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Role of *Proteus mirabilis* in Caffeine Degradation – A Preliminary Bioinformatics Study

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Abstract

An attempt to find the role of *Proteus mirabilis* in caffeine degradation using bioinformatics tools has been made here. Soils from coffee industries were taken and the bacterium was isolated and found to degrade caffeine. Identification of the bacterium through Sangers dideoxy sequencing of 16S rDNA was done and its genome taken from online database was used for homology modeling of the enzyme to identify regions of similarity and enzyme structure prediction. Also attempts to secondary structure prediction and protein threading has been done to study the enzyme and compare the enzymes of *Proteus mirabilis* with that of other reported caffeine degrading organisms.

Keywords: Caffeine degradation, bioinformatics, homology modeling, protein threading, secondary structure prediction.

Introduction

Caffeine, a plant product has shown its occurrence in beverages like tea, coffee and soft drinks and cocoa[1,2] and due to its regular intake by individuals and its prevalent adverse effects, demand for decaffeinated beverages has been growing nowadays. Its side-effects include toxicity in excess of consumption, high adrenal simulation, irregular muscular activity, cardiac arrhythmia and high heart output and even mutations[3]. Thus need for caffeine degradation is eminent. With this aim, several approaches have been reported earlier, that involve chemical, microbial and enzymatic techniques. Chemical approaches like water, solvent and super critical fluid extraction have been found to be non specific and expensive. They also involve the use of toxic solvents[4]. Hence enzymatic methods have promising advantages in decaffeination, due to their safety and advantage over microbial decaffeination of not affecting food sensory quality[3,5].

Successful decaffeination would allow coffee husks to be available as animal feed and manure[6,7]. It would also reduce pollution caused by caffeinated products in water bodies[8]. Also in food industry, decaffeinated products would reduce the risk of caffeine dependence and side-effects. Microorganisms degrading caffeine have been identified to be mostly *Aspergillus* or *Pseudomonas spp*[4]. But recently a few new organisms like *Paenibacillus marcerans* have also found to degrade caffeine[5].

In a similar approach another isolate taken from soils near coffee industry was found to degrade caffeine. The isolate showed reduced levels of caffeine by UV spectroscopy and was found to be *Proteus mirabilis SNBS* from 16S rDNA sequencing results. Since the isolate has not been reported of caffeine degradation, bioinformatics' tools were employed to check the presence of any similar proteins as the other caffeine degradations organisms, using homology modeling and secondary structure prediction. These techniques would help assist in the validation of the fact that *Proteus mirabilis SNBS* strain has the potential to degrade caffeine. Also a significant problem that exists with previously reported microorganisms is that the final end products of their caffeine pathways are toxic to the environment[4] and potent carcinogens. Hence an enzyme distinct from other enzymes including caffeine dehygenase or caffeine methylase might result in a different product, which would be less harmful than the products obtained from the latter.

Material and Methods

Screening and Isolation: Soil sample obtained from a coffee factory in Chittoor was taken and serially diluted. Caffeine enriched media or CEM was prepared using Lauryl sulphate HiVeg Broth (30.0g/l), anhydrous caffeine (0.3g/l), sodium chloride (0.5g/l) and coffee husk extract (0.5% w/v). Using pour plate technique, the diluted samples were incubated for 48hrs at 37°C. After incubation, isolates of common morphology were streaked in CEM media separately. The isolates were also tested for Gram's staining.

UV-Visible Spectroscopy: The isolate found to be growing and forming zones in CEM was then grown in broth containing CEM for 24 hrs at 37°C in an orbital shaker. The sample was drawn at two intervals – 24 hours and 48 hours. Centrifugation at 12000rpm yielded supernatant which was subjected to UV-visible absorbance at 275nm taking non-caffeinated media as blank. UV-visible absorption of CEM before decaffeination was also taken to compare the readings with decaffeinated media[9].

Biochemical Identification: The isolates were tested for biochemical identification using Advanced Bacterial Identification Software (ABIS)[10]. Since all the isolates had a common morphology, one of them was tested and identified. This was performed to obtain the basic idea of the genus of the isolate and to ensure that it was not one of the earlier reported caffeine degrading bacteria.

16S rDNA Sequencing: The isolate was then sequenced of 16S partial rDNA sequence using Sangers' dideoxy sequencing method. BioEdit Sequence Alignment Editor (Version 7.1.3.0) was used for assembly method of the sequences. Using the sequenced data, Basic Local Alignment Search Tool (BLAST) using BLASTIN 2.2.27 software was employed in 16S ribosomal RNA bacterial database to decipher the type of the organism.

Sequence Similarity Identification: The caffeine dehydrogenase protein[11] has been sequenced for *Pseudomonas sp. strain CBB1*. These sequences were aligned with the proteins in PDB database[12] of *Proteus mirabilis*. The protein sequence with the highest similarity was taken for homology modeling[13]. The protein selected was Chain A of crystal structure of Amidohydrolase Pmi1525 (Target Efi-500319) From *Proteus mirabilis Hi4320*[14]. The template chosen for homology modeling had a 43% similarity and 16% query coverage. The multiple sequence alignment[15] was carried out for secondary structure analysis and fold recognition[16]. This was done because the similarity between the caffeine dehyrdogenase proteins and *Proteus mirabilis* proteins is very less. Thus, this helped us in increasing the efficiency of the structure predicted.

Homology Modeling: The caffeine dehydrogenase sequence of Pseudomonas sp. strain CBB1was used and the query sequence was used as the template. The software used was Modeller V9.10[17]. Five most probable structures were predicted and comparing their DOPE (Discrete optimized protein energy) score and GA431 score, the best suitable model was selected. This model was validated to explain how well the model conforms to common refinement restraint values. Validation was done using the WHAT-IF program[18].

Secondary Structure Prediction and Fold Recognition: Since, the model predicted by homology modeling was not very accurate, the basic secondary structure was predicted using different online programs. The query used was the multiple sequence alignment between the caffeine dehydrogenase and the *Proteus mirabilis* enzyme.

Results and Discussion

Screening and Isolation: Screening of the soil samples resulted in the formation of zones of degradation which could be that of caffeine due to excess presence of anhydrous caffeine and caffeine husk. Hence the microbes were isolated from the corners of these zones were taken and sub-cultured to yield pure isolates. Colonies with a common, smooth morphology were identified after incubation. These also showed swarming motility and were identified Gram negative. The colonies gave a slimy exudate and did not give single colonies.

UV-Visible Spectroscopy: The UV-visible absorption results at 275nm showed that the levels of caffeine had decreased after 24 hours and further after 48 hours (figure.1). This gave a preliminary idea that the isolate is capable of degrading caffeine.

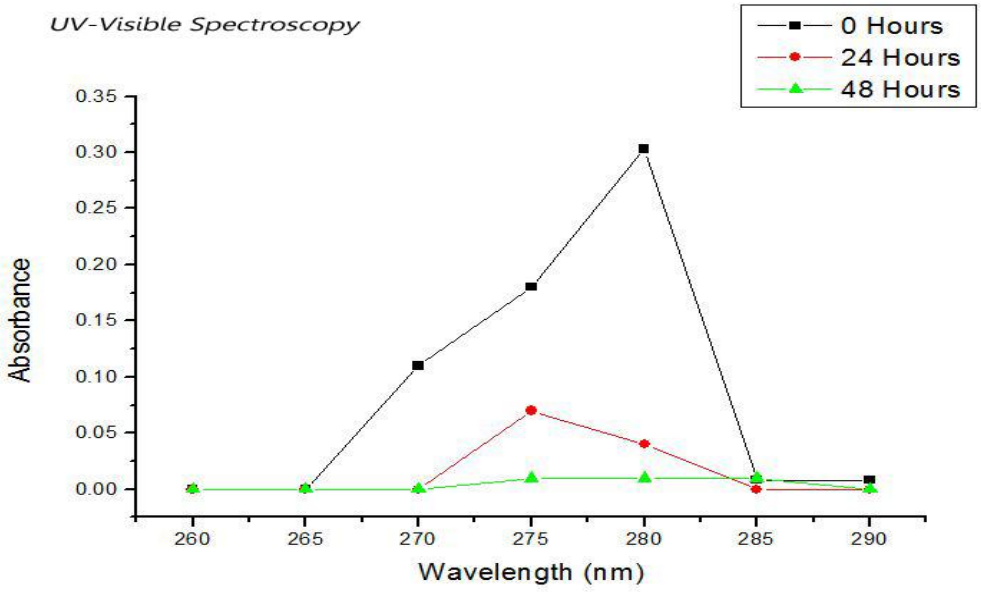

Figure-1UV-
Visible Absorbance results showing reduced absorption at 275nm, thus giving a possibility of caffeine degradation

Biochemical Identification: The results of the biochemical tests are shown in table 1, which were then entered in ABIS software to give the probable species which could be present (table 2). These microorganisms have not been reported earlier for caffeine degradation. Hence the next step of species identification using 16S rDNA sequencing was done.

Table-1
Results of the biochemical tests of the microorganism isolated

Biochemical Tests	Positive (+) or Negative (-)
Motility	+
Catalase	+
Oxidase	-
Ornithine decarboxylase	-
Methyl red	+
Indole	-
Voges- Proskauer	-
Hydrogen sulfide	+
Urea hydrolysis	+
Maltose Fermentation	-
Gas from Glucose	+
Sucrose Utilisation	+
Xylose Utilisation	+

Table-2
Identification of the probable species of isolate by ABIS software

Probable Microbe	Probability (%)	Accuracy (%)
Proteus mirabilis	82	23
Citrobacter freundii	82	23
Proteus penneri	81	23
Citrobacter werkmanii	81	23

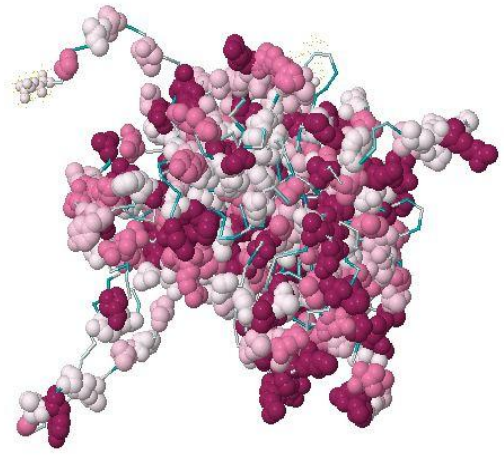

Figure-2
Most probable structure of the enzyme of *Proteus mirabilis* using Homology modeling

16S rDNA Sequencing: The partial, assembled sequence of 16S rDNA – 1363 base pairs, was successfully processed and obtained. BLAST results showed that the sequence was 99% identical to *Proteus mirabilis* NCTC11938 (Accession Number: NR_0.43997.1). The sequenced was further submitted to GenBank – BankIt database as *Proteus mirabilis* SNBS and with JX974560 as the accession number.

Homology Modeling: The most suitable structure for the caffeinase enzyme of *Proteus mirabilis* was predicted and shown in figure 2.

The validation results are given as an overall summary of the quality of the structure as compared with available reliable structures. Structure Z-scores[19], positive is better than average:

Table-3
Gives the features and their resolution obtained. Higher positive values denote better resolution

Feature	Resolution
Resolution read from PDB file	-1.000
1st generation packing quality	-3.813 (poor)
2nd generation packing quality	-4.361 (bad)
Ramachandran plot appearance	-4.122 (bad)
Chi-1/chi-2 rotamer normality	-4.347 (bad)
Backbone conformation	-6.217 (bad)
Inside/Outside distribution	1.205 (unusual)

Table-4
Shows the RMS values of the conformation of the structure obtained by homology modeling

Feature	RMS Z-score (should be close to 1.0)
Bond lengths	1.097
Bond angles	1.677
Omega angle restraints	1.236
Side chain planarity	0.592 (tight)
Improper dihedral distribution	1.682 (loose)

Secondary Structure Prediction and Fold Recognition: The secondary structure prediction is performed using DSSP[20] and PSIPRED[21]softwares. The secondary structure for each sequence was represented by a colour. If a sequence in the alignment had no colour assigned, this means that either there is no information available, or that no prediction was possible for that sequence. The gi_330689734 represents sequence of caffeine dehydrogenase, and the gi_403399746 represents sequence of Amidohydrolase of *Proteus mirabilis.* The colour assignments are given in the figure 3 and 4. To identify conserved domains, the conservation scoring was performed by PRALINE software[22]. The scoring scheme works from 0 for the least conserved alignment position, up to 10 for the most conserved alignment position. The colour assignments were given in the figure 5 and 6. To check for hydrophobicity, the hydrophobicity scale used was from Eisenberg *et al* (1984)[23]. The colour assignments from hydrophobic to hydrophilic are given in the figure 7 and 8.

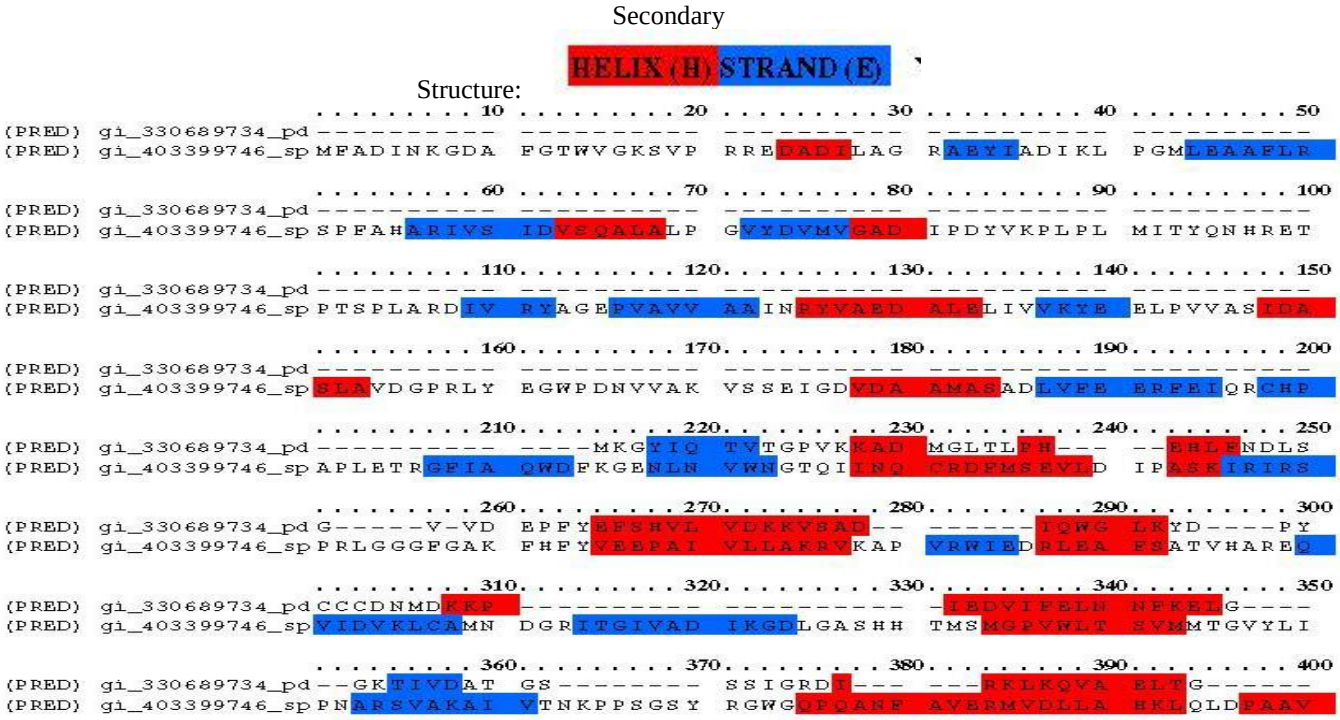

Figure-3
Secondary Structure prediction of the first 400 base pairs

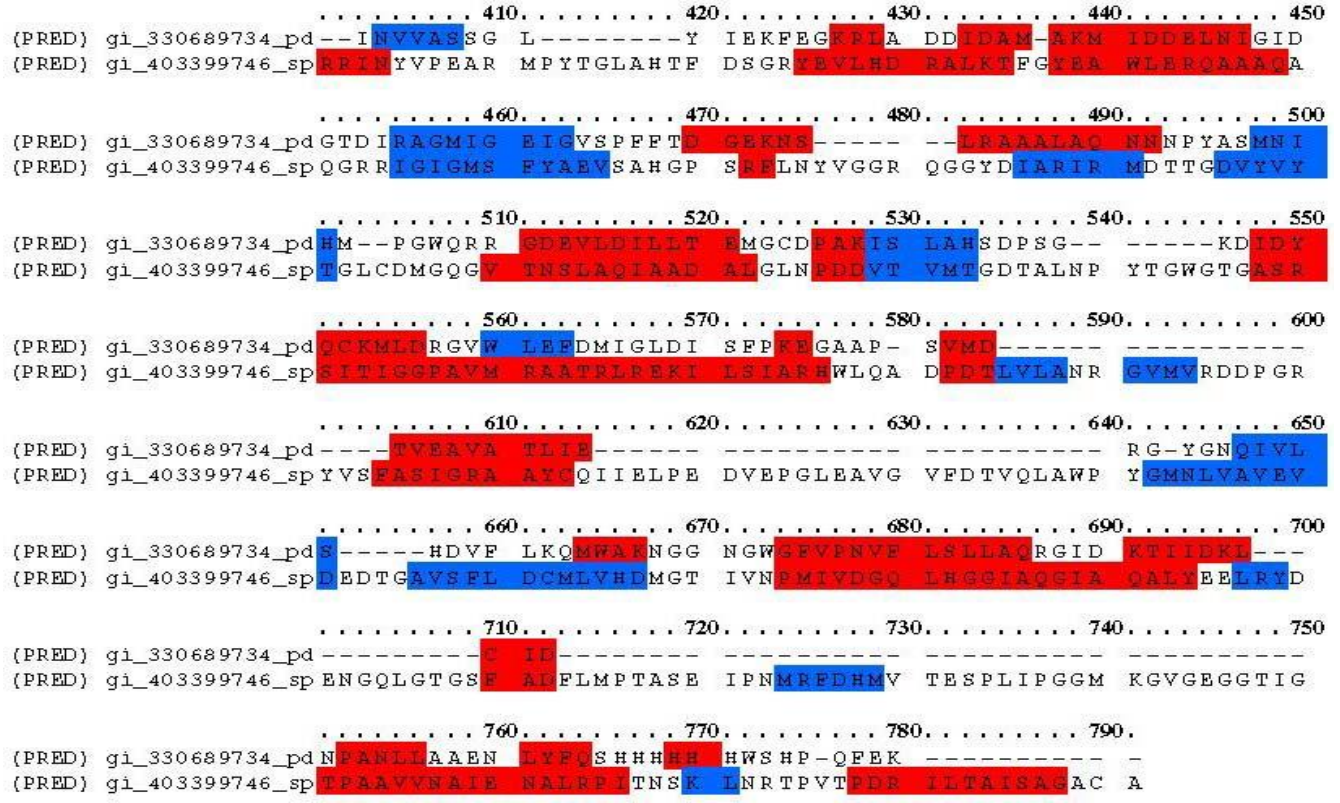

Figure-4
Secondary Structure prediction of the 401 to 791 base pairs

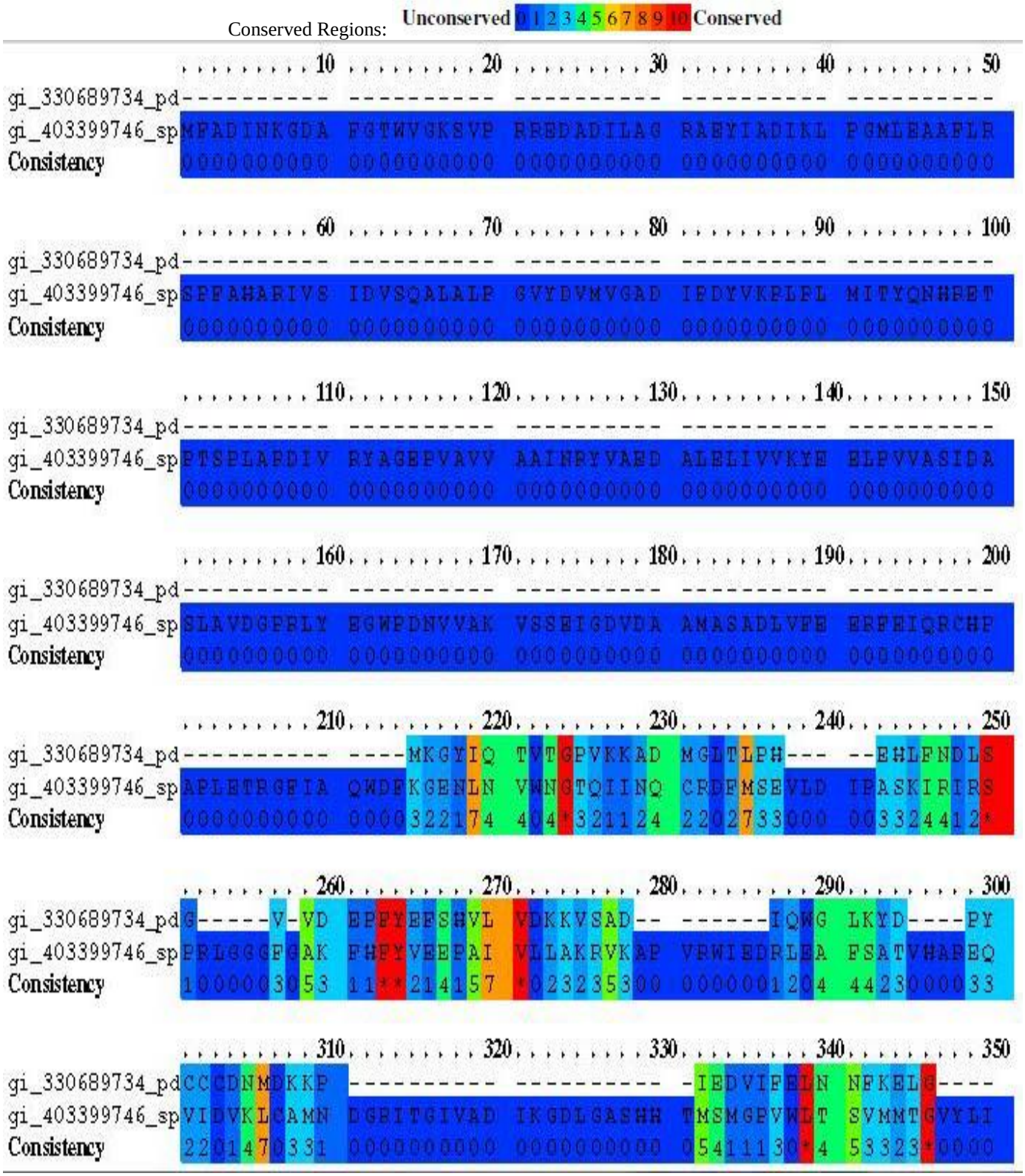

Figure-5
Conserved regions predicted of the first 350 base pairs

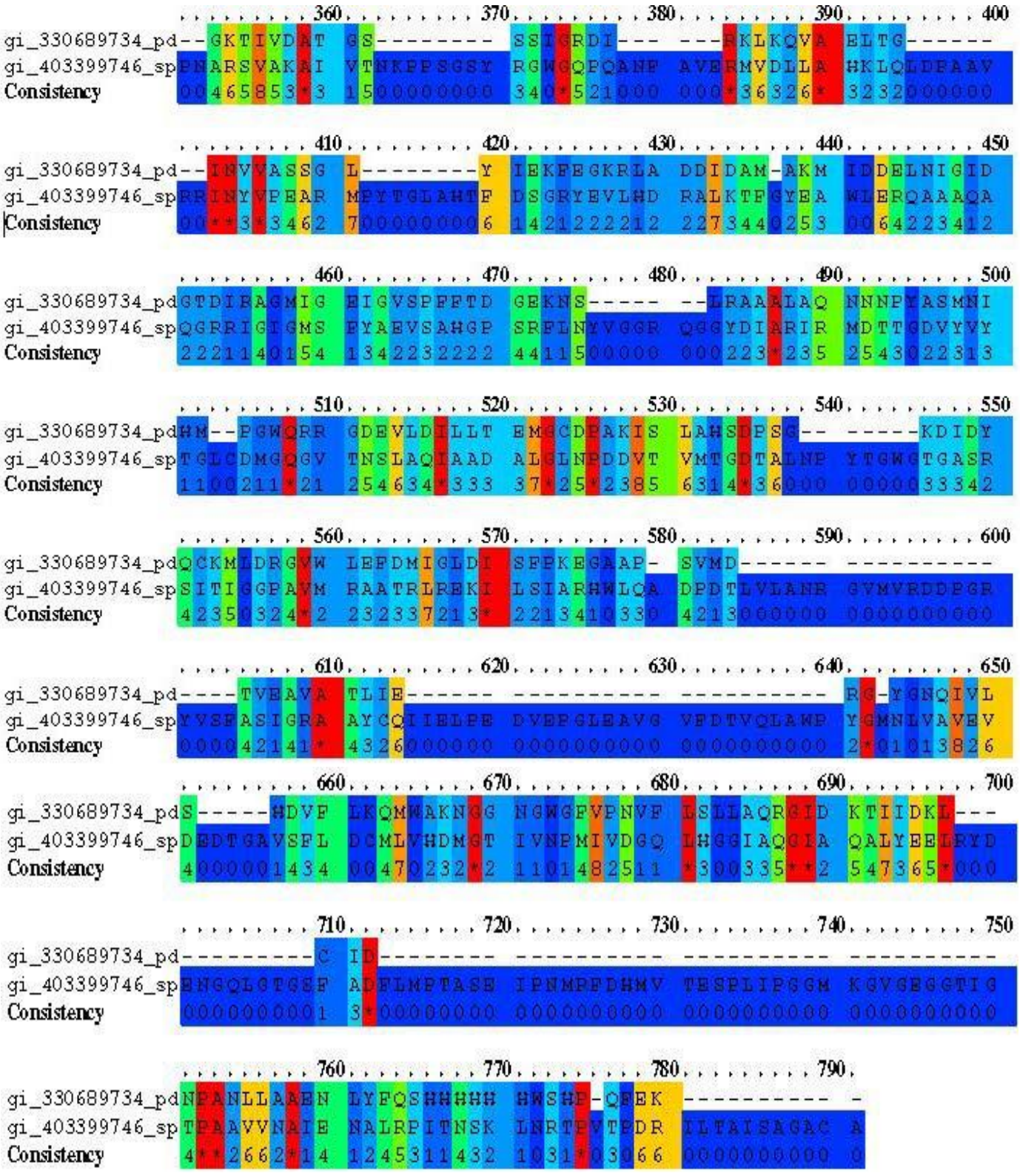

Figure-6
Conserved regions predicted of the 351 to 791 base pairs

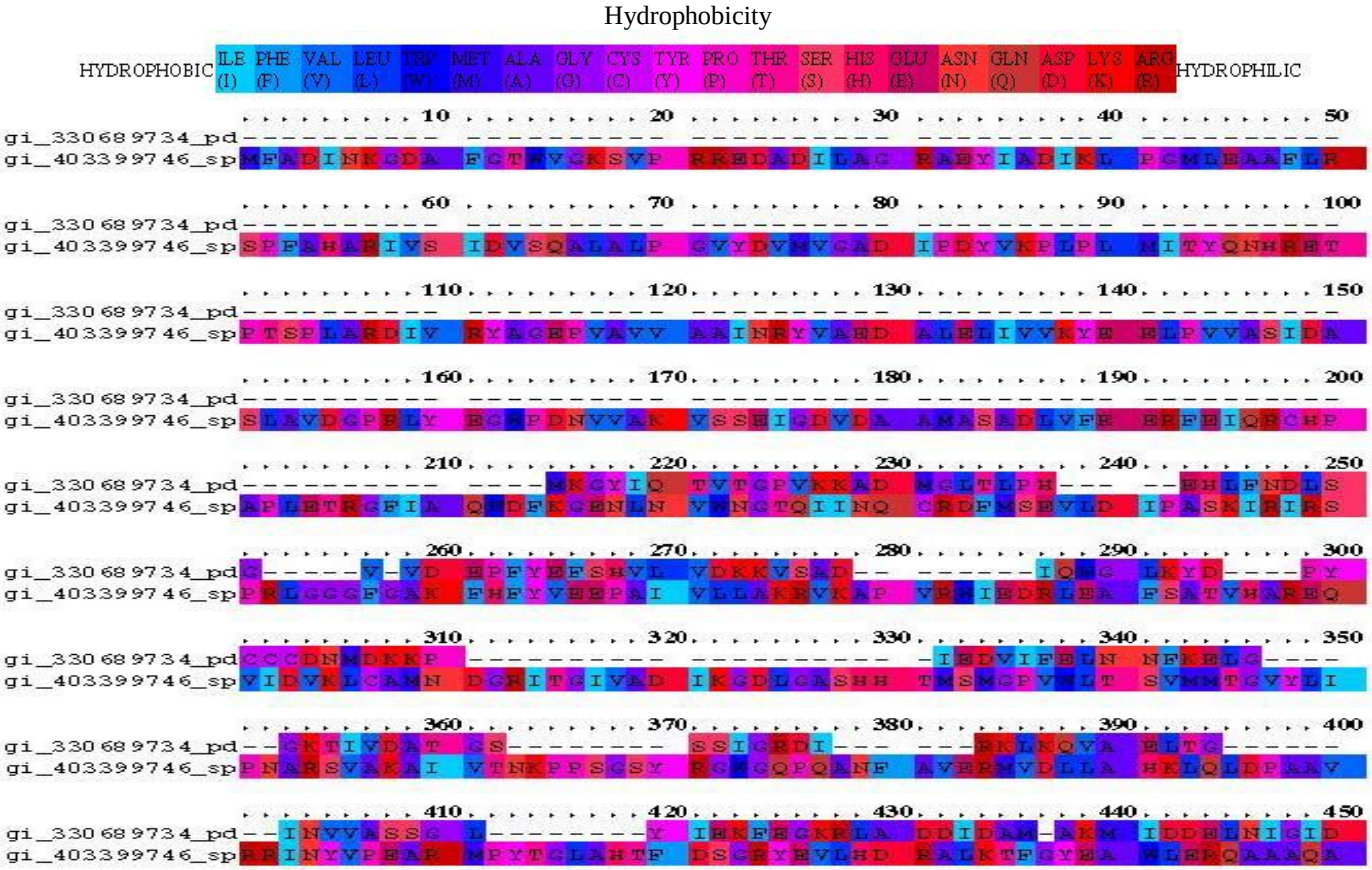

Figure-7
Extent of hydrophobicity predicted of the first 450 base pairs

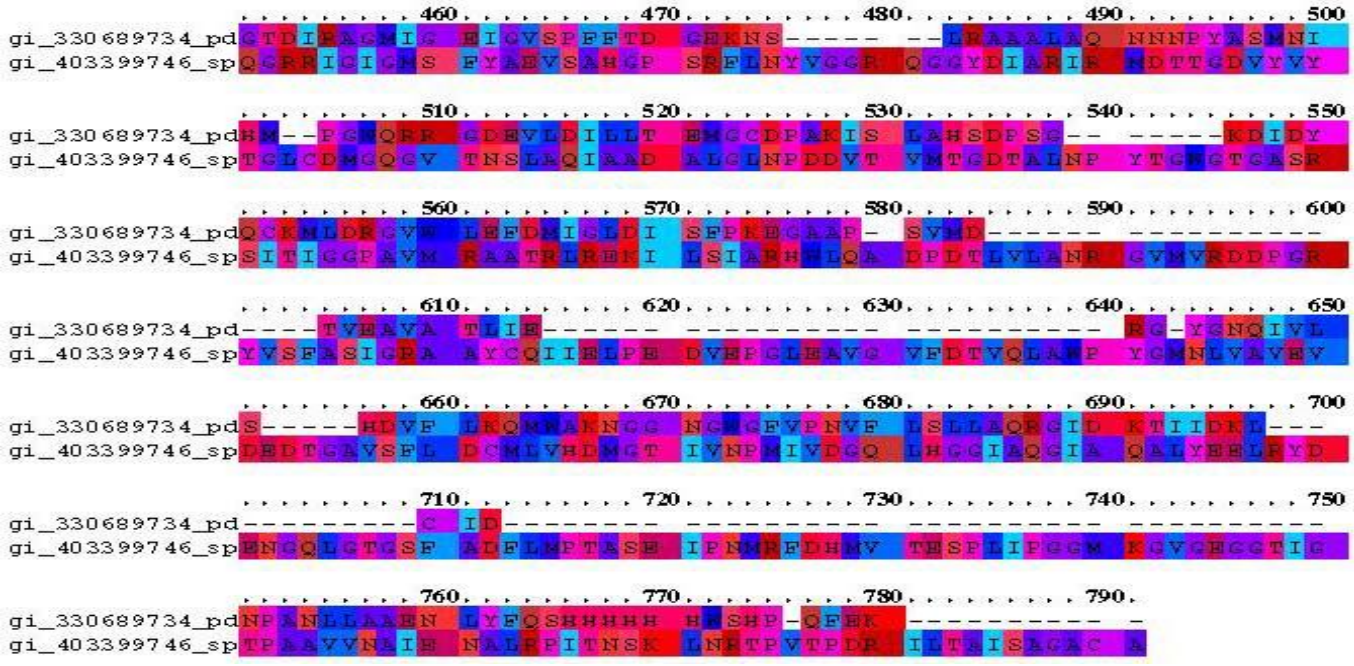

Figure-8
Extent of hydrophobicity predicted of the 451 to 791 base pairs

Comparing these results we can understand and visualize the structure of the caffeinase enzyme. This can help us in carry out protein-protein interaction and molecular docking experiments.
Since the *Proteus mirabilis* protein considered is similar to the caffeine dehydrogenase enzyme, and the regions are conserved it can be said that the function would be the same. Thus, the caffeinase enzyme structure predicted should degrade caffeine.

Conclusion

Preliminary study of caffeine degradation by *Proteus mirabilis SNBS* has been done, including the use of homology modeling in predicting the probable structure of its caffeinase enzyme and determining its conserved sequences, by comparing it with caffeine dehydrogenase sequence. The conserved sequences could represent active sites of the enzyme, which can be proved using docking studies. Also the new enzyme may yield in a different, non-toxic metabolite that could prove to be most suitable for caffeine degradation. Hence further studies on the organism to prove its caffeine degrading ability and on the enzyme to find out its exact nature and action would be a promising scope in the future.

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