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Research Article

Insilico Analysis on Secondary Structural and Functional Predictions of Chitinase Enzyme In *Brassica Juncea*

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Abstract

Andaman and Nicobar Islands consists about 132 species of mustards belonging to 59 genus and much more remain unreported. Determine whether a charged histidine side chain affects Alpha helix stability only when histidine is close to one end of the helix or also when it is the central region, we substitute a single histidine residue at many positions in two reference peptide and measure helix stability and histidine. The fold deviation for the amino acid residues present in all chain of chitinase protein is represented in the form of plot and the structure of fold deviated region in the protein structure was predicted. The Hydrophobic Cys, Phe, Iso, Leu, Met, Val, Try and Hydrophilic Gly, Ser, Thr, Cys, Asp, Glu, Tyr, Pro, Arg, Lys, Gln amino acid residues plays an important role in helix stability. Ten folded region were found in protein structure and the type of fold is predicted as Alpha + Beta class. The fold predicted is based on the disulphide bond present in the structure. *Brassica Juncea* is that it helps in the growth and development of the plant and prevents the plant from fungal attack. In this studies can be used in the future to find the pesticide to prevent the fungal attack and the Bio–fertilizers can be manufactured using chitinase enzyme.

Key Words: Chitinase enzyme, Helix Stability, Hydrophobicity, Motif, Protein Folding, Secondary Structure.

Introduction

The Andaman and Nicobar group of Islands are a vast repository of plant *Brassica juncea* situated 1200 km away from the mainland India in the emerald blue sea of Bay of Bengal. Indian Sub-Continent account for over a thousand species of *Brassica japonica*, *Brasica arvensis*, *Brassica rapa* and *Brassica tournefortii* out of 10,000 to 12,000 Brassica species found world over (Duke, J. A., et.al., 1981and Knowles, P. F., et al., 1981 and Leung, A.Y., 1980). Majority of Brassica species about (150 species) are found in phyto-geographically interesting north eastern hill region. As per the present records, Andaman and Nicobar Islands consists about 132 species of mustards belonging to 59 genus and much more remain unreported. During the survey trips taken the four genus and viz., SIPADAN, MABUL, KAPALAI and BORNEO which have so far not been found in mainland India (Maity, P. K., et al., 1980 and Patel, J. R.., et al., 1980 and Patel, L. M.., et al., 1989).

Brassica juncea, (Fig.1) also known as mustard greens, Indian mustard, Chinese mustard, and leaf mustard, is a species of mustard plant. Sub-varieties include Southern Giant Curled Mustard, which resembles a headless cabbage such as Kale, but with a distinct horseradish-mustard flavor (Elizabeth, Jeffery., et al., 2005 and Anamika, S. S. E., et al., 2009). It is also known as green mustard cabbage. It is a tetraploid having four set of chromosomes in each cell of the plant. The Seeds are globular, dark-brown and rarely yellow (Begum, F., et al., 1995 and Choudhary, B. R., et al**.,** 2008 and Chrungu, B., et al., 1999). This plant flowers in June-July and bears fruits in August-September, after ripening seeds don't germinate. Seeds begin to germinate in the spring. In deeper soil layers seeds remain viable for up to 5 years (Duke, J. A., et al**.,** 2002 and Erickson, L. R., et al**.,** 1983). As day length increases, mustard bolts up with a 3 ft (0.9 m) stalk supporting bright yellow flowers that soon develop into sickle-shaped green seed pods. It can withstand temperatures as low as 20ºF (-4ºC) without damage. It cannot stand temperatures above 85ºF (29 ºC). Tolerates a pH in the range 4.3 to 8.3. The parts of the plants like leaves, seed, root and mustard oil are used in the treatment of disease. The plant seed have antibiotic effect which is used in the treatment of foot ache and tumor. The roots are used in the treatment of cold, fever, and stomach disorders. Leaves are applied in the fore head to relieve head ache (Fitz, John, R. G., et al., 2007 and Gleason, H. A., et al, 1991 and). The mustard oils are used to treat skin eruptions and ulcers (Halldén, C., et al**.,** 1987 and Kunakh, V. A., et al., 2008).

Chitinase was described for the first time in 1911 by Bernard as anti-fungal factor (Lefol, E., et al. 1997.and Moffat, A. S**.,** 1995). The chitinase protein helps plant to defend them against fungal attack .This protein can act directly on fungal and insect affected areas (Plieske, J., et al., 2001). Enzymes present in the protein can be used as free or immobilized form to kill fungi and insects affected areas in plant (Prakash, S, et al., 1980 and Rao, G. U., et al**.** 1996). Most of the chitinase enzyme classes of protein belong to the family glycoside hydrolase 18 and glycoside hydrolase19. The eukaryotic chitinase are grouped in glycoside hydrolase 18 and chitinases of higher plants are grouped in glycoside hydrolase 19. The two families in

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chitinase enzyme contain both endo and exo chitinase. Endochtinases cleaves the chitin molecules randomly and Exochitinases cleaves off chitobiose and these enzymes play a major role in carbohydrate metabolism. Chitinase can attack the chitin molecule which is the main structural component in fungal cell wall and insect's skeleton. In nature, Chitin is found to be in the form of complex with other molecules such as carbohydrate and protein. These proteins are isolated from all organs in plant, tobacco hornworm and several other insect species. The chitinase protein is also being used for the improvement and development of plant. Protein folding is the physical process by which a polypeptide folds into its characteristic and functional three-dimensional structure from random coil.

The mechanism of protein folding is mainly used to describe the single domain protein and it also determines the stability of secondary structural elements and hydrophobicity of the protein sequence. Folding mainly involves two models (Roy, N. N**.,** 1984) Diffusion collision model, Nucleation condensation model, Proteins that follow this mechanism fold in a stepwise manner and involve growing of secondary structure elements (Song, K., et al. 1995 and Song, X., et al., 2010). These elements then collide, combine, strengthen and pack tightly together to form the diffusion collision model. Example: Barnase is the protein that folds in step wise manner and follows the diffusion collision model (Srivastava, A., et al., 2001). Protein following these model have been seen to fold from an unstructured denature state with weak nucleus. The nucleus gets stabilized by amino acid residues and then the primary and secondary structures are formed simultaneously. The nucleus condenses until the folding is complete. For instance, Chymotrypsin inhibitor 2 proteins have been shown to follow the nucleation – condensation model. The most abundant helix type in protein is the alpha helix, accounting for about 31% of amino acid secondary structure state (Vaughan, J. G., et al., 1973 and Velasco, L., et al. 1998). Alpha helices are stabilized because amino acids in the helix can form hydrogen bonds with their amide groups to amide groups directly above and below them in the helix but in aqueous solution these amide bonds would form hydrogen bond with water. So, forming an alpha helix provides little additional stability for the folded state of the structure. The chemical environment around the alpha helix also plays a big role in stabilizing an alpha helix's structure (Alberts, Bruce., et al., 2002).

The hydrophobicity character is the property of a side chain to be less soluble in water than in a polar solvent that is repelled from a mass of water. Hydrophobic materials are used for oil removal from water (Anfinsen, C. 1972 and Robson, B., et al., 2008). The hydrophobic amino acids like Leucine, Isoleucine, Methionine, etc., are often fully buried within the core of the protein, but this happens in frequently for the polar amino acids (Chiti, F., et al., 2006 and Berg, B., et al., 1999). This analysis strongly indicated that the non-polar residues do tend to cluster together within the cores of protein and this provides hydrophobic driving energy for the folding process of protein structure (Lienqueo, M. E., et al., 2002 and Mahn A. et al., 2005).The energy to transfer the side chain of each amino acid from a polar to a non-polar solvent (water to ethanol).Identification of *Brassica juncea* chitinase enzyme.Prediction of protein structure and finding the motif region in the chitinase enzyme (Kyte, J., et al., 1982 and Rose, G., et al., 1985). Analysis of different types of motif in the chitinase enzyme.Finding the hydrophobic and hydrophilic residues region in the protein. Determination of folding classification and it's residues of hydrophobic charged side chain (polar side chain, cysteine side chain and interpretation of force field responsible residues atoms (Cornette, J., et al., 1987).

Fig. 1: Morphological structure of *Brassica Juncea* plant with flowers

Material and Methods

Identifying the Motif Region

Motif region for the chitinase enzyme was identified using Motif 3D and Motif search. The motif 3D was used to identify motif region, polar and non polar group. Motif 3D score for the predicted structure of chitinase enzyme was also analyzed. Motif search was used to find number of motif present in the sequence, motif position and to identify the specific prosite pattern. The protein structure for the chitinase enzyme was predicted using the database PDB SUM (Protein Data Bank). This database provides detailed information about the protein with their structure and function (Alberts, Bruce., et al., 2002 and Anfinsen, C. 1972). Helix wheel and amino acid composition for the protein sequence was being identified for the predicted protein structure using BIOEDIT software. The PDB SUM database is used for the prediction of protein structure. The Helix, Motifs, Disulphide bonds and domains present in the chitinase enzyme structure was analyzed (Robson, B., et al., 2008 and Chiti, F., et al., 2006 and Berg, B., et al., 1999).

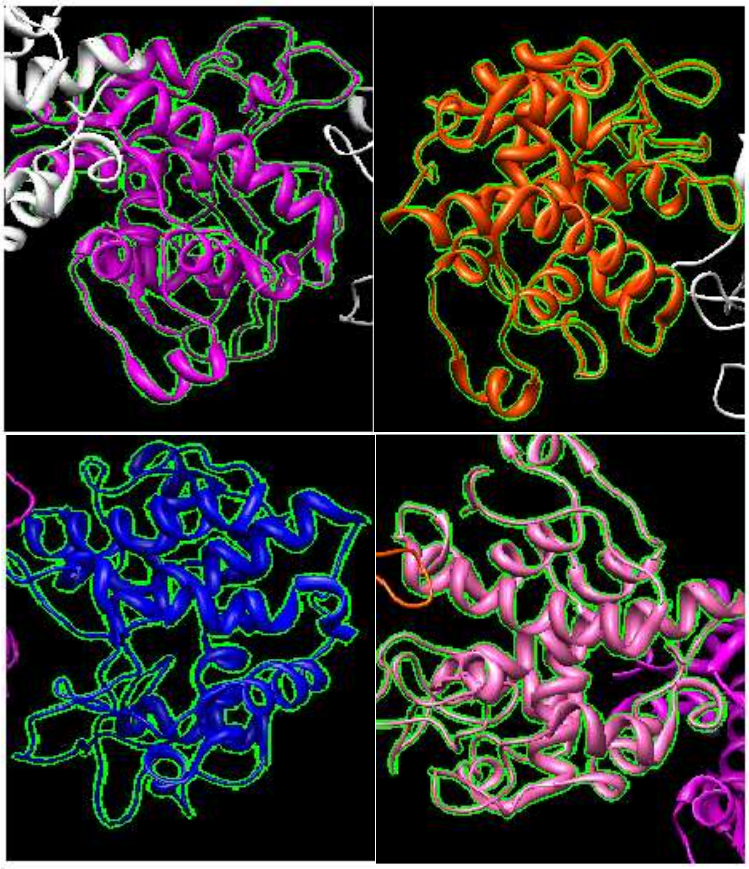

Fig2: Hydrophobic region present in A,B,C, and D chain in the chitinase protein structure. Rose color-Hydrophobic region of A-Chain, Red color-Hydrophobic region of B-Chain, Blue color - Hydrophobic region of C-Chain, Pink color-Hydrophobic region of D-Chain.

Finding the Hydrophobic and Hydrophilic residues region

The 3D structure of the protein was viewed using GENEIOUS 3.5.6 program. The hydrophobic amino acid residues present in the protein was found and were represented in the form of Histogram plot. Hydrophobicity frequency statistics for the non polar residues present in the structure was also analyzed. GENTHREADER is used to find the hydrophobic regions present in the chitinase enzyme. Three different colors in which RED color represent the residues which are highly hydrophobic, BLUE color represents the residues which are hydrophilic (Fig.2). The HELIQUEST program used to find the polar and non – polar residues present in the helical region of protein secondary structure. Helix sequence was retrieved using MOLSOFT software and given as input from this polar and non–polar residues present in the chitinase enzyme helical region was analyzed (Lienqueo, M. E., et al., 2002 and Mahn, A. et al., 2005).

Protein folding

The secondary structure of chitinase protein was predicted. The folded region in the protein was predicted using FOLD pro Recognition tool.It was visualised using MOLSOFT software. The secondary structure of chitinase enzyme was predicted using Chou & Fasman algorithm (Kyte, J., et al., 1982).This is one of the traditional method used to predict secondary structure of protein. Using this algorithm the total number of helices,sheets,turns and coils were found along with it's percentage (Rose, G., et al., 1985). Fold index is a tool which shows the graphical representation of folded, unfolded, Hydrophobic and charged region present in the protein (Fig.3).

Results

Brassica juncea chitinase is an endo-acting, pathogenesis-related protein that is classified into glycoside hydrolase family 19, with highest homology (50–60%) in its catalytic domain to class I plant chitinases. the active-site residues are identified as Glu212 and Glu234. Glu212 is believed to be the catalytic acid in the reaction, whereas Glu234 is thought to have a dual role both activating a water molecule in its attack on the anomeric carbon, and stabilizing the charged intermediate, Chitin, an insoluble homo polymer of β-(1→4)-linked units of *N*-acetyl glucosamine, is one of the most abundant polysaccharides on earth. It is found, for example, in the exoskeletons of insects and in fungal cell walls. Chitinase (EC 3.2.1.14) catalyze the random cleavage of internal glycosidic linkages in chitin chains. In insects, the primary roles of chitinase seem to lie in growth and morphogenesis, because they can break down and so enable rebuilding of chitin-containing structures, as reviewed earlier. In other organisms such as fungi, bacteria and animals, chitinases

appear to have their most important functions in catabolism and development. In plants, these enzymes represent the single largest group of pathogenesis-related proteins; they are strongly upregulated under pathogen attack and in response to other stresses (heat, cold, etc.), and so are thought to represent a vital part of plant defense mechanisms. The Hydrophobic, Hydrophilic amino acid residues their charged side chain with position, Motifs, Type of motif and amino acid residues involved, Protein folding type and classification, Fold deviation, Half life, Functional site of protein, Mutation and Helix stability was analyzed for chitinase protein taken from the plant *Brassica juncea* using different bioinformatics tools and softwares. During these studies the following results were found. The best motif region present in the chitinase enzyme was predicted as (IAKFTAIWFWM). The type of motif with position was also analyzed.

The Hydrophobic (Cys, Phe, Iso, Leu, Met, Val, Try) and Hydrophilic (Gly, Ser, Thr, Cys, Asp, Glu, Tyr, Pro, Arg, Lys, Gln) amino acid residues plays an important role in helix stability. Ten folded region were found in protein structure and the type of fold is predicted as Alpha+Beta class. The fold predicted is based on the disulphide bond present in the structure. The fold deviation also occurs in the chitinase protein and scores for the deviated fold was analyzed for all the amino acid residues present in the protein sequence (Charton, M and Charton, B.I., 1982). The disadvantage of chitinase enzyme is that it has very low half–life because of more than three pest sites in the structure. The half–life of Chitinase enzyme is decreased and it cannot withstand not more than two days. But the chains A, B, C, and D in the Chitinase protein structure have same functional site. The mutation also affects the stability of the structure .The mutation mainly occurs by the change in single amino acid residue in the protein sequence. The Ala, Gly, Asn, Tyr, Phe are the amino acids involved in the chitinase enzyme and makes the protein secondary (helix) structure stable. Force field energy is calculated to find the energy for both the favorable and unfavorable region in the chitinase enzyme structure. The Cys residue at the position 230 has maximum energy in A,B,C and D chain which occurs in favorable region of the chitinase protein this shows that the structure have best fold region at that position. The stability is also more in that region of protein structure.

The force field was used to find energy and stability of protein secondary structure based on fold.The best predicted disulphide bond is at the position (23-85) (97-106) (205-237). As the maximum probability score (0.98227, 0.99541, 0.60965) and distance (5.83,10.81,4.30) for the bonds are at the position (23-85) (97-106) (205-237). Hence the structure is more stable at these position because of protein folding.The helix structure is stable at these position. PDB database was used to find the value of individual amino acid residues when it deviates from its actual fold. The score and position for all the 244 amino acid residues present in chitinase enzyme was found. The fold deviation for the aminoacid residues present in A and B chain of chitinase enzyme is represented in the form of plot and the structure of fold deviated region in the protein structure was also predicted using PDB database. The peaks in the plot represents the fold deviation of each amino acid residue from it's actual fold. The fold deviation for the amino acid residues present in "C" and "D" chain of chitinase enzyme is represented in the form of plot and the structure of fold deviated region in the protein structure was also predicted using PDB database (Fig4). The peaks in the plot represents the fold deviation of each aminoacid residue from its actual fold.

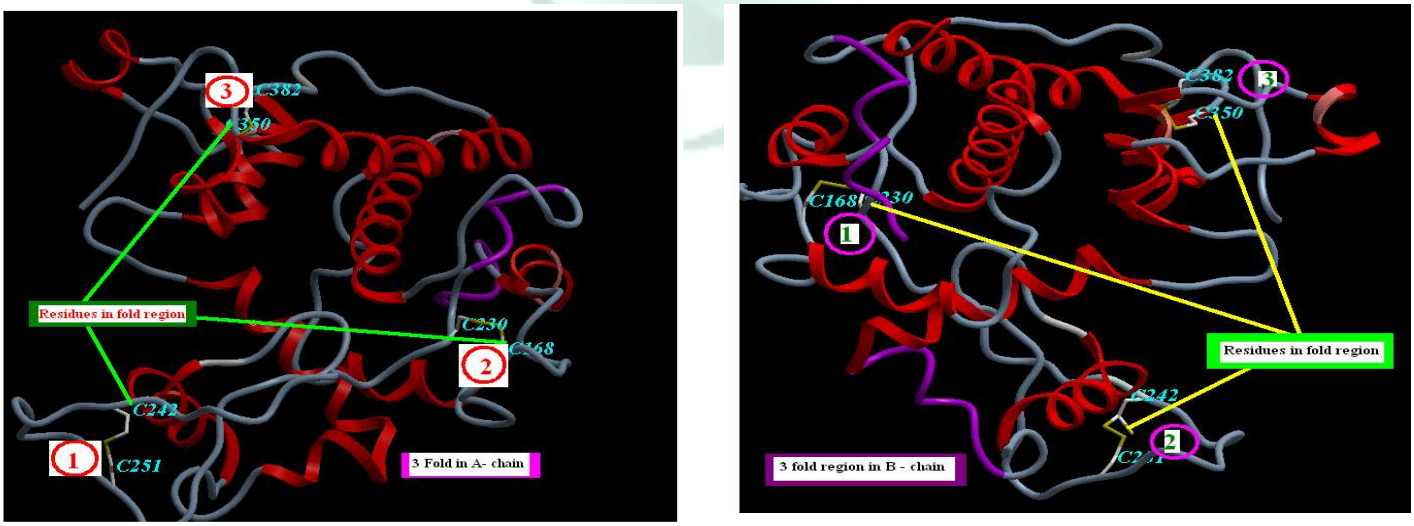

Fig. 3: Disulphide bond and cysteine residues with their position formed between these fold region was also being found.

Conclusion

The *Brassica juncea* is a plant which grows large amount in Andaman and Nicobar Islands. The most of the varieties of *Brassica juncea* grown here are not found in other parts of the country. The infection of this plant is mainly due to the fungus and bacteria. The main symptoms of the diseased plant show the presence of holes in the leaves and black color formation leads to blackleg disease caused by the fungus pythium species. Blackleg can attack at any stage of development. When the symptoms appear it is usually too late and any healthy roots and leaves in the plant are dead. Chemical applications of Subdue MAXX, Banrot are effective in the prevention of Blackleg disease but they affect the growth of the plant. The role of chitinase enzyme in the plant *Brassica juncea* is that it helps in the growth and development of the plant and prevents the plant from fungal attack. The results from the above studies can be used in the future to find the pesticide to prevent the fungal attack and the Bio-fertilizers can be manufactured using chitinase enzyme.

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References

1) Duke, J. A. and Wain, K. K.1981. Medicinal plants of the world, J.Hum.Genet.**3**: 234- 237.

2) Knowles, P. F., Kearney, T. E., and Cohen, D. B. 1981**.** Species of rapeseed and mustard as oil crops in California, AOCS Monograph, and American Oil Chemists' Society. Champaign, IL. **7:** 255–268.

3) Leung, A.Y. 1980. Encyclopedia of common natural ingredients used in food, drugs, John Wiley & Sons. New York. **2:** 23 – 27.

4) Maity, P. K., Sengupta, A. K., and Jana, P. K. 1980. Response of mustard variety varuna (*Brassica juncea*) to levels of irrigation and nitrogen, Indian Agriculturist **24(1):**43–47.

5) Patel, J. R., Parmar, M. T., and Patel, J. C**.** 1980. Effect of different sowing dates, spacings, and plant populations on yield of mustard, FEMS microbial. **25(3):** 526–527.

6) Perry, L. M.. 1989. Medicinal plants of east and Southeast Asia, MIT Press, Cambridge. **4**:20 –22.

7) Elizabeth, Jeffery. 2005. Maximizing The Anti-Cancer Power Of Broccoli, Science Daily, University of Cambridge, **22(4):** 56-59.

8) Anamika, S. S. E. and M. H. Fulekar. 2009. Phytoremediation of cadmium, lead and zinc by *Brassica juncea* ***L***. Czern and Coss, J. Appl. Biosci. **13:**726–736,

9) Begum, F., et al., 1995. Somatic hybrids between *Brassica juncea* (L.) Czern. and *Diplotaxis harra* (Forsk.) Boiss. and the generation of backcross progenies, Theor. Appl. Genet. **91:**1167–1172.

10) Choudhary, B. R.., et al**.,** 2008. Cytogenetics of *Brassica juncea* × *Brassica rapa* hybrids and patterns of variation in the hybrids derivative, Pl. Breed. (New York) **121:**292–296.

11) Chrungu, B., et al., 1999. Production and characterization of interspecific hybrids between *Brassica maurorum* and crop brassicas, Theor. Appl. Genet. **98:**608–613.

12) Duke, J. A., et al**.,** 2002. CRC Handbook of medicinal herbs. (CRC MedHerbs ed2) 23 – 28.

13) Erickson, L. R., et al**.,** 1983. Restriction patterns reveal origins of chloroplast genomes in *Brassica* amphiploids. Theor. Appl. Genet. 65:201–206.

14) Fitz, John, R. G. et al., 2007. Hybridization within *Brassica* and allied genera: evaluation of potential for transgene escape. Euphytica **158:**209–230.

15) Gladis, T. and K. Hammer. 1992. Die Gaterslebener *Brassica*-Kollektion - *Brassica juncea*, *B. napus*, *B. nigra* und *B. rapa*, Feddes Repert. **103:**469–507.

16) Gleason, H. A. and A. Cronquist. 1991. Manual of vascular plants United States and adjacent Canada, ed. 2. (Glea Cron ed2) 34 – 38.

17) Halldén, C., et al**.** 1987. Distribution and evolution of a tandemly repeated DNA sequence in the family Brassicaceae. J. Molec. Evol. **25:**318–323.

18) Kunakh, V. A., et al., 2008. Mixoploidy in wild and cultivated species of Cruciferae capable of hybridizing with rapeseed *Brassica napus*. Cytol. & Genet. **42:**204–209.

19) Lefol, E., et al. 1997. Sexual hybridisation in crosses of cultivated *Brassica* species with the crucifers *Erucastrum gallicum* and *Raphanus raphanistrum*: potential for gene introgression, Euphytica **95:**127–139.

20) Moffat, A. S**.** 1995. Plants proving their worth in toxic metal cleanup**.** Science 269:303.

21) Plieske, J. and D. Struss. 2001. STS markers linked to *Phoma* resistance genes of the *Brassica* B-genome revealed sequence homology between *Brassica nigra* and *Brassica napus*, Theor. Appl. Genet. **102:**483–488.

22) Prakash, S. and K. Hinata. 1980. Taxonomy, cytogenetics and origin of crop brassicas, a review, Opera Bot. **55:**14–16.

23) Rao, G. U., et al**.** 1996. Production of hybrids, amphiploids and backcross progenies between cold-tolerant wild species, *Erucastrum abyssinicum* and crop brassicas, Theor. Appl. Genet. **92:**786–790.

24) Roy, N. N**.** 1984. Interspecific transfer of *Brassica juncea*-type high blackleg resistance to *Brassica* napus, Euphytica **33:**295–303.

25) Song, K., et al. 1995. Rapid genome change in synthetic polyploids of *Brassica* and its implications for polyploid evolution, Proc. Natl. Acad. Sci. U.S.A. **92:**7719–7723.

26) Song, X., et al. 2010.Potential gene flow of two herbicide-tolerant transgenes from oilseed rape to wild *B. juncea* var. *gracilis*, Theor. Appl. Genet. 234 – 237.

27) Srivastava, A., et al. 2001. AFLP-based genetic diversity assessment amongst agronomically important natural and some newly synthesized lines of *Brassica juncea*, Theor. Appl. Genet. **102:**193–199.

28) Vaughan, J. G. and E. I. Gordon. 1973. A taxonomic study of *Brassica juncea* using the techniques of electrophoresis, gas-liquid chromatography and serology, Ann. Bot. (Oxford) **37**:167–184.

29) Velasco, L., et al. 1998. Variability for the fatty acid composition of the seed oil in a germplasm collection of the genus *Brassica*.Genet, Resources Crop Evol. **45:**371–382.

30) Alberts, Bruce, Alexander Johnson, Julian Lewis, Martin Raff, Keith Roberts, and Peter Walters. 2002. The Shape and Structure of Proteins. Molecular Biology of the Cell*;* Fourth Edition. New York and London: Garland Science**. 3:** 23 – 26.

31) Anfinsen, C. 1972. The formation and stabilization of protein structure. Biochem. J.. **128 (4):** 737–49.

32) Robson, B. and Vaithilingham, A. 2008, Protein Folding Revisited. Progress in Molecular Biology and Translational Science, Molecular Biology of Protein Folding. Elsevier Press/Academic Press **84**:161-202.

33) Chiti, F. and Dobson, C. 2006. Protein misfolding, functional amyloid, and human disease, Annual review of biochemistry **75**: 333–366.

34) Berg, B., Ellis, J., and Dobson, C. 1999. Effects of macromolecular crowding on protein folding and aggregation. The EMBO Journal **18**: 6927-6933.

35) Lienqueo, M. E., Mahn, A.V., Asenjo, J.A. 2002. Mathematical Correlations for Predicting Protein Retention Time in Hydrophobic Interaction Chromatography, J. Chromatogr. A **978:**71 – 79.

36) Mahn A. and Asenjo, J. A. 2005. Prediction of protein retention in Hydrophobic Interaction Chromatography, Biotechnol Adv **23 (5):** 359 – 368.

37) Kyte, J. and Doolite, R. 1982. A Simple Method for Displaying the Hydropathic Character of a Protein, J. Mol Biol. **157 :** 105-132.

38) Rose, G., Geselowitz, A., Lesser, G., Lee, R. and Zehfus, M. 1985. Hydrophobicity of Amino Acid Residues in Globular Proteins, Science **229:** 834-838.

39) Cornette, J., Cease, K. B., Margalit, H., Spouge, J. L.,Berzofsky J.L. and DeLisi., C. 1987. Hydrophobicity Scales and Computational Techniques for Detecting Amphipathic Structures in Proteins, J. Mol. Biol. **195:** 659-685.

40) Charton, M and Charton, B.I.. 1982. The Structural Dependence of Amino Acid Hydrophobicity Parameters, J. theor. Biol. **996:** 29-644.

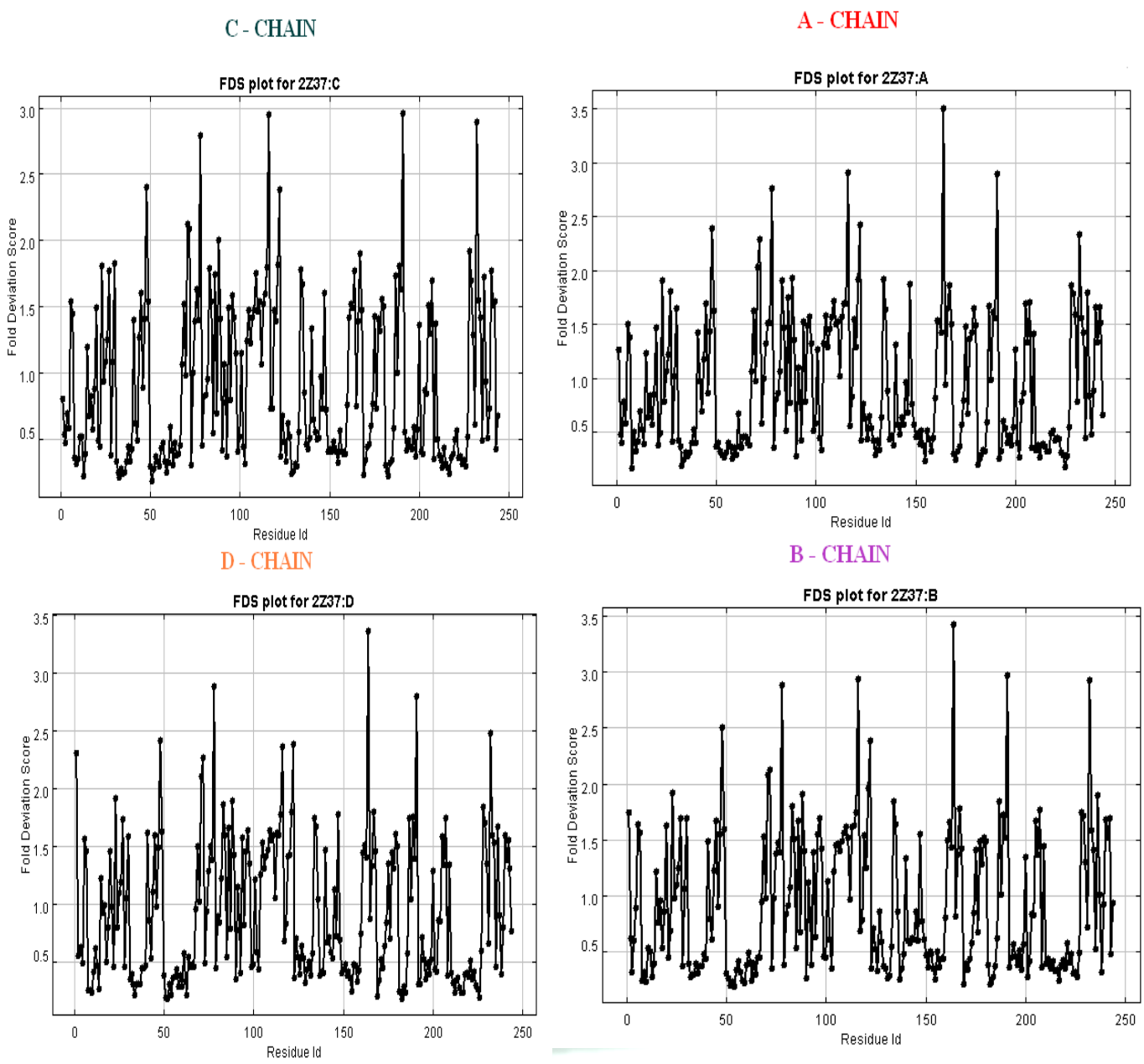

Fig. 4: Protein fold deviation plot for chitinase enzyme