Amino acid frequency and domain features serve well for random forest based classification of thermophilic and mesophilic protein; a case study on serine proteases

Jithin S. Sunny*, Lilly M. Saleena*

*Department of Biotechnology, SRM Institute of Science and Technology, SRM Nagar, Kattankulathur, 603203, Kanchipuram, Chennai TN, India

*Corresponding author: saleenam@srmist.edu.in

Abstract

Thermostability is an important prerequisite for enzymes employed for industrial applications. Several machine learning based models have thus been formulated for protein classification based on this particular trait. These models have employed features derived from sequences, structures or both resulting in a >93% accuracy based on a 10-fold cross-validation. Besides using various proteins from a wide range of organisms, such studies also rely on hundreds of features. In the present study, an enzyme specific classification model was created using significantly less number of features that provides a similar accuracy of classification for thermophilic and non-thermophilic enzyme serine proteases. For building the classifier, 219 thermophilic and 200 mesophilic bacterial genomes were mined for their respective serine protease sequences. Features were extracted for 800 sequences followed by feature selection. We deployed a random forest based classifier that identified thermophilic and non-thermophilic serine proteases with an accuracy of 95.71%. Knowledge of thermostability along with amino acid positional shifts can be vital for downstream protein engineering techniques. Thus, to emphasize the real time application of the enzyme specific classification model, a web platform has been designed. Combining the sequence data and the classification model, this prototype can allow users to align their query serine protease sequence against the custom database and identify its thermophilic nature.

Keywords: Serine protease, Thermophilic, Random Forest, Web tool
1. Introduction

Data-driven approaches for screening specific enzyme properties are being constantly developed. These methods require information derived from sequences such as co-evolution, substitutions, etc., to develop an accurate predictive model [1]. This method of quantifying the behavior of the data in-hand can be undertaken by machine learning (ML). A ML approach uses knowledge from the information-rich protein sequences and guide targeted protein engineering endeavors. Naturally found enzymes or wild type enzymes as they are known, function optimally in their actual environment. This limits their use in specific applications desired for industrial or scientific purposes. A combined approach involving ML and experimental techniques can be applied to address this limitation. Two prominent experimental strategies are rational design and directed evolution [2]. While directed evolution requires high throughput screening which in-turn demands large resources and intensive time scales, rational design is limited by the large gap that currently exists between sequence and structure knowledge availability. By combining ML based predictions, valuable prerequisites can be included in the above two enzymes engineering approaches.

The predictions that ML based approaches provide are based on several enzyme properties amongst which thermostability is of prime importance [3]. An enzyme is primarily a sequence of amino acids that are arranged in a specific order. Since this order also determines their thermophilic nature, knowledge of sequences can be integrated to build accurate models which can then fetch useful predictions for enzyme engineering processes [4, 5]. Over the past, protein classification based on this property has been performed using multiple machine learning methods such as support vector machines, deep neural networks and especially random forest (RF) classifiers [6]. While Wu et al [7] used 111 protein features based on primary secondary and tertiary structural parameters, Zuo et al [8] used only amino acid content as an input feature for a dataset of more than 3000 thermophilic protein from various sources. Similarly, Hao et al [9] used 900+ sequences and used the popular amino acid composition and dipeptide features. All the studies reported >90% accuracy for their classification models. These classifiers have been trained on hundreds of diverse thermophilic proteins from various species belonging to Archaea, Bacteria and other vertebrates and invertebrates. Feature extraction in such processes is
primarily based on amino acid composition and physicochemical properties imparted by the amino acid such as hydrophobicity, polarity, etc. Several studies conducted across multiple species have revealed unique amino acid level signatures. Residues such as Val, Ile, Glu, Arg were observed to be higher, and Gly, Met, Gln were lower [10]. Besides these residues, a higher occurrence of salt-bridges is also observed [11, 12]. Other important parameters include protein domains, protein binding regions, evolutionary residue conservation of residues, relative position of amino acids amongst others. Domain annotations of proteins have revealed conserved regions of proteins. These regions have often been shown to possess more theoretical sensitivity when it comes to identification than the sequence similarity strategy [13]. Characteristics from the protein binding region can be another significant feature. A protein complex may be composed of multiple binding regions which is dependent on the residue atoms of the interface [14] which is yet another determining factor for the protein functioning. Similarly, a wide range of conservation scores can be given to each residue in a protein which can be an important measure for its function [15]. Such parameters may have a significant role in determining the predictions of thermostability.

In the present study we aim to introduce an enzyme specific thermophilic classification model using the above discussed features. We use amino acid composition along with iso-electric point and several domain features to build a RF classifier. RF has been previously tested in similar works yielding highly accurate classification models. We lay out a workflow for a bacterial serine protease classification model (Fig 1). Serine proteases (SPs) are alkaline proteases, a hydrolase driven by nucleophilic core with a wide range of applications in industry [16]. SPs are mostly isolated from bacterial species and based on their source, they may display a variety of features. In the present study we make use of this diversity to analyze the enzyme further. Serine proteases isolated from thermophilic bacterial species display high resistance to temperatures above 60°C making it suitable for a variety in industrial processes [17, 18]. Establishing a classification model for this protein can serve both rational and directed evolution protein engineering endeavors. Moreover, this workflow can be utilized for other enzyme classes involving thermostability as classifying criteria.
2. Materials and Methods

2.1 Protein sequence mining

Initially, online repositories such as NCBI [19], PDB [20] and other biological databases were searched for SPs sequences from thermophilic bacterial genomes. First, thermophilic bacterial genomes were downloaded from NCBI which were later uploaded into RAST annotation server [21]. For manual curation of SPs, an Entrez application programming interface (API) was used [22]. An in-house python script was used to assist manual curation by integrating PubMed search with ESearch API. Additionally, relevant literature with the enzymes was searched manually also (Fig 2). The sequence data was curated in fasta format. These stored sequences were processed
using BLAST+ standalone tool to generate a custom database. Non-thermophilic SPs were also extracted from NCBI protein database.

Fig 2. Literature review pipeline

2.2 Feature extraction

Amino acid percentage was extracted from both thermophilic and non-thermophilic SPs. These frequencies of 20 amino acids were stored for all the SPs. Next, the pI for each sequence was evaluated using the pI calculator from BioPerl [23]. The third set of features we used was the number of residues belonging to the protein domain region. This prediction was based on InterPro results. Domain ranges were fetched for both thermophilic and non-thermophilic SPs.
using this online tool. Next, the number of protein binding sites for each sequence was predicted using the online tool PredictProtein [24]. Besides protein binding, this tool also predicts disordered regions, conserved residues, buried and exposed regions. The number of these regions spanning each sequence was used as a feature set. To remove the redundant information from the feature set, Pearson’s correlation was performed. First, the normalization was performed using the equation:

\[ x_i^n = \frac{x_i - \bar{x}}{x_{\text{max}} - \bar{x}} \]  \hspace{1cm} (1)

In the above equation, \( x_i \) represents the \( i \)-th value in the feature set. \( x_{\text{max}} \) and \( \bar{x} \) represents the maximum and minimum values of the feature set. Following normalization, Pearson’s correlation was used for estimating correlations between feature pairs.

2.3 Building a classifier

Next, a training set was generated using the SPs sequences retrieved above and their mesophilic counterparts. Labels 1 (thermophilic) and 0 (non-thermophilic) were placed for each sequence. For training and prediction, the extracted features were provided to the random forest model. The python library Sklearn was used for running the classifier. The standard random forest generates a collection of decision trees by randomly selecting subspaces from different sets of training data. The final prediction from the model is determined by majority vote.

2.4 Evaluating the model performance

Sensitivity (\( Se \)), Specificity (\( Sp \)) and accuracy (\( Acc \)) were used to evaluate the performance of the classifier.

\[ Se = \frac{TP}{TP+FN} \times 100\% \]  \hspace{1cm} (2)

\[ Sp = \frac{TN}{TN+FP} \times 100\% \]  \hspace{1cm} (3)

\( Se \) and \( Sp \) represents the performance of the classifier. \( Se \) represents the number of SPs sequences identified as thermophilic. Here, TP represents the thermophilic SPs and FN is the
number of thermophilic SPs predicted as non-thermophilic. Similarly, TN represents a number of non-thermophilic SPs. FP shows the number of non-thermophilic SPs identified as thermophilic. The third parameter was Acc.

\[
ACC = \frac{TP + TN}{TP + TN + FP + FN}
\]  

(4)

The accuracy reflects the ability of prediction of the RF classifier using the test-set.

2.5 Developing a model protein engineering platform

We built a prototype sequence based classifier tool of heat stable enzyme for SPs. The two features of the tool are SPs BLAST and the built-in classifier. The SPs sequences retrieved from the thermophilic sequences were first used to create a custom database which later was assigned to the tool. The user provided sequence can be submitted and the resulting alignment with top 10 thermophilic SPs within the cut-off e-value, percentage identity and other alignment parameters can be visualized in a separate output page. The second tool is the classifier itself. Similar to the BLAST input, users can submit their SP sequence to identify the thermophilic nature.

3. Results and Discussion

Thermophilic bacterial genomes from NCBI were annotated and the protein files were analyzed. Manual curation also produced SPs reported from experimental studies. The Esearch API generated PubMed IDs for the corresponding SPs study. The output was parsed and the protein sequences were extracted. A total of 470 SPs sequences were extracted from 291 thermophilic bacterial species and literature survey (Supplementary file 1). Similarly, 300 non-thermophilic SPs sequences were also downloaded from NCBI. All the sequences were retrieved in fasta format.

Pearson’s correlation revealed 3 amino acids ALA, GLY and VAL to be positively correlated (0.71). Only GLY was retained in the feature set. None of the physicochemical properties; domain range, protein binding site residues, isoelectric point (pI), conserved residues were observed to be correlated. The reduced feature set tested with a random forest algorithm showed more than 90 % value for all three indicators. The accuracy indicators Se, Sp and ACC were
95.53%, 96.01% and 95.71% respectively. The model under study has comparatively higher accuracy than previous studies that have performed classification of thermophilic and non-thermophilic protein. It can be observed that enzyme specific data could require less features for classification based on thermophilic nature.

Amino acid frequency, pI, number of residues in each domain, residues with lowest evolutionary conservation scores, number of protein binding regions, exposed and buried regions formed the classification features. Parameters for almost all studies till date have utilized amino acid composition, multiple sequence alignment profiles, pseudo amino acid composition [25]. These studies involve prediction and classification problems involving proteins with significantly less homology. With the 24 features the RF presented accuracy comparable to the RF classifier of Changli et al. which is 95.69% [11]. In perhaps one of the most accurate study yet, they tested thermophilic proteins belonging to a wide range of organisms. Similarly, the decision tree used by Li et al. [7] involved 111 features for 580 thermophilic proteins and their study involved both sequence and structural data as a feature set. Another major study in this field was conducted by Yong et al., and Hao et al. While a K-nearest neighbor classifier had an accuracy of 91% Support vector machine yielded 93.27% accuracy (Fig 3). All of the above studies base their classification on various classes of proteins belonging to different species. The present study however tries to showcase that a specific classification model built using only serine proteases requires significantly less number of features to achieve similar accuracy.
Fig. 3 Comparative accuracy for Machine learning algorithms used for thermophilic and non-thermophilic protein classification

Web tool prototype

First, the BLAST tool allows users to align their SP sequence against the in-built library of 400+ sequences. The easy access allows the user to submit and retrieve the results in a separate page with sequences alignment and their corresponding scores. Since thermophilic nature is highly dependent on the sequence, this alignment feature can be very useful for designing user-defined sequences. The alignment tool can further aid the users in finding similar SPs to their query. Thus unknown/novel proteins showing SP behavior can be identified using this interface. Second and perhaps the significant part of this platform is the classifier interface. The thermophilic property of SP is heavily dependent on the pattern of amino acid arrangement. This exact property is being used by the classifier to predict whether the input SP sequence exhibits a
thermophilic nature or not. Upon submitting the SP sequence, the RF classifier will generate the result as a binary classification of either “0” or “1”, where “1” shows that the sequence provided is indeed thermophilic in nature. The built-in classifier currently predicts with an accuracy of 95.71%. Fig 4 Shows our proposed web page design for the tool.

Fig. 4 Prototype of thermophilic Serine protease analysis tool
4. Conclusion

Thermophilic serine protease is a highly desired enzyme that is required for various industrial applications. Hence, availability of a unified resource platform for its sequence analysis can prove vital. To engineer thermostable enzymes for industrial usage, there exist a variety of pre-requisites. By combining a machine learning approach we have successfully built an accurate model to classify serine protease based on its thermophilic nature. Further, the same study model can be applied to any class of enzyme for its protein engineering studies.

Acknowledgements

The authors are thankful to SRM-IST dept. of Biotechnology for their support.

Conflict of interest

Authors declare no competing interests.

Author’s contributions

Jithin S. Sunny: Data collection, analysis, manuscript writing

Lilly M. Saleena: Conceptualization, Data collection, Review
References

[1] Chaparro-Riggers, J.F., Polizzi, K.M. and Bommarius, A.S., 2007. Better library design: data-driven protein engineering. *Biotechnology Journal: Healthcare Nutrition Technology*, 2(2), pp.180-191.

[2] Siedhoff, N.E., Schwaneberg, U. and Davari, M.D., 2020. Machine learning-assisted enzyme engineering. *Methods in Enzymology*, 643, pp.281-315.

[3] Bruins, M.E., Janssen, A.E. and Boom, R.M., 2001. Thermozymes and their applications. *Applied biochemistry and biotechnology*, 90(2), pp.155-186.

[4] Y. Wang, X. Hu, L. Sun, Z. Feng, H. Song, (2014) Predicting enzyme subclasses by using random forest with multicharacteristic parameters. *Protein and peptide letters*, 21(3), pp.275-284.

[5] E Ibrahim, N. and Ma, K., 2017. Industrial applications of thermostable enzymes from extremophilic microorganisms. *Current Biochemical Engineering*, 4(2), pp.75-98.

[6] Fan, G.L., Liu, Y.L. and Wang, H., 2016. Identification of therophilic proteins by incorporating evolutionary and acid dissociation information into Chou's general pseudo amino acid composition. *Journal of Theoretical Biology*, 407, pp.138-142.

[7] Wu, L.C., Lee, J.X., Huang, H.D., Liu, B.J. and Horng, J.T., 2009. An expert system to predict protein thermostability using decision tree. *Expert Systems with Applications*, 36(5), pp.9007-9014.

[8] Zuo, Y.C., Chen, W., Fan, G.L. and Li, Q.Z., 2013. A similarity distance of diversity measure for discriminating mesophilic and therophilic proteins. *Amino acids*, 44(2), pp.573-580.

[9] Lin, H. and Chen, W., 2011. Prediction of therophilic proteins using feature selection technique. *Journal of microbiological methods*, 84(1), pp.67-70.

[10] Panja, A.S., Bandopadhyay, B. and Maiti, S., 2015. Protein thermostability is owing to their preferences to non-polar smaller volume amino acids, variations in residual physico-chemical properties and more salt-bridges. *PloS one*, 10(7), p.e0131495.

[11] Feng, C., Ma, Z., Yang, D., Li, X., Zhang, J. and Li, Y., 2020. A Method for Prediction of Thermophilic Protein Based on Reduced Amino Acids and Mixed Features. *Frontiers in Bioengineering and Biotechnology*, 8, p.285.
[12] Taylor, T.J. and Vaisman, I.I., 2010. Discrimination of thermophilic and mesophilic proteins. *BMC structural biology, 10*(1), pp.1-10.

[13] Bouchot, J.L., Trimble, W.L., Ditzler, G., Lan, Y., Essinger, S. and Rosen, G., 2013. Advances in machine learning for processing and comparison of metagenomic data. *Computational Systems Biology: From Molecular Mechanisms to Disease*, pp.295-329.

[14] Guo, F., Zou, Q., Yang, G., Wang, D., Tang, J. and Xu, J., 2019. Identifying protein-protein interface via a novel multi-scale local sequence and structural representation. *BMC bioinformatics, 20*(15), pp.1-11.

[15] Malhis, N., Jones, S.J. and Gsponer, J., 2019. Improved measures for evolutionary conservation that exploit taxonomy distances. *Nature communications, 10*(1), pp.1-8.

[16] A. Jablaoui, A. Kriaa, N. Akermi, H. Mkaouar, A. Gargouriri, E. Maguin, M. Rhimi, (2018) Biotechnological applications of serine proteases: a patent review. *Recent patents on biotechnology, 12*(4), pp.280-287.

[17] A. Razzaq, S. Shamsi, A. Ali, Q. Ali, M. Sajjad, A. Malik, M. Ashraf, (2019) Microbial proteases applications. *Frontiers in bioengineering and biotechnology, 7*, p.110.

[18] M. Sharma, Y. Gat, S. Arya, V. Kumar, A. Panghal, A. Kumar, (2019) A review on microbial alkaline protease: an essential tool for various industrial approaches. *Industrial Biotechnology, 15*(2), pp.69-78.

[19] National Center for Biotechnology Information (NCBI)[Internet]. Bethesda (MD): National Library of Medicine (US), National Center for Biotechnology Information; [1988] – [cited 2017 Apr 06]. Available from: https://www.ncbi.nlm.nih.gov/

[20] Berman, H.M., Westbrook, J., Feng, Z., Gilliland, G., Bhat, T.N., Weissig, H., Shindyalov, I.N. and Bourne, P.E., 2000. The protein data bank. *Nucleic acids research, 28*(1), pp.235-242.

[21] Aziz, R.K., Bartels, D., Best, A.A., DeJongh, M., Disz, T., Edwards, R.A., Formsma, K., Gerdes, S., Glass, E.M., Kubal, M. and Meyer, F., 2008. The RAST Server: rapid annotations using subsystems technology. *BMC genomics, 9*(1), pp.1-15.
[22] Entrez Programming Utilities Help [Internet]. Bethesda (MD): National Center for Biotechnology Information (US); 2010-. Available from: https://www.ncbi.nlm.nih.gov/books/NBK25501/

[23] Stajich, J.E., Block, D., Boulez, K., Brenner, S.E., Chervitz, S.A., Dagdigian, C., Fuellen, G., Gilbert, J.G., Korf, I., Lapp, H. and Lehväslaiho, H., 2002. The Bioperl toolkit: Perl modules for the life sciences. *Genome research, 12*(10), pp.1611-1618.

[24] Yachdav, G., Kloppmann, E., Kajan, L., Hecht, M., Goldberg, T., Hamp, T., Hönigschmid, P., Schafferhans, A., Roos, M., Bernhofer, M. and Richter, L., 2014. PredictProtein—an open resource for online prediction of protein structural and functional features. *Nucleic acids research, 42*(W1), pp.W337-W343.

[25] Liu, B., 2019. BioSeq-Analysis: a platform for DNA, RNA and protein sequence analysis based on machine learning approaches. *Briefings in bioinformatics, 20*(4), pp.1280-1294.
Below is the list of thermophilic bacterial species whose genomes were analyzed

| Acetomicrobium faecale | Anoxybacter fermentans | Bacillus thermoto lerans | Alicyclob acillus pomorun m | Coprotherm obacter platensis | Fervidobacterium thiand rens | Kyridia tusciae | Thermus aquaticus |
|------------------------|------------------------|--------------------------|-----------------------------|-------------------------------|-----------------------------|----------------|------------------|
| Acetomicrobium flavidum | Apibacter mensalis     | Bacillus vallismor tis    | Alicyclob acillus sacchari  | Coprotherm obacter protetoli cious | Fontimon as thermonap hilus | Laceyella sacchari | Thermus arci formis |
| Achromobacter aloeverae | Aquitalea pelogenes    | Balneari um lithotrop hicum | Alicyclob acillus sendai ensis | Crenotalae thermo phila | Fournier ella massili en sis | Laceyella sediminis | Thermus brock ianus |
| Acidimicrobium fer rooxidans | Arcobacter canalis   | Belliline a coldifista lae | Alicyclob acillus tenghon gensis | Deferribacte r desulfurica ns | Geobacilli us galactosi dus | Lebetimon as natsushima e | Thermus caldif ontis |
| Acidithiobacillus cal dus | Arcobacter cibarius | Bogorrell a caseilypica | Alicyclob acillus vulcana lis | Deferrisoma camini | Geobacilli us iictianus | Levilin eara thermo boles | Thermus cal diertees |
| Acidobacterium aitaau | Arcobacter halotis | Brevibaci llus borsten sis | Alicyclob acillus vulcanalis s | Deferrisoma camini | Geobacilli us iictianus | Lihuaxuea thermophil a | Thermus filiformis |
| Acidothermus cellulolyticus | Ardenticaten a maritima | Brockia lithotrop hica | Alloiococcus otitidis | Defluviatile a raffinose de ns | Geobacilli us jurassicu s | Limisphaera ngatamari kens | Thermus ignitere a |
| Acinetobacter celticu s | Athalassotoga saccharophil a | Caenibacilli us caldisapo nyliticus | Alteri bacillus perspoleon sis | Desulfacinu m hydrotherm ale | Geobacilli us kausph ilus | Litorilina aeropil a | Thermus oshimai |
| Acinetobacter colisticae resistens | Aurantimonas coralicie da | Caldalka libacillus thermaru m | Alteromonn as addita | Desulfacingu m infernum | Geobacilli us stea rothep hil a | Lysinbacilli us sphaericus | Thermus parvatiensis |
| Acinetobacter defluvi i | Aureimonas alamirensis | Caldanae robacter subterra neus | Alteromonn as oesturii vivens | Desulfosem a caldarium | Geobacilli us subterra neus | Mahella australiensis | Thermus scotoductus |
| Acinetobacter equi | Bacillus alcalophilus | Caldanae robus fijenissis | Alteromonn as genoven sis | Desulfother mus okinawensis | Geobacilli us thermant arcicus | Marinither mus hydrotherm alis |
| Acinetobacter seiferti | Bacillus altitudinis | Caldanae robus polysacc haroliticus | Alteromonn as oceani | Desulfotom a aculum australicum | Geobacilli us thermoc ater philus | Meiother mus chlorphil us |
| Actinobaculum suis | Bacillus alveayuensis | Caldanae roviga acetigigen ens | Alteromonn as stellipola ris | Desulfotom a aculum carboxydivo rans | Geobacilli us thermode nirifican s | Moorell a glycerini |
| Actinomadura formosensis | Bacillus amylophil anus | Calderiha bians mariti munis | Aminiphil us circumsp rictus | Desulfotom a aculum hydrotherm ale | Geobacilli us thermole ovrans | Moorell a humiferrea | Thermus aquaticus |
| Actinomadura rubrobrunea | Bacillus aquimaris | Caldibacillus debilis | Ammonifex degensii | Desulfotomaculum nigrificans | Geobacillus toebii | Moorella mulderi |
|--------------------------|-------------------|----------------------|-------------------|-----------------------------|------------------|------------------|
| Actinomyces ruminicola   | Bacillus atrophaeus| Caldibacillus ulosiruptor acetigenus | Ammonifex thiophilus | Desulfotomaculum profundus | Geobacillus usulcani | Moorella stansii |
| Actinopolyspora mortvallis| Bacillus azotoformans | Caldibacillus ulosiruptor balcanica | Amphibacillus cullus jilinensis | Desulfotomaculum putei | Geobacillus zahalae | Moorella thermoacetica |
| Aeribacillus compositi   | Bacillus bataviensis | Caldibacillus ulosiruptor changbaiensis | Amphibacillus cullus sediminis | Desulfotomaculum thermocisternum | Geothermobacter ehrlichii | Persephone lila marina |
| Aeribacillus pallidus    | Bacillus coagulans | Caldibacillus ulosiruptor hydrothermals | Amphibacillus cullus sylanus | Desulfotomaculum acutilabens | Geothermobacter subterreus | Natranaerobius biaus thermophilus |
| Aeromonas bivalvium      | Bacillus cohnii   | Caldibacillus ulosiruptor kristjansonii | Amycolatopsis metriothenomophilus | Desulfotomaculum mesophilum | Geothermobacter ehrlichii | Persephone lila marina |
| Aeromonas media          | Bacillus cytotoxicus | Caldibacillus ulosiruptor lactoaceticus | Amycolatopsis urazovskyi | Desulfotomaculum acutilabens | Halobacillus alkaliphilus | Pseudoalteromonas thermoresistens |
| Aeromonas molluscorum    | Bacillus drentensis | Caldibacillus ulosiruptor owensensis | Anaerobacillus hydrogeniformans | Desulfotomaculum acutilabens | Heliobacter modesticaldum | Pseudoalteromonas thermoresistens |
| Aeromonas sanarellii     | Bacillus drentensis | Caldibacillus ulosiruptor owensensis | Anaerobacillus hydrogeniformans | Desulfotomaculum acutilabens | Herbinia hemicellulosilytica | Rhodothermus profundus |
| Aeromonas simiae         | Bacillus firmus    | Caldibacillus ulosiruptor saccharolyticus | Anaerobacillus saccharolyticus | Desulfotomaculum acutilabens | Herbinia hemicellulosilytica | Rhodothermus profundus |
| Aeromonas taiwanensis    | Bacillus furcatioli | Caldibacillus ulosiruptor oshimai | Anaerobacillus saccharolyticus | Desulfotomaculum acutilabens | Hippea arvini | Thermocrinis albus |
| Afipia birghiae          | Bacillus galactosidilyticus | Caldilinea aerophila | Anaerobacillus galactosidilyticus | Desulfotomaculum acutilabens | Hippea jasoniae | Thermocrinis marinae |
| Afipia massiliensis      | Bacillus halosaccharovorans | Caldisalicibus anartha | Anaerobacillus halosaccharovorans | Desulfotothrix indicum | Hippea marina | Thermocrinis ruber |
| Albidovulum inexpectatum | Bacillus indicus    | Calditerricola satsumae | Anaerobicoccus thermophilus | Desulfotomaculum acutilabens | Hydrogenibacillus schlegelii | Thermodesulfatator autotrophicus |
| Albidovulum xiamenense   | Bacillus indicus    | Calditerricola satsumae | Anaerobicoccus thermophilus | Desulfotomaculum acutilabens | Hydrogenibacillus schlegelii | Thermodesulfatator autotrophicus |
| Alcanivorax dieselolei    | Bacillus jeotgalii | Caldisalicibus anartha | Anaerobicoccus thermophilus | Desulfotomaculum acutilabens | Hydrogenibacillus schlegelii | Thermodesulfatator autotrophicus |
| Alcanivorax dieselolei    | Bacillus jeotgalii | Caldisalicibus anartha | Anaerobicoccus thermophilus | Desulfotomaculum acutilabens | Hydrogenibacillus schlegelii | Thermodesulfatator autotrophicus |
| Chenkov | Alophil | Hilus | Hydrogen | Thermosulf | Urimonas | Dismutans |
|---------|---------|-------|----------|------------|-----------|-----------|
| Alcanivorax nanhaiicus | Bacillus licheniformis | Caloramator fervidus | Anaeromaibacter bairesiensis | Enterobacter mori | Hydrogenophaga pseudoflavus | Thermosulfurimonas dismutans |
| Alicyclobacillus acidophilus | Bacillus marisflavi | Caloramator michellei | Ancylobacter pratisalii | Enterococcus cacaoe | Hydrogenophaga taeniopsidis | Thermotoga maritima |
| Alicyclobacillus acidocaldarius | Bacillus methanolicus | Calorana erobacter azoresensis | Aneurini bacillus danicus | Enterococcus crotali | Hydrogenothermus marinus | Thermotoga naphthophila |
| Alicyclobacillus acidoterrestris | Bacillus mojavensis | Calorana erobacter azoresensis | Aneurini bacillus thermophilus | Fervidicella mettallireducens | Inmirana thermophilica | Thermotoga neapolitana |
| Alicyclobacillus contaminans | Bacillus novalis | Calorana erobacter ferrireducens | Anoxybacillus amylolyticus | Fervidicola ferrireducens | Keratinibacter paraluntense | Thermovibrio abulorum |
| Alicyclobacillus herbarius | Bacillus smithii | Caminibacter mediatotalis | Anoxybacillus flavithermus | Fervidobacterium changbaicum | Kosmopteragama arenocollina | Thermovibrio ammonificans |
| Alicyclobacillus hesperidum | Bacillus sonorensis | Caminieilla sporogenes | Anoxyba cillus pushchinensis | Fervidobacterium gondwanense | Kosmopteragama olearia | Thermovibrio guaymasensis |
| Alicyclobacillus kakegawensis | Bacillus tequilensis | Clostridium clariflavum | Anoxybacillus tepidamans | Fervidobacterium islandicum | Kosmopteragama pacifica | Thermovibrio guaymasensis |
| Alicyclobacillus macrosporangiids | Bacillus thermostephandyi | Clostridium straminis olvens | Anoxybacillus thermarum | Fervidobacterium nodosum | Kroppeptedtia pulmonis | Thermus antranikianii |
| Alicyclobacillus montanus | Bacillus thermocopriae | Clostridium thermoce-lium | Anoxybacillus vitaminophilus | Fervidobacterium pennivorans | Kyrpiediaspormannii | Thermus antranikianii |
