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., IQSTYPGYMSGTSMATPHVAGVAA 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 worldwide 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°C to 70°C [8]. For applications in detergents and tanning processes, alkaline proteases with an optimal temperature of 50–70°C 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°C [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's ProtParam tool.

| 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 metchnikovii      | CAAB2213.1           |
| 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 | EDO01828.1           |
| gamma proteobacterium IMCC3088 | EGG29355.1        |
| Streptomyces coelicolor A3(2) | NP_629848.1         |
| Croceibacter atlanticus HTCC2559 | YP_00314792.1   |
| Pseudoalteromonas tunicata D2 | ZP_0113525.1      |
| Vibrio harveyi HY01      | ZP_01984231.1       |
| Streptomyces roseosporus NRRL 15998 | ZP_06588036.1 |
| Bacteroides dorei5_1_38/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           |
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 AEQ76892.1 and 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, AIA22212.1, 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 ABL26331.1 (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 (pl) 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 (pl) 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 hydrophilia (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]. Alkaline proteases of Bacillus megaterium (WP_014462137.1), Bacillus gibsonii (CAE48242.15), Bacillus alcalophilus (AAA22212.1), Bacillus clausii KSM-K16 (YP_174261.1), Brachyspira pilosicoli and gamma proteobacterium IMCC3088 (EGG29355.1) were 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°C [23] and at pH 7.4 [24]. The protease from Bacillus cereus exhibited 100% activity at temperature 60°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°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 cereus 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%              |
|   | Protein ID   | % Similarity | % Identity | % Query Coverage | % Template Coverage |
|---|--------------|--------------|------------|------------------|---------------------|
| 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% |
Table 3. Physicochemical properties of alkaline proteases of different bacterial sources.

| Source bacteria          | Accession number | Number of amino acids | Molecular 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_00539 5360.1   | 677                   | 71225.0          | 4.59           | 76                                                      | 41                                                      | 29.87             | 75.33          | -0.287                          |
| Aeromonas hydrophila     | ACl49707.1        | 391                   | 40075.0          | 6.65           | 26                                                      | 25                                                      | 31.68             | 85.91          | -0.026                          |
| Vibrio parahaemolyticus  | WP_01531 2848.1   | 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_02962 8642.1   | 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 metschinkovii     | CAA82213.1        | 547                   | 58997.1          | 5.80           | 49                                                      | 41                                                      | 29.34             | 83.86          | -0.214                          |
| Sinozobium fredii HH103  | YP_005192 926.1   | 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                          |
| Species                          | Accession Number | E Values | Physiological Temperature | Physical Characteristics |
|---------------------------------|------------------|----------|--------------------------|--------------------------|
| **Shewanella benthica KT99**    | EDQ01828.1       | 555      | 56409.9                  | 4.31                     |
| **gamma proteobacterium**       | EGG29355.1       | 646      | 70282.8                  | 4.60                     |
| **Streptomyces coelicolor A3(2)**| NP_629848.1      | 413      | 42712.8                  | 5.00                     |
| **Croceibacter atlanticus**     | YP_003714.1      | 408      | 42170.1                  | 4.58                     |
| **Pseudoalteromonas tunicate D2**| ZP_011345.25    | 697      | 73527.3                  | 4.98                     |
| **Vibrio harveyi HY01**          | ZP_019842.31     | 791      | 82334.1                  | 4.62                     |
| **Streptomyces roseosporus NRRL 15998** | ZP_065880.36    | 400      | 40400.1                  | 4.52                     |
| **Bacteroidesdorei 5_1_36/D4**  | ZP_087957.57     | 564      | 62292.0                  | 4.81                     |
| **Bacillus sp.**                | CAE51830.1       | 374      | 38286.5                  | 4.60                     |
| **Bacillus alcalophilus**        | AAA22212.1       | 380      | 38853.0                  | 4.68                     |
| **Bacillus licheniformis**       | AEU12640.1       | 379      | 38774.7                  | 8.90                     |
| **Bacillus subtilis**            | AGV98709.1       | 449      | 49587.9                  | 6.08                     |
| **Bacillus clausii**             | ABI26631.1       | 361      | 38106.6                  | 6.07                     |
| **Bacillus cereus**              | KGT43836.1       | 397      | 42333.8                  | 7.72                     |
| **Bacillus megaterium**          | WP_014462137.1   | 415      | 45011.0                  | 8.83                     |
| **Bacillus gibsonii**            | CAE48424.15      | 383      | 39976.3                  | 4.53                     |
| **Bacillus circulans**           | AEQ76892.1       | 442      | 47857.8                  | 5.14                     |
| **Bacillus lehensis**            | AFK08970.1       | 378      | 38804.2                  | 4.72                     |
| **Bacillus sp. B001**            | ADK62564.1       | 375      | 38634.1                  | 4.00                     |
| **Bacillus lehensisG1**          | AIC93003.1       | 375      | 38684.3                  | 4.09                     |
| **Bacillus sp.YAB**              | P20724.1         | 378      | 38793.1                  | 4.66                     |
| Organism                                | Accession | 442 | 48089.3 | 5.98 | 54 | 48 | 31.85 | 80.07 | -0.360 |
|----------------------------------------|-----------|-----|---------|------|----|----|-------|-------|--------|
| Bacillus amyloliquefaciens             | WP_02095  |    |         |      |    |    |       |       |        |
|                                        | 5853.1    | 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 |
| Thermocatninomyces sp.Gus2-1           | KFZ40693.1|389 | 40445.8 | 4.93 | 38 | 24 | 25.26 | 80.82 | -0.192 |
| Streptomyces albus                      | AIA06556.1|403 | 41088.3 | 5.88 | 49 | 42 | 19.26 | 72.95 | -0.352 |
| Streptomyces fulvissimus                | WP_01561  | 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 K16            | ZP_019897.49.1|677 | 71039.2 | 4.82 | 70 | 43 | 27.40 | 77.80 | -0.221 |
| Bacillus clausii KSM-K16               | WP_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 |
| Brachyspirapiloscoli                   | WP_01527  | 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 |
Fig 1: Multiple sequence alignment by ClustalW2, showing common motif with green color.
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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