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In silico analysis to elect superior bacterial alkaline protease for detergent and leather industries

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ABSTRACT

Alkaline protease contributes 40% of the total worldwide enzyme sales. Alkaline protease that is stable at very high temperature and pH is massively desirable for detergent industry and leather industry specially in tanning process. So the present study aims to elect superior bacterial alkaline protease (high temperature and pH stable) as compared to the alkaline proteases of currently industrially used bacteria (*Bacillus subtilis* and *Bacillus cereus*). A total of 50 protein sequences of alkaline proteases of different bacteria were analyzed through *in silico* characterization. ProtParam result revealed that isoelectric point and aliphatic index of alkaline protease of *Bacillus megaterium* were 8.83 and 93.35 respectively. In case of alkaline protease of *B. megaterium*, these two properties were significant in comparison to alkaline proteases of industrially used bacteria and other considered bacteria. A common motif of 28 amino acid residues i.e., IQSTYPGEDYEYMSGTSMATPHVAGVAA was found using MEME software in 46 protein sequences. It can be concluded that alkaline protease of *Bacillus megaterium* may be superior to alkaline proteases of industrially used bacteria and other considered bacteria. In addition, obtained common motif indicates its probable role in catalytic function and structure of alkaline proteases.

Indexing terms/Keywords

Alkaline protease, Isoelectric point, Aliphatic index, Common motif.

Academic Discipline And Sub-Disciplines

Biotechnology

SUBJECT CLASSIFICATION

Enzyme selection

TYPE (METHOD/APPROACH)

Computational

INTRODUCTION

Proteolytic enzymes play a specific catalytic role in the hydrolysis of proteins. They are widespread in all living organisms as they are essential for cell growth and differentiation [1]. Proteases are the most important industrial enzymes that perform a wide variety of functions and have various important biotechnological applications [2]. Proteases alone form approximately 60% of the total world-wide enzyme production [3]. Among the various proteases, high alkaline proteases which alone account for about 40% of the total worldwide enzyme sales [4], proved predominantly suitable for industrial uses. This is mainly due to their high stability and activity under harsh conditions. Alkaline proteases are of special interest as they are used in leather processing and manufacturing of detergents, food, pharmaceuticals [5, 6].

Proteases with high activity and stability at high alkaline range and high temperatures are interesting for bioengineering and biotechnological applications [7]. Most of the alkaline proteases that play a role in industries are thermostable as their optimal activity lies between 50^0C to 70^0C [8]. For applications in detergents and tanning processes, alkaline proteases with an optimal temperature of 50–70^0C and an optimal pH of 9-12 are desirable [9, 10, 11].

Microorganisms represent an attractive source of proteases, owing to the distinct advantages they offer over plant and animal proteases [12]. Among them, bacteria are the most dominant group of alkaline protease producers. Currently, a large number of commercially available alkaline proteases are derived from the *Bacillus* species because of their high pH and temperature stability [1]. The protease enzyme from *Bacillus subtilis* is found to contribute in maximum softness in leather [13]. Protease of *Bacillus cereus* used in commercial laundry detergents is found to be superior over the proteases in comparison to the enzyme stability during the washing at higher temperature, e.g., 40–50^0C [14].

As high thermostable and pH stable alkaline proteases are extremely demanding in leather specially in tanning process and detergent industry [9], so the present study performs *in silico* characterization of 50 full length bacterial protease protein sequences representing alkaline protease and alkaline serine protease to elect superior enzyme comparing with alkaline proteases of currently industrially used bacteria.

MATERIALS AND METHODS

A total of 50 protein sequences of alkaline proteases of different bacteria were retrieved from the NCBI (http://www.ncbi.nlm.nih.gov/). The accession numbers of alkaline protease protein sequences along with the source bacteria are listed (see table 1). The protein sequences of alkaline proteases were aligned using ClustalW2 (http://www.ebi.ac.uk/tools/clustalw2). The Molecular Evolution Genetic Analysis (MEGA), version 5.2 was utilized in this study for phylogenetic tree construction using neighbor joining method. The self-optimized prediction method with alignment (SOPMA) tool at ExPASy server was exploited for comparative secondary structure analysis. The MEME (http://meme.nbcr.net/meme/) software was used to elect common motif from the protein sequences. Different physicochemical properties of alkaline proteases were computed using ExPASy'sProtParam tool.

Table 1. List of different bacterial sources of alkaline proteases with respective accession numbers.

Source bacteria	Accession number
Alteromonas sp	2004286A
Vibrio alginolyticus	WP_005395360.1
Aeromonas hydrophila	ACI49707.1
Vibrio parahaemolyticus	WP_015312848.1
Vibrio vulnificus	BAH82872.1
Vibrio cholerae	WP_029628642.1
Colwellia psychrerythraea 34H	AAZ26780.1
Vibrio metschnikovii	CAA82213.1
Sinorhizobium fredii HH103	YP_005192926.1
Thermoactinomyces sp. E79	AAB36499.1
Pseudoalteromonas sp. AS-11	BAB61726.1
Desulfovibrio magneticus RS-1	BAH77090.1
Stigmatella aurantiaca DW4/3-1	EAU64253.1
Shewanella benthica KT99	EDQ01828.1
gamma proteobacterium IMCC3088	EGG29355.1
Streptomyces coelicolor A3(2)	NP_629848.1
Croceibacter atlanticus HTCC2559	YP_003714792.1
Pseudoalteromonas tunicata D2	ZP_01134525.1
Vibrio harveyi HY01	ZP_01984231.1
Streptomyces roseosporus NRRL 15998	ZP_06588036.1
*Bacteroidesdorei*5_1_36/D4	ZP_08795757.1
Bacillus sp.	CAE51830.1
Bacillus alcalophilus	AAA22212.1
Bacillus licheniformis	AEU12640.1
Bacillus subtilis	AGV98709.1
Bacillus clausii	ABI26631.1
Bacillus cereus	KGT43836.1
Bacillus megaterium	WP_014462137.1
Bacillus sp. Y	BAD36788.1
Bacillus circulans	AEQ76892.1
Bacillus lehensis	AFK08970.1

Bacillus sp. B001	ADK62564.1
*Bacillus lehensis*G1	AIC93003.1
Bacillus sp. YAB	P20724.1
Bacillus amyloliquefaciens	WP_020955853.1
Bacillus gibsonii	CAE48424.15
Geobacillus stearothermophilus	AAK29176.1
Thermoactinomyces sp. Gus2-1	KFZ40693.1
Streptomyces albulus	AIA06556.1
Streptomyces fulvissimus	WP_015612234.1
Shewanella oneidensis MR-1	NP_718668.1
Vibrio parahaemolyticus 10329	EGF40976.1
Vibrio parahaemolyticus AQ3810	ZP_01989749.1
Bacillus clausii KSM-K16	YP_174261.1
Pseudomonas aeruginosa	AAX84042.1
Streptomyces sp.	CAA52206.1
Staphylococcus massiliensis S46	EKU45981.1
Brachyspira pilosicoli	WP_015274969.1
Bacillus pumilus	BAE79641.1
Pseudomonas fluorescens	BAA36461.1

RESULTS AND DISCUSSION

The 50 protein sequences of alkaline proteases retrieved from NCBI were characterized for biochemical features, multiple sequence alignment, phylogenetic tree construction, common motif and secondary structure analysis using various computational tools.

Multiple sequence alignment by ClustalW2 represents significant alignment pattern (see figure 1). The multiple sequence alignment of these protein sequences exposed a range of similarity score of 77-98% among WP_005395360.1, WP_015312848.1, BAH82872.1, ZP_01984231.1, EGF40976.1 and ZP_01989749.1. 81-98% similarity score was revealed by YP_174261.1 with CAE51830.1, AAA22212.1, AFK08970.1 and P20724.1. AFK08970.1 revealed a range of similarity score of 80.25-98.94% with AAA22212.1 and P20724.1. The protein sequence of WP_020955853.1 showed a range of similarity score of 70.36-81.45% with AEQ76892.1 and AGV98709.1. 91.25% similarity score was found between ZP_06588036.1 and WP_015612234.1. In addition, the remaining similarity scores of protein sequences were very low (below 30%).

A total of 50 alkaline protease sequences were subjected to phylogenetic tree construction by neighbor joining method (see figure 2). Through phylogenetic tree analysis, it was found that *Bacillus cereus* (KGT43836.1) along with AAB36499.1, KFZ40693.1, AAK29176.1 and WP_014462137.1 positioned in same cluster. Besides, *Bacillus subtilis* (AGV98709.1) along with AEQ76892.1 and WP_020955853.1 located in same cluster. Therefore, properties of alkaline proteases of AAB36499.1, KFZ40693.1, AAK29176.1, WP_014462137.1 and *Bacillus cereus* (KGT43836.1) may be similar. Also, alkaline protease of *Bacillus subtilis*(AGV98709.1) may show similarity in properties with AEQ76892.1and WP_020955853.1.

Secondary structure analysis was done using SOPMA software (see table 2). The secondary structure designates whether a given amino acid lies in a helix, strand or coil [15, 16]. The secondary structure prediction indicated that random coil was dominant in all the alkaline proteases except CAE51830.1, AAA22212.1, AEU12640.1, AGV98709.1, CAE48424.1, AFK08970.1, AIC93003.1, P20724.1, WP_015274969.1 and KFZ40693.1 in which α-helix was dominant. In case of all the alkaline proteases, it was clearly noticed that β-turns showed less percentage of conformation (below 16%). Extended strands were ranging from 16.15-30.04% in all the alkaline proteases sequences.

The common motif of 28 amino acids found in 46 protein sequences is shown with green color in alignment (see figure 1). Motif analysis of different alkaline proteases using MEME with a maximum of ten motif hits presented that motif-1 of 28 amino acid residues, i.e., IQSTYPGEDYEYMSGTSMATPHVAGVAA was uniformly distributed in 46 protein sequences. As the similarity scores obtained from ClustalW2 result were very low in most of the protein sequences, so the resulting common motif may be responsible for catalytic function and structure of the alkaline proteases.

Physicochemical properties of alkaline proteases determined by using ProtParam tool (see table 3). The physicochemical properties showed that molecular weight was highest in NP_718668.1 (84386.2 Da) and lowest in ABI26631.1 (38106.6 Da). Secondary structure analysis exhibits that the instability index is used to measure in vivo half-life of a protein [17]. The proteins which have been reported as in vivo half-life of less than 5 h showed instability index greater than 40, whereas those having more than 16 h half-life [18] have instability index of less than 40. Instability index of all the protein sequences was found less than 40 except BAH77090.1, EGG29355.1 and NP_629848.1.

The Grand Average hydropathy (GRAVY) indices of all alkaline proteases were ranging from -0.437 to -0.002 except BAH77090.1, YP_003714792.1, CAE51830.1, AEU12640.1, AFK08970.1 and P20724.1. This low range of value indicates the likelihood of better interaction with water [19]. As a result, in industrial sector the extraction of protease is easy since it does not bind to hydrophobic membrane.

Isoelectric point (pI) is the pH at which the surface of protein is covered with charge but net charge of the protein is zero. Proteases from alkalophilic *Bacillus* sp. with very high isoelectric points (pI) can withstand higher pH ranges [20]. Isoelectric points of alkaline proteases of *Bacillus megaterium* (WP_014462137.1), *Bacillus licheniformis* (AEU12640.1), *Bacillus pumilus, Bacillus cereus* (KGT43836.1), *Staphylococcus massiliensis* S46 (EKU45981.1), *Aeromonas hydrophila* (ACI49707.1), *Vibrio cholerae* (WP_029628642.1) and *Bacillus subtilis* (AGV98709.1) were found 8.83, 8.90, 8.67, 7.72, 7.78, 6.65, 6.27 and 6.08 respectively. Isoelectric points of remaining protein sequences were found below 6.08. On the basis of isoelectric point, alkaline proteases of *Bacillus megaterium*, *Bacillus pumilus* and *Bacillus licheniformis* can provide stability at high pH.

Aliphatic index of protein measures the relative volume occupied by aliphatic side chains of the amino acids (Alanine, valine, leucine and isoleucine). Globular proteins with high aliphatic index have high thermostability and an increase in aliphatic index increases protein thermostability [21, 22]. From our analysis, it was observed that very high aliphatic index for the alkaline proteases of *Bacillus megaterium* (WP_014462137.1), *Bacillus gibsonii* (CAE48424.15), *Bacillus alcalophilus* (AAA22212.1), *Bacillus clausii* KSM-K16 (YP_174261.1), *Brachyspira pilosicoli* and *gamma proteobacterium* IMCC3088 (EGG29355.1) was found 93.35, 91.93, 90.68, 91.18, 92.08 and 93.81. Rest of the alkaline proteases revealed the aliphatic index with a range of 66.22-88.67. Higher aliphatic index of alkaline proteases of *Bacillus megaterium* and *gamma proteobacterium* IMCC3088 can enhance their constancy at high thermal condition.

The isoelectric point of alkaline protease of *Bacillus licheniformis*was higher but aliphatic index was lower as compared to alkaline protease of *Bacillus megaterium* while alkaline protease of *gamma proteobacterium* IMCC3088 provided reciprocal measure for these parameters. So, considering both the parameters we found that alkaline protease of *Bacillus megaterium* was comparatively significant. In addition, experimental data demonstrated that 100% stability of alkaline protease from *Bacillus subtilis* was in the temperature range of 35–55^{0}C [23] and at pH 7.4 [24]. The protease from *Bacillus cereus* exhibited 100% activity at temperature 60^{0}C and maintained over 80% of its original activity between pH 8 and 11 [25]. On the other hand, the protease from *Bacillus megaterium* showed 100% activity in the temperature up to 80^{0}C and good stability (~95%) in the pH range of 7.0–8.5, with 100% stability at pH 7.5 [26].

Moreover, the microbes which have rapid growth are preferred as sources of proteases [27]. Faster growth of bacteria depends on short generation time [28]. The generation time of *Bacillus megaterium* is 25 min. [29] that is shorter than the generation time of 28-36 min. and 120 min. of *Bacillus cerus* and *Bacillus subtilis* respectively [30, 31]. So, from the present study and the experimental data, we can state that the alkaline protease of *Bacillus megaterium* may be promising in harsh conditions.

Table 2. Secondary structure of alkaline proteases.

Serial no.	Accession No	Alpha helix (Hh) (%)	Extended strand (Ee) (%)	Beta turn (Tt) (%)	Random coil (Cc) (%)
1	2004286A	19.32%	29.63%	11.43%	39.61%
2	WP_005395360.1	22.75%	25.85%	9.45%	41.95%
3	ACI49707.1	34.27%	20.72%	7.16%	37.85%
4	WP_015312848.1	23.04%	26.00%	10.19%	40.77%
5	BAH82872.1	26.85%	26.41%	11.13%	35.61%
6	WP_029628642.1	22.71%	30.04%	11.72%	35.53%

7	AAZ26780.1	24.14%	28.90%	13.14%	33.83%
8	CAA82213.1	28.88%	21.76%	10.97%	38.39%
9	YP_005192926.1	29.42%	16.15%	9.62%	44.81%
10	AAB36499.1	29.43%	23.18%	13.54%	33.85%
11	BAB61726.1	18.28%	27.82%	8.90%	44.99%
12	BAH77090.1	29.08%	18.73%	10.56%	41.63%
13	EAU64253.1	33.28%	18.94%	9.56%	38.23%
14	EDQ01828.1	20.36%	29.01%	14.59%	36.04%
15	EGG29355.1	32.66%	20.28%	9.44%	37.62%
16	NP_629848.1	27.12%	20.10%	8.23%	44.55%
17	YP_003714792.1	26.47%	26.47%	11.03%	36.03%
18	ZP_01134525.1	24.39%	27.55%	11.48%	36.59%
19	ZP_01984231.1	22.12%	28.07%	12.26%	37.55%
20	ZP_06588036.1	27.00%	22.00%	9.25%	41.75%
21	ZP_08795757.1	27.84%	26.42%	11.70%	34.04%
22	CAE51830.1	34.76%	21.12%	12.83%	31.28
23	AAA22212.1	37.63%	18.68%	9.74%	33.95%
24	AEU12640.1	32.72%	25.07%	11.08%	31.13%
25	AGV98709.1	36.08%	18.71%	10.69%	34.52%
26	ABI26631.1	28.81%	24.10%	13.85%	33.24%

27	KGT43836.1	23.93%	24.18%	13.35%	38.54%
28	WP_014462137.1	25.30%	24.10%	10.60%	40.00%
29	CAE48424.1	36.29%	24.28%	10.44%	28.98%
30	AEQ76892.1	34.16%	19.46%	10.41%	35.97%
31	AFK08970.1	33.07%	20.90%	13.76%	32.28%
32	ADK62564.1	33.60%	21.33%	10.40%	34.67%
33	AIC93003.1	34.67%	21.60%	13.60%	30.13%
34	P20724.1	33.86%	21.16%	13.49%	31.48%
35	WP_020955853.1	28.05%	21.27%	12.44%	38.24%
36	BAD36788.1	24.06%	27.57%	13.03%	35.34%
37	AAK29176.1	28.43%	27.68%	14.46%	29.43%
38	KFZ40693.1	37.02%	18.25%	12.60%	32.13%
39	AIA06556.1	27.79%	20.84%	8.68%	42.68%
40	WP_015612234.1	30.50%	20.75%	10.25%	38.50%
41	NP_718668.1	22.06%	25.40%	10.41%	42.13%
42	EGF40976.1	23.04%	26.14%	10.19%	40.62%
43	ZP_01989749.1	23.04%	26.00%	10.19%	40.77%
44	YP_174261.1	33.95%	20.26%	10.53%	35.26%
45	WP_015274969.1	40.64%	19.66%	9.64%	30.06%
46	AAX84042.1	31.52%	23.17%	12.73%	32.57%
47	CAA52206.1	29.12%	25.52%	10.31%	35.05%

48	BAA36461.1	21.99%	30.08%	11.00%	36.93%
49	BAE79641.1	25.85%	22.72%	14.62%	36.81%
50	EKU45981.1	26.40%	20.56%	11.93%	41.12%

Table 3. Physicochemical properties of alkaline proteases of different bacterial sources.

Source bacteria	Accession number	Number of amino acids	Moleclar weight	Theoretical pI	Total number of negatively charged residues (Asp + Glu)	Total number of positively charged residues (Arg + Lys)	Instability index	Aliphatic index	Grand average of hydropathicity
Alteromonas sp.	2004286A	621	63962.0	4.60	52	31	32.06	76.99	-0.183
Vibrio alginolyticus	WP_005395360.1	677	71225.0	4.59	76	41	29.87	75.33	-0.287
Aeromonas hydrophila	ACI49707.1	391	40075.0	6.65	26	25	31.68	85.91	-0.026
Vibrio parahaemolytics	WP_015312848.1	677	71023.2	4.82	70	43	26.99	77.95	-0.217
Vibrio vulnificus	BAH82872.1	674	70860.3	4.93	68	42	27.32	81.62	-0.167
Vibrio cholerae	WP_029628642.1	546	58484.0	6.27	50	47	31.18	76.67	-0.380
Colwellia psychrerythraea 34H	AAZ26780.1	609	62536.8	4.50	66	38	23.27	70.43	-0.154
Vibrio metschnikovii	CAA82213.1	547	58997.1	5.80	49	41	29.34	83.86	-0.214
Sinorhizobium fredii HH103	YP_005192926.1	520	55356.2	5.85	56	49	34.59	79.54	-0.259
Thermoactinomyces sp. E79	AAB36499.1	384	40132.5	6.04	25	21	32.99	78.57	-0.124
Pseudoalteromonas sp. AS-11	BAB61726.1	629	65189.5	4.55	51	29	35.39	72.18	-0.198
Desulfovibrio magneticus RS-1	BAH77090.1	502	51915.0	5.71	40	33	43.58	84.16	0.038
Stigmatella aurantiaca DW4/3-1	EAU64253.1	586	60586.7	5.03	64	45	23.94	84.66	-0.049

Shewanella benthica KT99	EDQ01828.1	555	56409.9	4.31	58	26	22.16	66.22	-0.116
gamma proteobacterium IMCC3088	EGG29355.1	646	70282.8	4.80	81	46	40.56	93.81	-0.226
Streptomyces coelicolor A3(2)	NP_629848.1	413	42712.8	5.00	51	40	40.09	83.85	-0.155
Croceibacter atlanticus HTCC2559	YP_003714792.1	408	42170.1	4.58	41	23	26.29	86.57	0.048
Pseudoalteromonas tunicate D2	ZP_01134525.1	697	73527.3	4.98	55	39	18.46	66.54	-0.373
Vibrio harveyi HY01	ZP_01984231.1	791	82334.1	4.62	82	45	28.16	75.95	-0.223
Streptomyces roseosporus NRRL 15998	ZP_06588036.1	400	40400.1	4.52	52	27	21.81	75.30	-0.202
Bacteroidesdorei 5_1_36/D4	ZP_08795757.1	564	62292.0	4.81	71	48	36.61	81.38	-0.271
Bacillus sp.	CAE51830.1	374	38286.5	4.60	35	17	24.75	88.48	0.040
Bacillus alcalophilus	AAA22212.1	380	38853.0	4.68	38	18	33.80	90.68	-0.004
Bacillus licheniformis	AEU12640.1	379	38774.7	8.90	27	31	13.00	82.96	0.033
Bacillus subtilis	AGV98709.1	449	49587.9	6.08	57	53	32.87	68.40	-0.432
Bacillus clausii	ABI26631.1	361	38106.6	6.07	31	26	32.91	88.67	-0.150
Bacillus cereus	KGT43836.1	397	42333.8	7.72	30	31	19.28	74.71	-0.341
Bacillus megaterium	WP_014462137.1	415	45011.0	8.83	29	34	27.84	93.35	-0.094
Bacillus gibsonii	CAE48424.15	383	39976.3	4.53	45	22	30.09	91.93	-0.028
Bacillus circulans	AEQ76892.1	442	47857.8	5.14	58	43	35.87	79.41	-0.365
Bacillus lehensis	AFK08970.1	378	38804.2	4.72	35	19	26.08	87.54	0.025
Bacillus sp. B001	ADK62564.1	375	38634.1	4.00	56	15	36.97	84.08	-0.144
*Bacillus lehensis*G1	AIC93003.1	375	38694.3	4.09	55	15	34.83	84.59	-0.129
Bacillus sp.YAB	P20724.1	378	38793.1	4.66	35	19	26.01	87.54	0.014

Bacillus amyloliquefaciens	WP_020955853.1	442	48089.3	5.98	54	48	31.85	80.07	-0.360
Bacillus sp.Y	BAD36788.1	798	84313.4	4.65	94	54	19.88	82.12	-0.105
Geobacillus stearothermophilus	AAK29176.1	401	42814.5	4.77	44	27	19.96	83.22	-0.159
Thermoactinomyces sp.Gus2-1	KFZ40693.1	389	40445.8	4.93	38	24	25.26	80.82	-0.192
Streptomyces albulus	AIA06556.1	403	41088.3	5.88	49	42	19.26	72.95	-0.352
Streptomyces fulvissimus	WP_015612234.1	400	40360.0	4.58	49	27	19.61	72.12	-0.207
Shewanella oneidensis MR-1	NP_718668.1	807	84386.2	5.57	64	51	26.84	73.52	-0.270
Vibrio parahaemolyticus 10329	EGF40976.1	677	71045.2	4.82	70	43	27.44	77.65	-0.224
Vibrio parahaemolyticus AQ3810	ZP_01989749.1	677	71039.2	4.82	70	43	27.40	77.80	-0.221
Bacillus clausii KSM-K16	YP_174261.1	380	38881.1	4.67	38	18	34.71	91.18	-0.002
Pseudomonas aeruginosa	AAX84042.1	479	50416.9	4.28	55	25	18.72	77.52	-0.217
Streptomyces sp.	CAA52206.1	388	39566.5	4.59	39	20	29.83	77.47	-0.020
Staphylococcus massiliensis S46	EKU45981.1	394	41957.7	7.78	37	38	22.13	73.02	-0.437
Brachyspirapilosicoli	WP_015274969.1	529	57931.4	4.89	76	53	38.63	92.08	-0.215
Bacillus pumilus	BAE79641.1	383	39450.1	8.67	25	28	26.34	80.81	-0.123
Pseudomonas fluorescens	BAA36461.1	482	50223.4	4.64	48	28	7.25	65.06	-0.334

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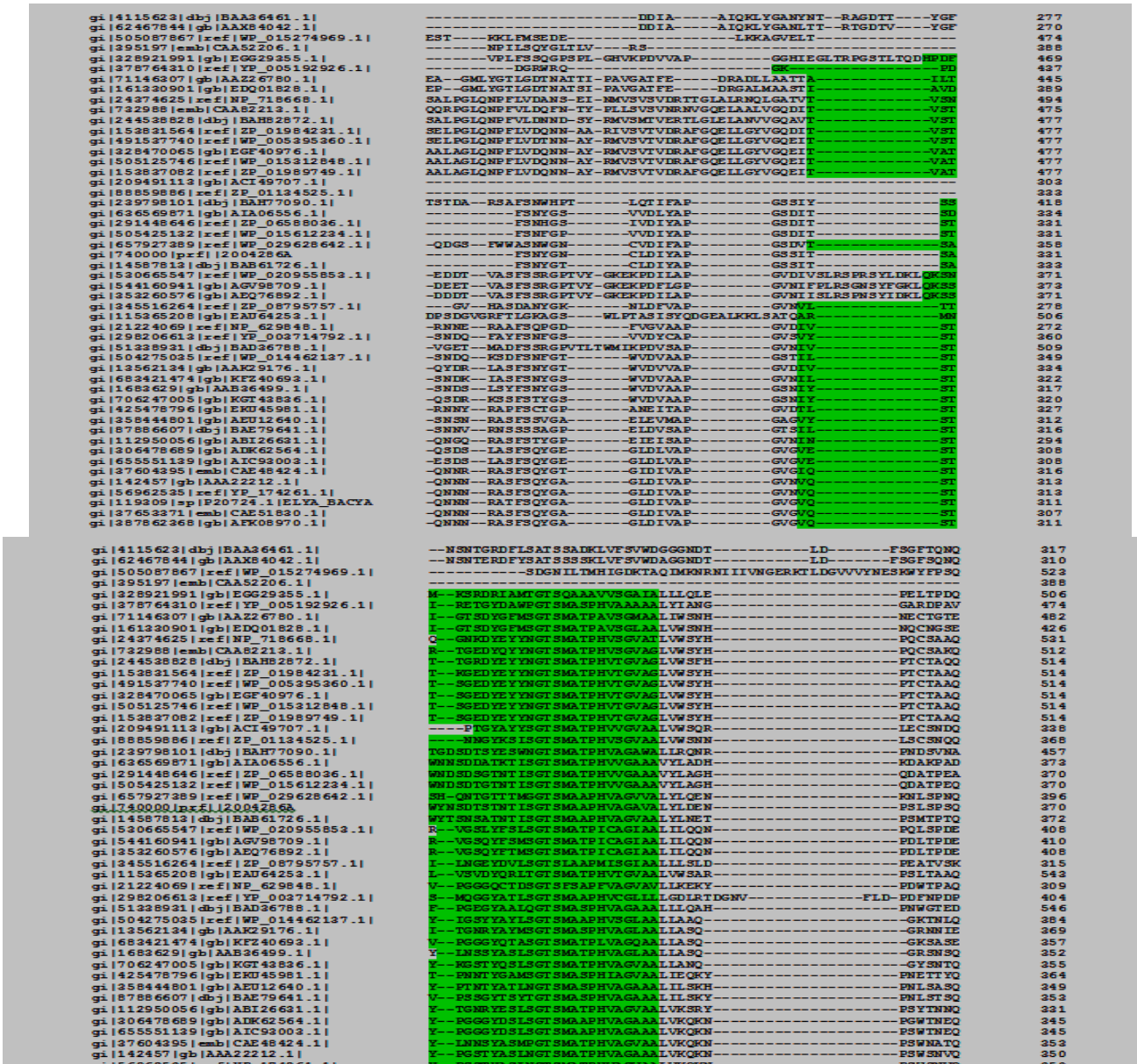

Fig 1: Multiple sequence alignment by ClustalW2, showing common motif with green color.

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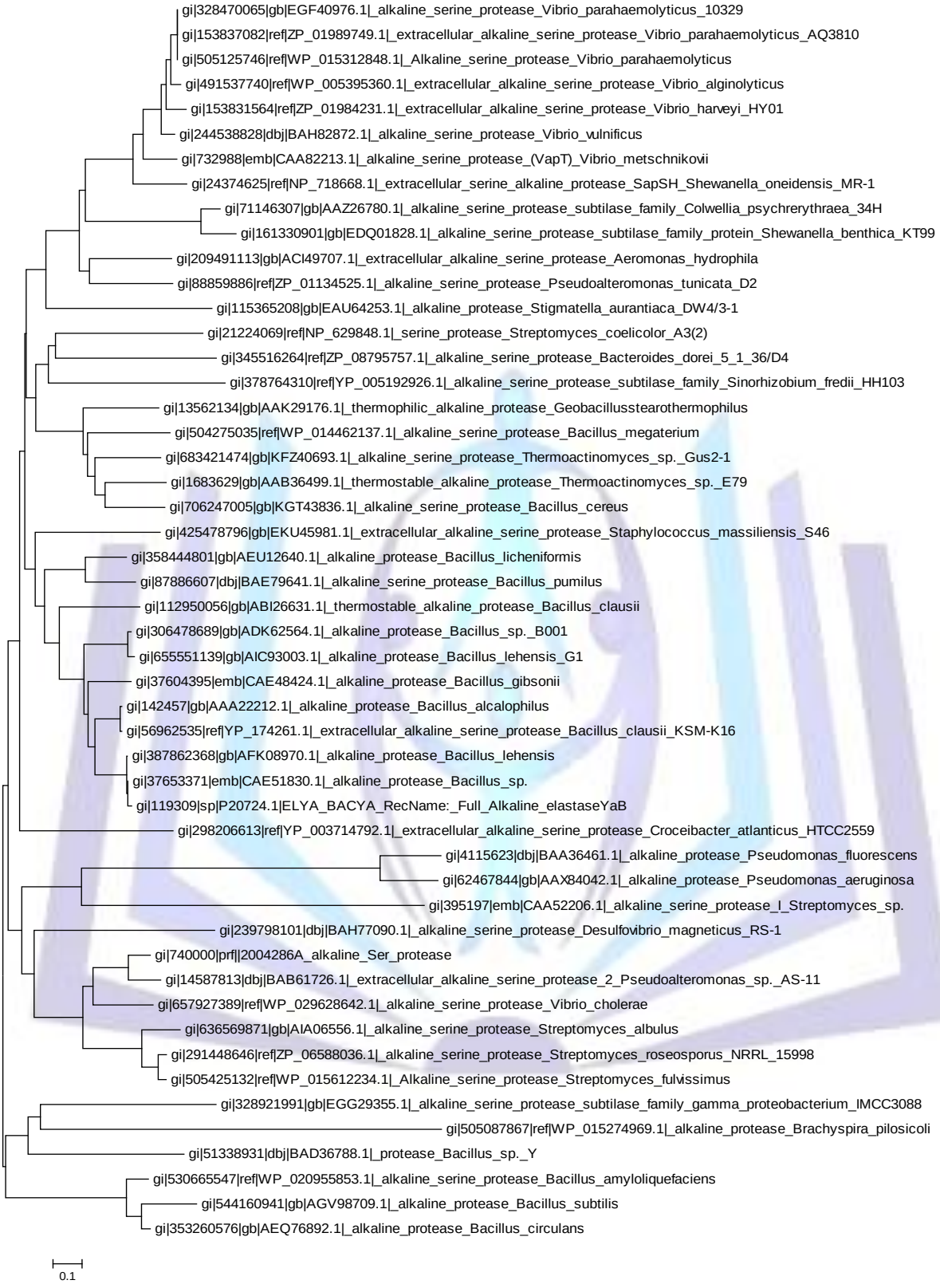

Fig 2: Phylogenetic tree constructed by NJ method based on alkaline protease protein sequences. Proteases of *Bacillus cereus, Thermoactinomyces* sp*, Bacillus_megaterium* and *Geobacillus stearothermophilus* are in one cluster. Also, another one cluster contains *Bacillus subtilis* and *Bacillus circulans.* Alkaline proteases located in one cluster, may show similarity in their properties.

CONCLUSION

As a final point we can say that alkaline protease of *Bacillus megaterium* may be superior to alkaline proteases of industrially used bacteria and other considered bacteria in view of the industrially relevant factors (high temperature and pH stability). Therefore, further studies need to be carried out for applying the selected alkaline protease in detergent and leather industries. In addition, another finding of this study is a common motif in 46 protein sequences. So, further research is required to determine the exact role of this common motif in catalytic activity and structure of the alkaline proteases. Besides, this common motif may be used for designing degenerate primers or probes for PCR-based amplification or hybridization-based detection of alkaline protease sequences from different organisms.

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