4	Tr|Q6HIZ4|Q6HIZ	Model#4	0.4373	0.3124
5	Tr|Q6HIZ4|Q6HIZ	Model#5	0.3501	0.2472
6	Tr|Q6HIZ4|Q6HIZ	Model#6	0.4175	0.3058
7	Tr|Q6HIZ4|Q6HIZ	Model#7	0.3668	0.2575
8	Tr|Q6HIZ4|Q6HIZ	Model#8	0.3732	0.2630
9	Tr|Q6HIZ4|Q6HIZ	Model#9	0.3882	0.2753
10	Tr|Q6HIZ4|Q6HIZ	Model#10	0.3780	0.2763
11	Tr|Q6HIZ4|Q6HIZ	Homology model	----------	----------

The final refined model structure of alkaline protease was considered for the evaluation of homology model, which revealed that 90.61% of the residues were in the core region in the Ramachandran map performed by MOE for the analysis of protein geometry structure. It contains the core score > 0.02 and the outlier score < 0.0005. However, only 1.39% of amino acid residues were reviled outside the allowed region (Figure 11).

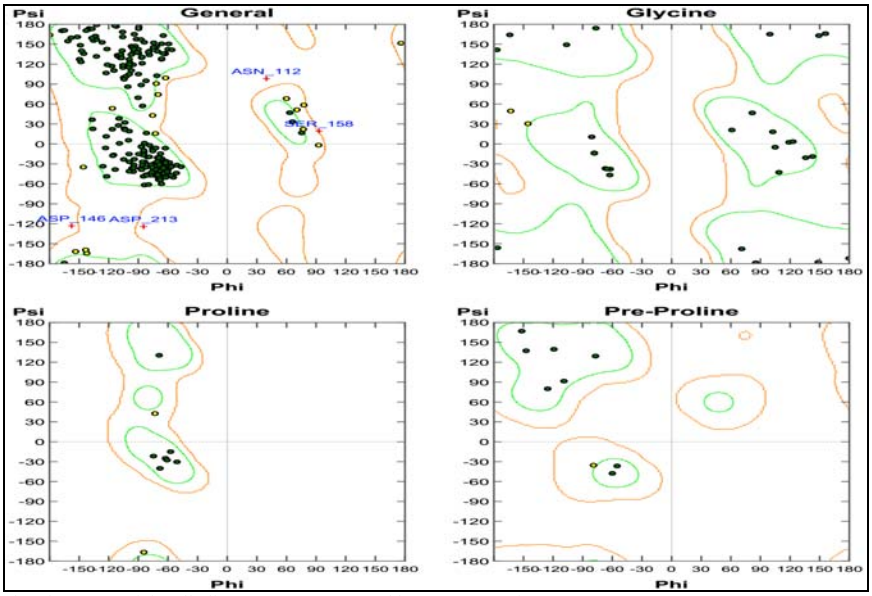

Figure 11. Ramachandran map of the alkaline protease generated by MOE (Version 2008.10).

Further, the results obtained from Verify 3D showed that about 96.40% of the residues had an average 3D-1D score ≥ 0.2, which revealed that the protein structure is acceptable. Besides, the ERRAT2 score values were used to check the quality of the modulated protein, which estimated the overall quality factor of about 86.617 (Figure 12). The overall quality factor is expressed as the percentage of protein for which the calculated error value falls below 95% rejection limit. The good high resolution structures generally produce the values around 95% or higher. For lower resolution (2.5 to 3Å), the average overall quality factor is around 91%. In the present study, the ERRAT score of 86.617% revealed that the predicted protein structure of alkaline protease is well accepted. Similarly, more than 96.4% of the amino acids with the Verify 3D score ≥ 0.2 revealed that the modeled structure is highly accurate and could be used for the molecular docking experiment.

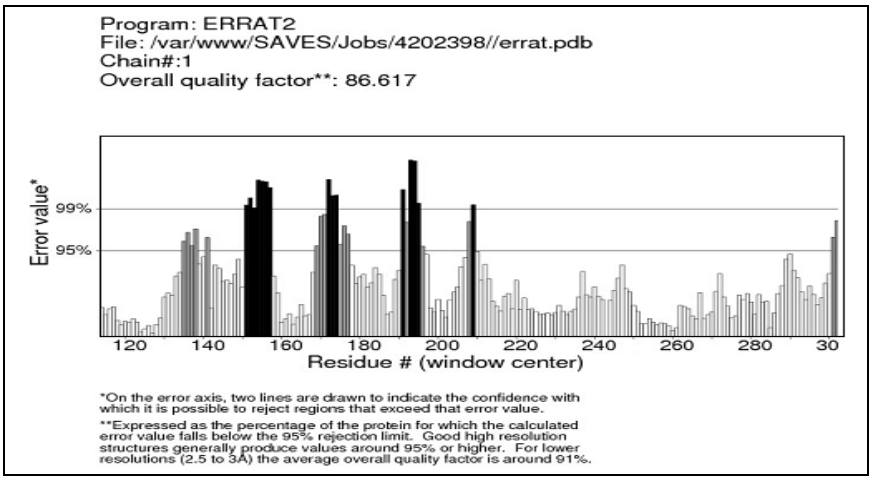

Figure 12. Overall quality factor of the protein generated by the ERRAT server.

The recent advances in bioinformatics with the rapid accumulation of 3D structures of protein complexes through X-ray crystallization provide insights into the protein-protein interactions facilitating rational drug design and treatment of diseases. However, all the protein complexes have not been crystallized and therefore various computational techniques have been developed to address such situation.

One of the promising approaches is protein docking where the structure of a complex between two proteins is predicted based on the independently crystallized structures of the components. The Z-DOCK program performs full rigid-body search of the docking orientations between the two proteins, which includes performance optimization and a novel pair-wise statistical energy potential. The ZDOCK searches all the possible binding modes in the translational and rotational space between the two proteins and evaluates each pose using energy based scoring function. The result of the ZDOCK server was then put into PatchDock to prognosticate the binding affinity with casein protein using PatchDock. PatchDock is geometry based molecular docking based on the shape complementarity principles, which aimed at finding the docking transformations that yield good molecular shape complementarity [55]. Such transformations when applied induce both wide interface areas and small amounts of steric clashes. A wide interface is ascertained to include several matched local features of the docked molecules that have complementary characteristics (Figure 13).

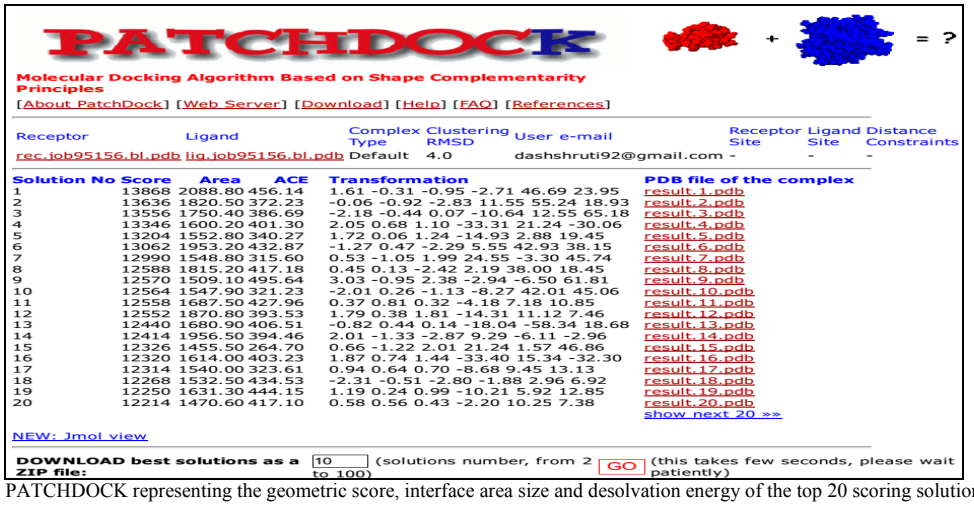

Figure 13. PATCHDOCK representing the geometric score, interface area size and desolvation energy of the top 20 scoring solutions.

PatchDock algorithm divides the Connolly dot surface representation of the molecules into concave, convex and flat patches [56]. Then, the complementary patches are matched in order to generate candidate transformations. Each candidate transformation is further evaluated by scoring function that considers both geometric fit and atomic desolvation energy [57]. Finally, RMSD (root mean square deviation) clustering is applied to candidate solutions to discard the redundant solutions. The main reason behind the PatchDock's high efficiency is its expeditious transformational search, which is driven by the local features matching rather than the brute force probing of the six-dimensional transformation space. Further, it expedites the computational processing time by utilizing the advanced data structures and spatial pattern detection techniques such as geometric hashing and pose clustering that were originally developed in the field of computer vision [55].

The protein complex with PatchDock score of 13868 with interface area of 2088.8 Å was considered as the probable site of interaction in absence of co-crystal structure between alkaline protease and casein (Figure 15). Conclusively, a complex structure was found, which showed that the alkaline protease binds with the casein protein to facilitate its degradation (Figure 14).

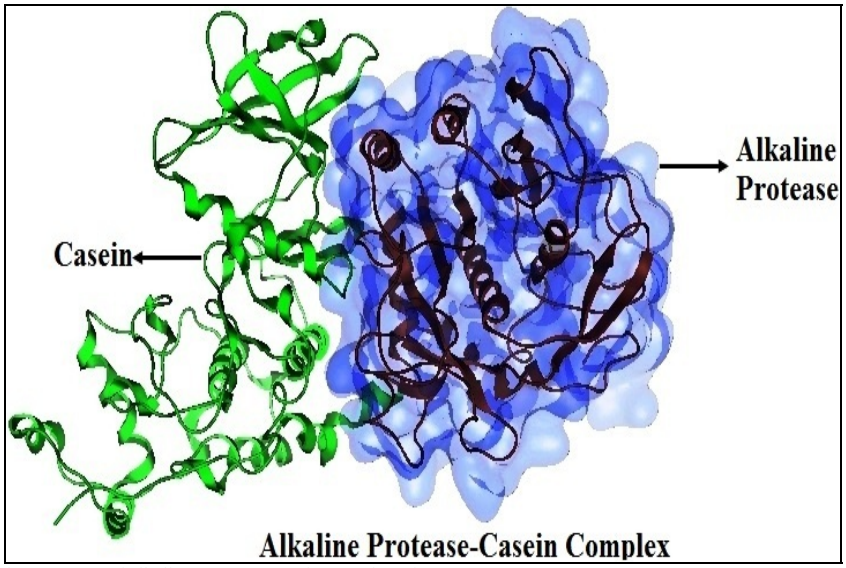

Figure 14. Molecular structure of alkaline protease-casein complex after PATCHDOCK.

III. CONCLUSION

It is evident from the study that the bacterium isolated form milkshed soil sample is considered to be the good source of alkaline protease production. The isolated bacterium is identified as *Bacillus* sp. based on the morphological, biochemical and molecular characterization. The isolated bacterium was found to exhibit closer resemblance with *Bacillus thuringiensis* based on 16S rDNA sequence comparison. The study revealed that the isolated bacterium exhibited relatively higher growth rate in alkaline medium (pH 9) at 37°C, which was considered to be the favorable conditions for higher protease activity. The sensitivity of bacterium was analyzed through antibiotics sensitivity test. Besides, the thermal death time of the bacterium was determined to be 4 hr at 60°C. Moreover, the molecular modeling suggested that the alkaline protease was being produced by the isolated bacterium has higher binding affinity with casein protein, which facilitate its degradation in soil. The study suggested that the isolated bacterium could be used as an alternative source for the commercial production of alkaline protease for industrial use.

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