

Expression, characterization, and activity optimization of a novel cellulase from the thermophilic bacteria *Cohnella sp.* A01

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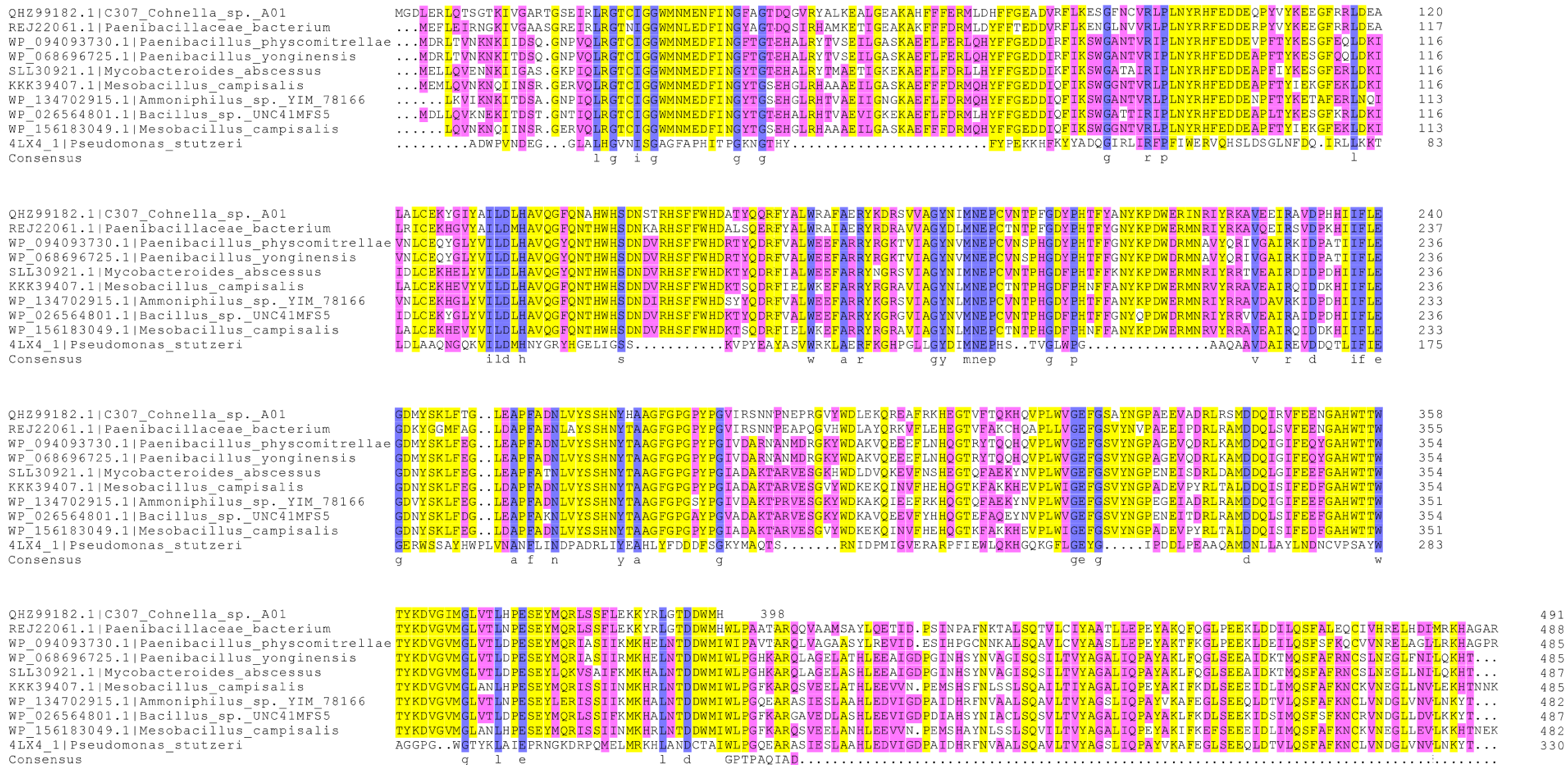


Figure S1. The results of multiple alignments of CelC307 amino acid sequence with some of the well-known cellulases (with the highest similarity) led to identifying the most conserved residues.

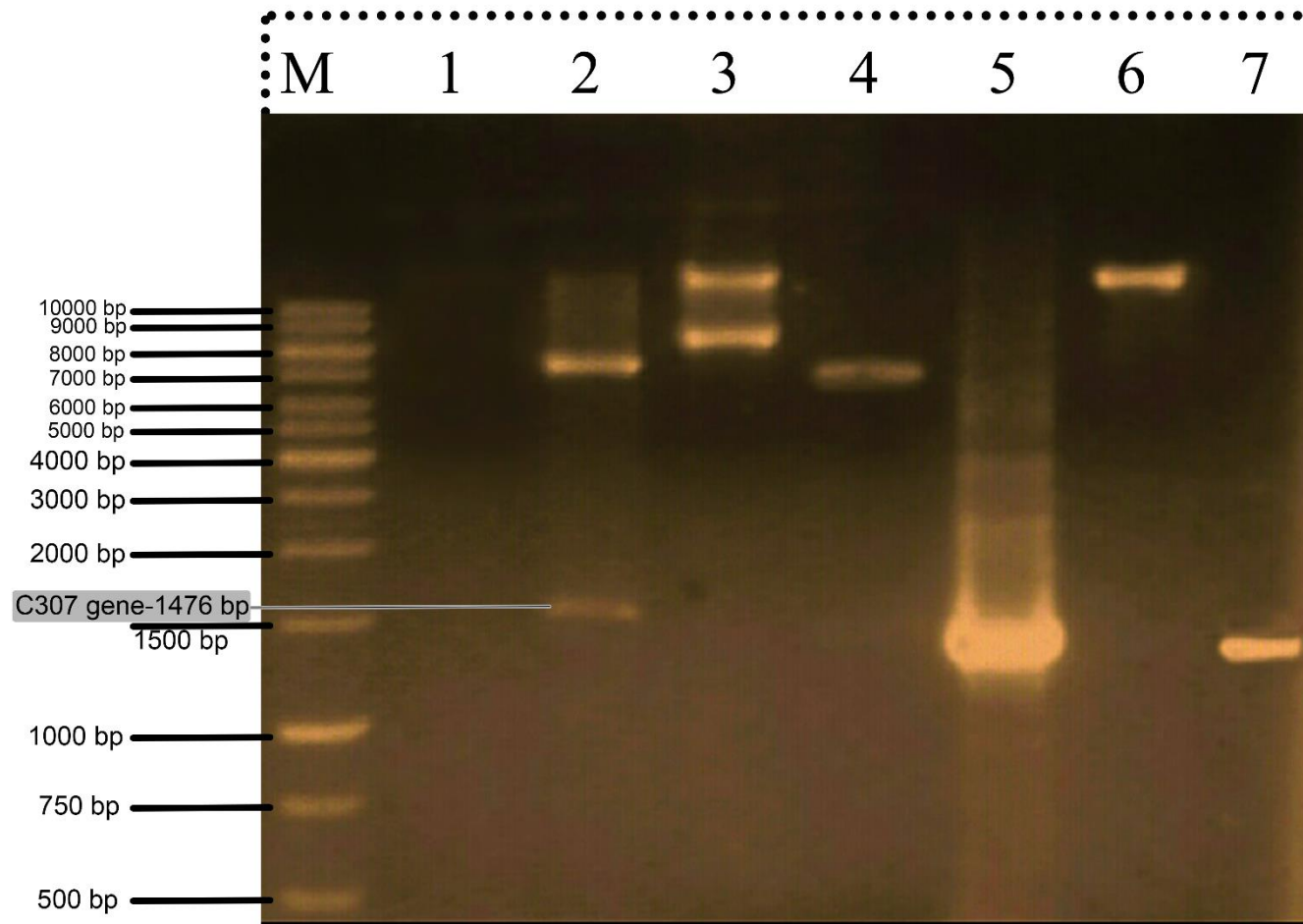


Figure S2. Analysis of cloning of recombinant CelC307. (A) M, DNA marker negative; 1, negative control; 2, Double digestion of recombinant pET26b(+) by *XhoI* and *NdeI* restriction enzyme; 3, empty pET26b(+) vector; 4, digested pET26b(+) vector; 5, PCR from genomic DNA of CelC307 sequence; 6, recombinant pET26b(+) containing CelC307 sequence; 7, PCR from recombinant pET26b(+) vector with specific primers for CelC307 sequence (colony PCR).

Table S1. Table of the Plackett-Burman design of seven factors.

Runs	Variables							Response (U/ml)
	pH	Temp. (°C)	Shack (rpm)	Inoculum conc. (OD₆₀₀)	Time (h)	Yeast extract (g/l)	Trypton (g/l)	
1	8	20	70	0.3	18	7.5	12.5	70
2	8	40	70	1	4	2.5	7.5	15
3	8	20	200	0.3	4	2.5	12.5	45.2
4	8	40	200	0.3	18	7.5	7.5	33.4
5	5	40	200	1	4	7.5	12.5	124.2
6	5	20	200	1	18	2.5	12.5	135
7	5	20	70	1	18	7.5	7.5	108.1
8	8	20	200	1	4	7.5	7.5	52.1
9	8	40	70	1	18	7.5	12.5	38
10	5	40	200	0.3	18	2.5	7.5	39.6
11	5	40	70	0.3	4	7.5	12.5	50.3
12	5	20	70	0.3	4	2.5	7.5	56.2

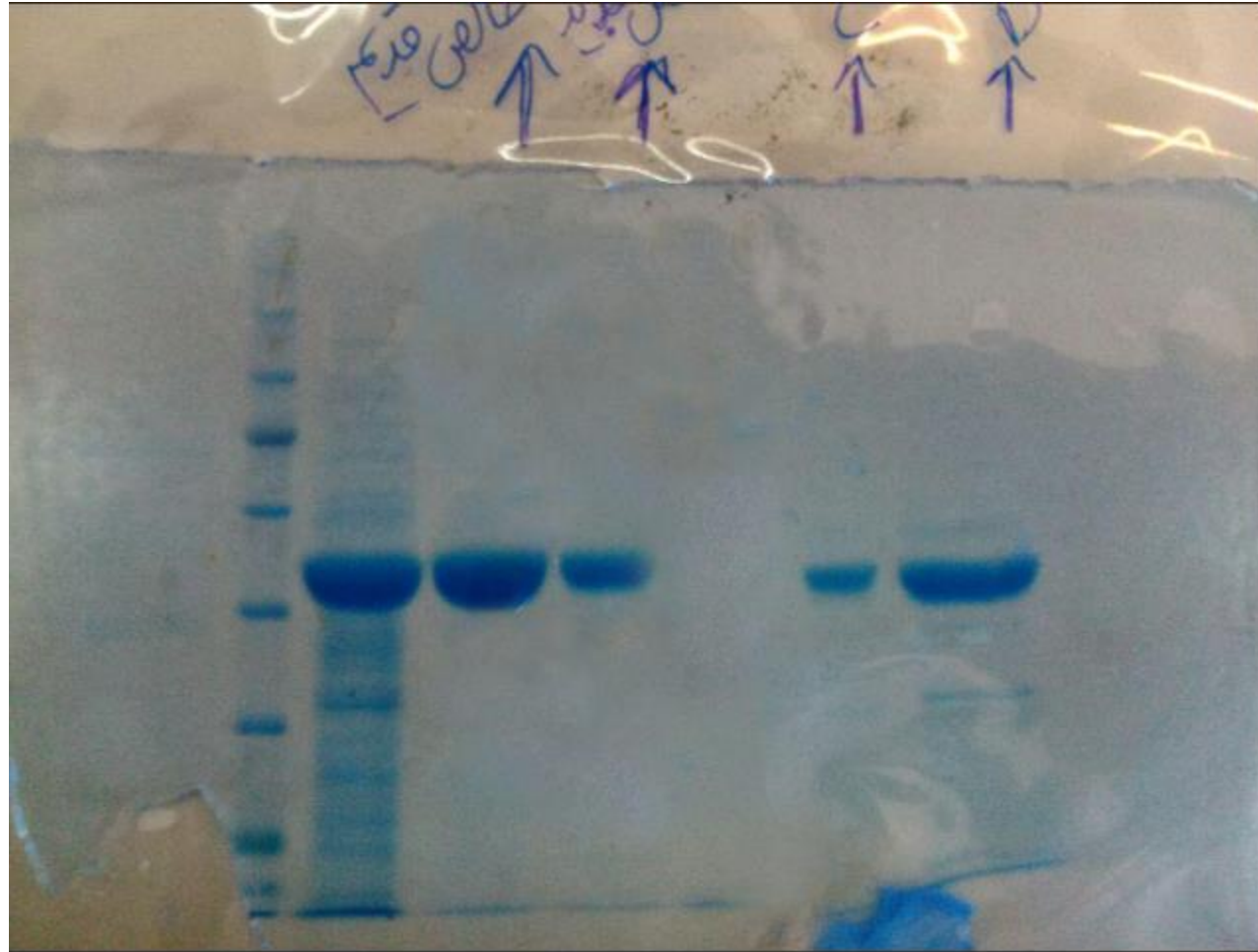
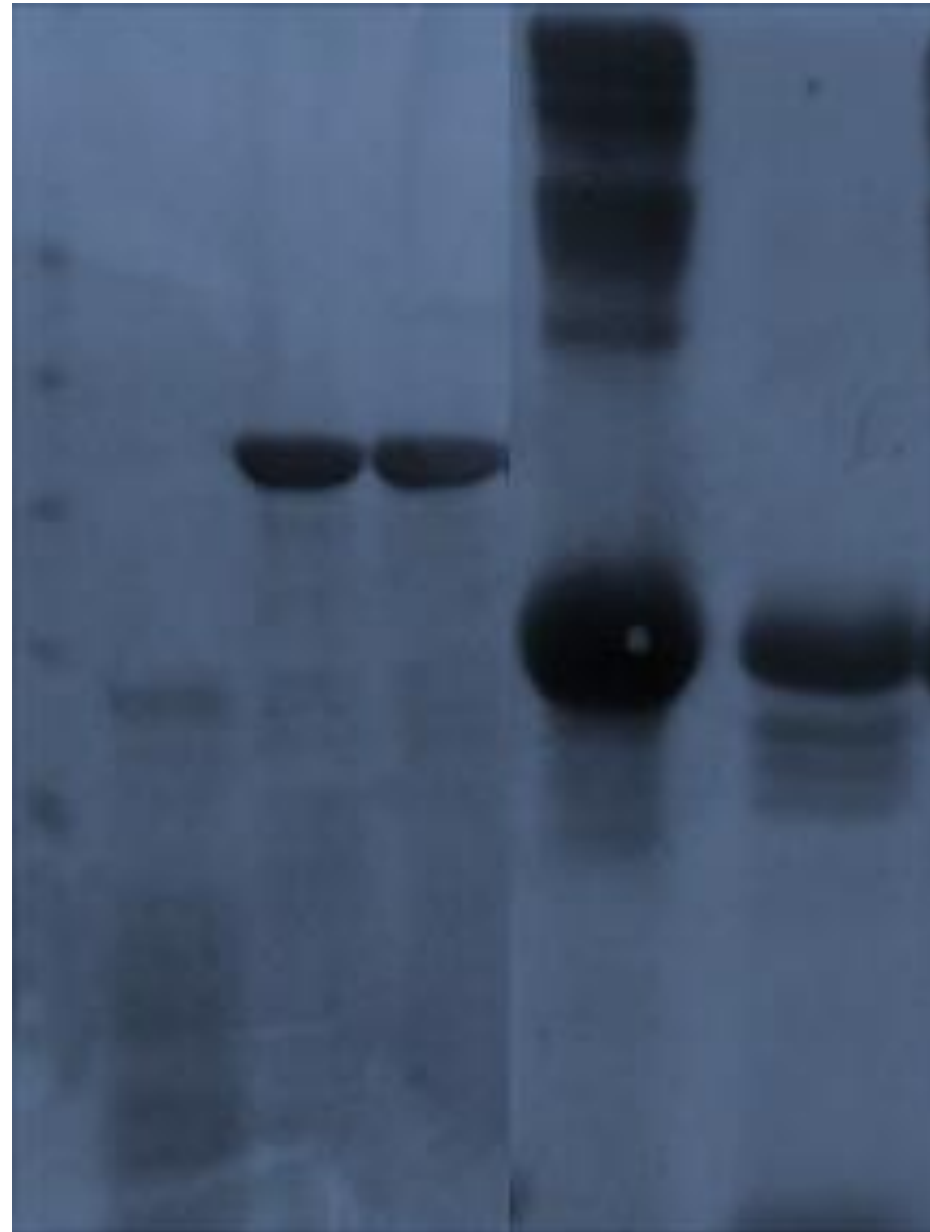


Figure S3. Full-length gel_Expression and Purification



**Figure S4. Full-length gel_Proteolytic digest
resistance**



Figure S5. Full-length gel_Zymography



Figure S6. Full-length picture_Assay