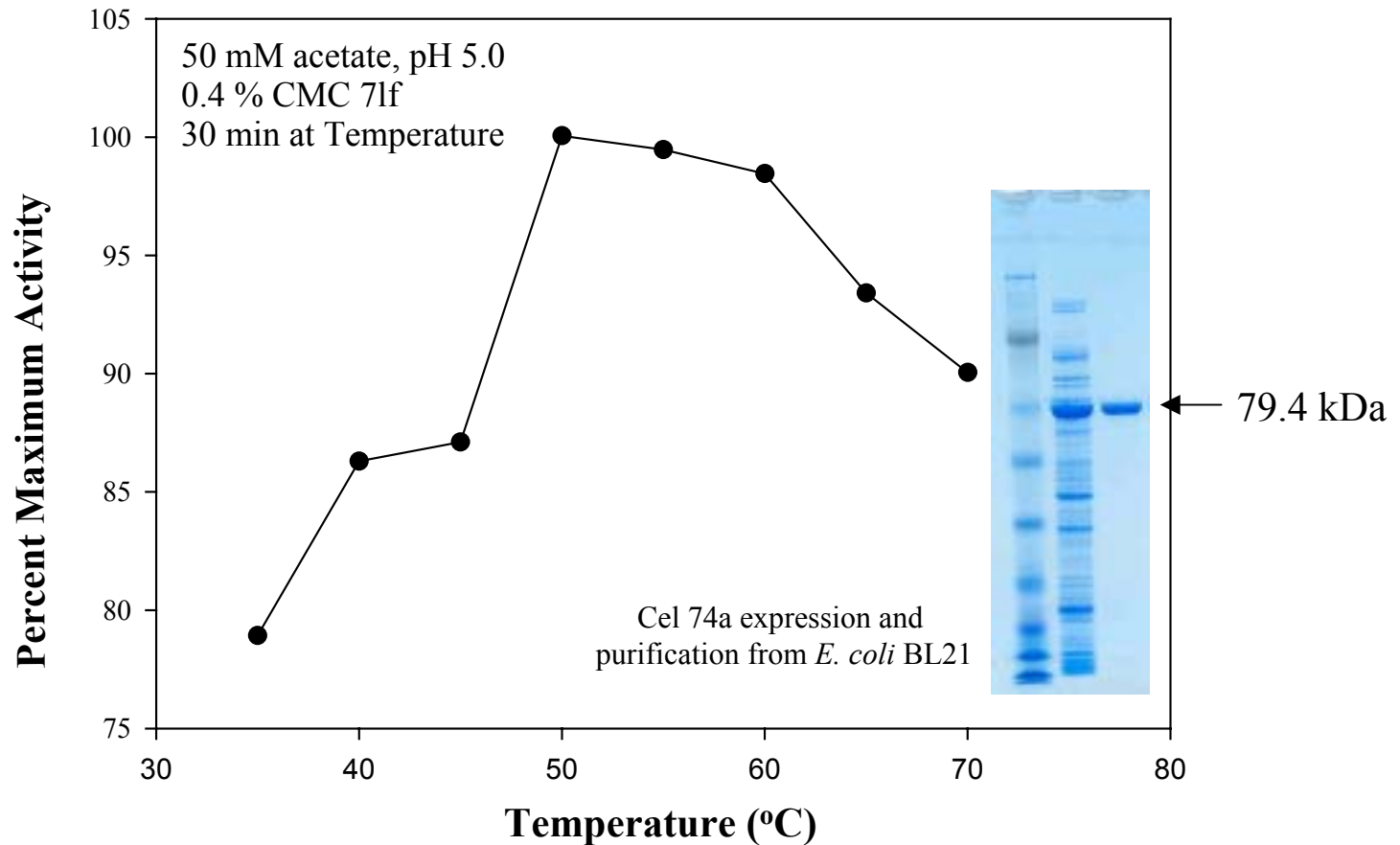
# Differential Scanning microCalorimetry of *A. cellulolyticus* Cel 12A cd



# pH Optimum of *A. cellulolyticus* Cel 12A cd

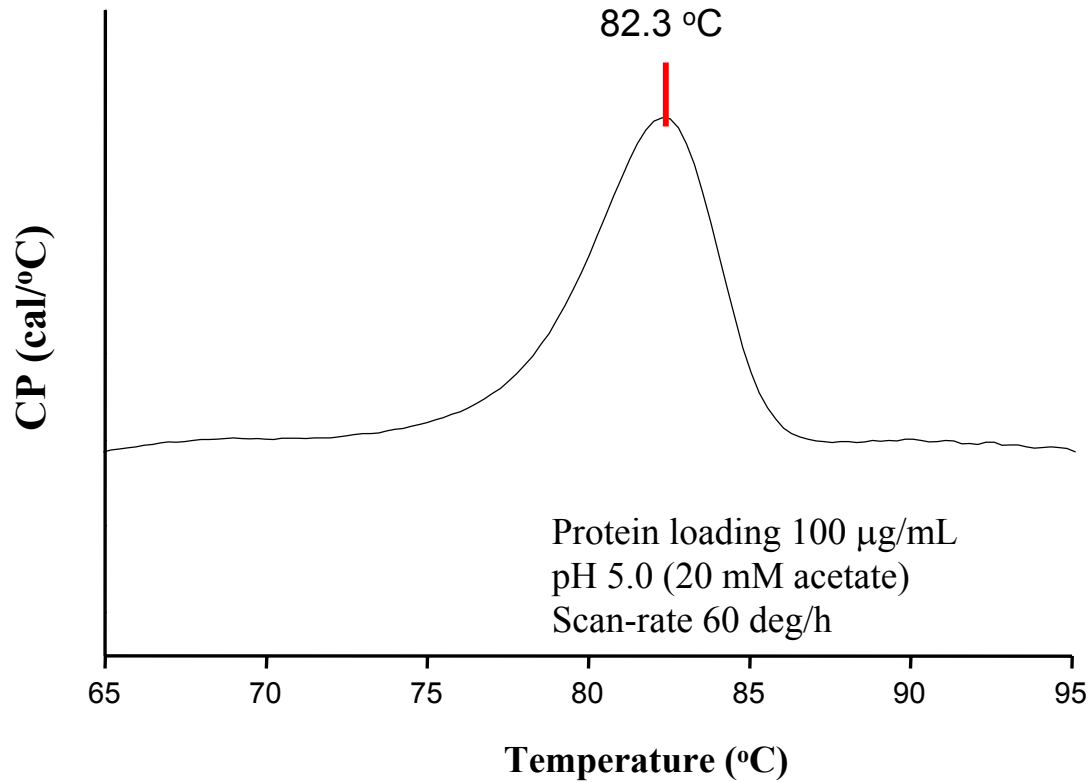


# Temperature Optimum of *A. cellulolyticus* Cel 74A cd

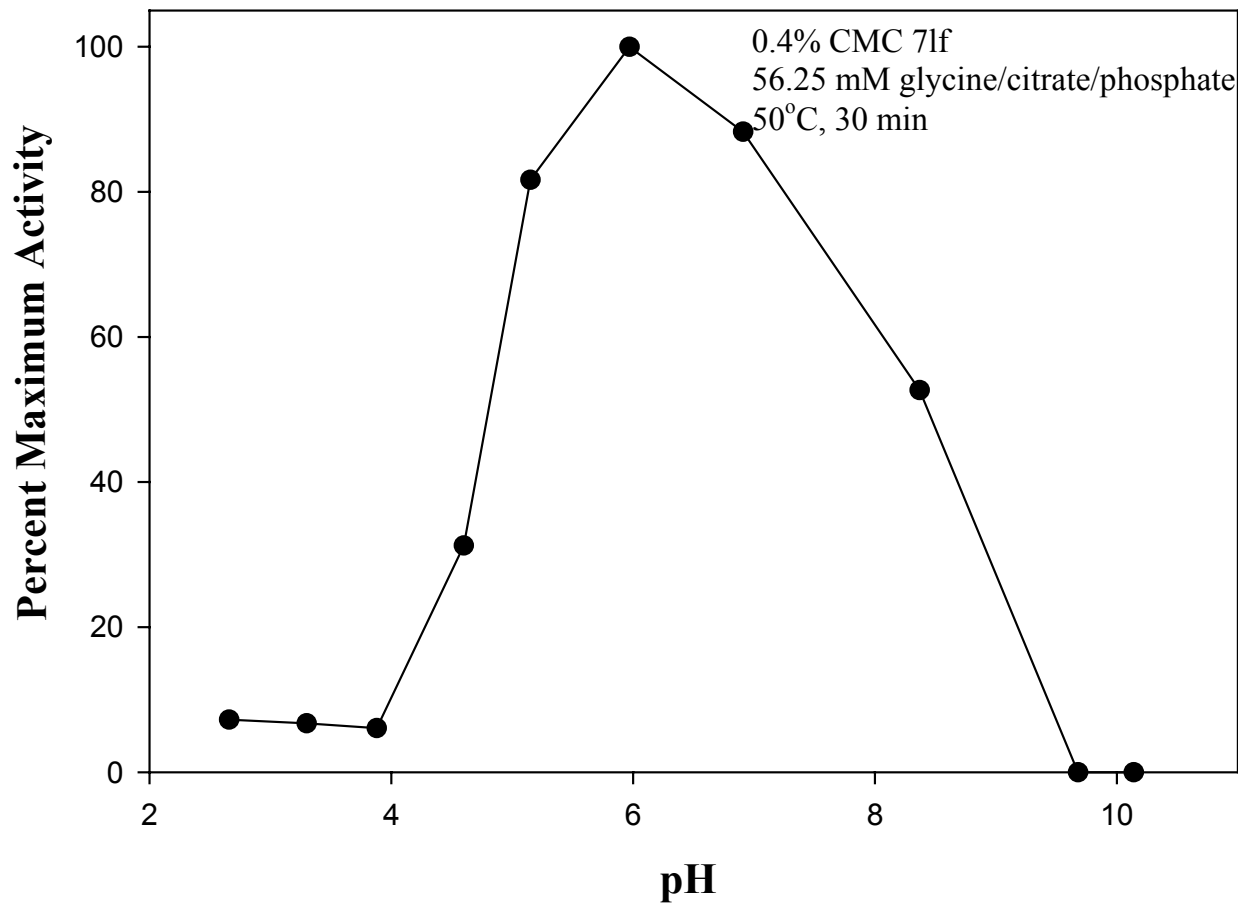




# Differential Scanning microCalorimetry of *A. cellulolyticus* Cel 74A cd



# pH Optimum of *A. cellulolyticus* Cel 74A Catalytic Domain



# Activity Comparison of *A. cellulolyticus* Glycosyl Hydrolases on Azurine-Crosslinked Insoluble Polysaccharides

AZCL-Substrate	Activity	cel5A 65°C	cel12A 65°C	cel74A 60°C
HE-Cellulose	<i>endo</i> -1,4- $\beta$ -glucanase	4+	4+	4+
Galactomannan	<i>endo</i> -1,4- $\beta$ -Mannanase	1+	-	-
Galactan	<i>endo</i> -1,4- $\beta$ -Galactanase	-	-	-
$\beta$ -Glucan	$\beta$ -Glucanase	4+	4+	1+
Curdlan	<i>endo</i> -1,3- $\beta$ -Glucanase	-	-	-
Arabinoxylan	<i>endo</i> -Xylanase	1+	2+	-
Xylan	<i>endo</i> -Xylanase	1+	3+	1+
Pachyman	<i>endo</i> -1,3- $\beta$ -Glucanase	-	-	-
Xyloglucan	<i>endo</i> -1,4- $\beta$ -glucanase	-	2+	4+
Dextran	<i>endo</i> -1,6- $\alpha$ -Dextranase	-	-	-
Amylose	$\alpha$ -amylase	-	-	-
Pullulan	Limit dextran	-	-	-

Activity = relative amount of dye released for each enzyme following a 1 hour incubation at temperature  
Table is meant to demonstrate the substrate range for each enzyme.

## Specific Activity ( $\mu\text{mol}/\text{min mg}^{-1}$ ) of *A. cellulolyticus* Catalytic Domain Enzymes on p-nitrophenol Linked Soluble Substrates

PNP linked substrate	cel5A	cel12A	cel74A
$\beta$ -D glucopyranoside	ND	ND	ND
$\beta$ -D cellobioside	2.61	20.59	ND
$\beta$ -D maltoside	ND	ND	ND
$\beta$ -D xylopyranoside	ND	ND	ND
$\beta$ -D mannopyranoside	ND	ND	ND
$\beta$ -D lactopyranoside	0.117	6.63	ND

ND = not detectable

50 mM acetate, pH 5.0, 1.0 mg/mL substrate

60°C, 30 min

# Conclusions

- Cellulase genes (*cel12a* and *cel74a*) from *Acidothermus cellulolyticus* were cloned by expression screening of a lambda genomic DNA library in *E. coli* using CMC.
- The catalytic domains from *cel 12a* and *cel74a* have been sequenced and expressed in *E. coli*. FASTA analysis of the catalytic domains classified the enzymes as a family 12 endoglucanase and a family 74 avicelase.
- The purified proteins are thermotolerant and their activities on soluble and insoluble dye-linked substrates were compared.

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