RESEARCH ARTICLE SCIENCE INTERNATIONAL

DOI: 10.5567/sciintl.2013.175.182

# *In silico* Study of Pectin Methylesterase in *Musa acuminata* for Delayed Ripening

[1]Charu Verma, [2]Shipra Gupta, [3]R.K. Singh, [1]Sanjay Mishra

[1]Department of Biotechnology and Microbiology, Institute of Foreign Trade and Management, IFTM Campus, Delhi Road, Moradabad 244001. UP, India

[2]Department of Bioinformatics, Biotech Park, Kursi road, Lucknow, 226015. UP, India

[3]Department of Electronics, Uttrakhand Technical University, Dehradun, UK, India

## ABSTRACT

How to delay fruit ripening is an important biological interest. During transport the fruits get harmed due to their early ripening. On fruit ripening there are many biochemical changes takes place like respiration, ethylene production and activity of cell wall degrading enzymes etc. There are many cell wall degrading enzymes which play an important role in fruit ripening namely PME, PG, PL etc. PME is one of the cell wall degrading enzymes which act on pectin, (a primary cell wall constituent) and releases methanol and hydrogen ions. The objective of this *in silico* study is to inhibit the activity of PME in *Musa acuminata* which leads to their delayed ripening and increases their more availability. Using cross species analysis, we found that banana has maximum homology with carrot. The validation and verification of the designed model has been performed and found that it is good quality model. This model is further used for docking studies. Knowledge about the site at which a ligand binds provides an important clue for predicting the function of a protein and is also often a prerequisite for performing docking computations in virtual drug design and screening. Docking results suggested that Green tea catechin and salicin are the best inhibitors having good interaction energies which binds at third motif i.e., Asp381 residue on active site of PME in *Musa acuminate*. It was found that Green tea catechin is better than salicin because it is a natural inhibitor, antioxidant and inhibits the activity of PME whereas salicin is a chemical compound inhibits ripening at some specific concentration only, although the binding energies of both the inhibitors have approximately similar. This study is used to increase the more availability of banana through delaying its ripening by inhibiting the activity of pectin methylesterase.

**Key words:** Pectin methylesterase, molecular docking, active site, banana, inhibitors, fruit ripening, banana ripening

Science International 1 (5): 175-182, 2013

## INTRODUCTION

The cell wall of the plant cell is composed of polysaccharides (pectins), enzymes and structural proteins. The Pectins includes heterogenous group of polymers namely homogalacturonans, rhamnogalacturonans I and II[1]. On demethylesterification of homogalacturonans, Pectin Methylesterase (PME) releases acidic pectins and methanols. PMEs act on homogalacturonans either randomly (as in fungi) or linearly (as in plants) along the chain of pectin[2]. When PMEs works randomly on homogalacturonans, the demethylesterification occur, this is responsible for the release of protons. Subsequently, the action of released proton towards endopolygalacturonases is responsible for loosening of cell wall[3]. While in case of plants, PME linearly acts on homogalacturonans that releases free carboxyl ions. These ions could interact with $Ca^{2+}$ that helps in creating a pectate gel[4].

PME is bound by electrostatic interactions to the cell wall in plants and the solubilization of the enzymes takes place with the help of high ionic strength solutions[5]. The extraction and purification of PME in different varieties of plants namely banana[6,7], tomatoes[8,9,10,11,12,13,14,15], Oranges[16,17,18,19,20,21,22,23,24], apples[25,26,27,28,29,30,31,32] and grapefruits[33,34,35] has already been done. Previously it has been reported that there are three forms of PMEs in banana namely PME1, PME2 and PME3[36]. It is also shown that the activity of the PME increase during the ripening of the banana[37], apple[38], avocado[39] and papaya[40].

Fruits play a vital role for good human health. Bananas are the rich source of starch on which a large

**Corresponding Author:** Sanjay Mishra, School of Biotechnology, IFTM University, Lodhipur Rajput, Delhi Road (NH-24), Moradabad 244 102, U.P., India

number of populations depends for their food. The biochemical changes such as respiration, ethylene production, activity of cell wall degrading enzymes, biosynthesis of carotenoids, essential oils, flavor and aroma components increases during fruit ripening[37,38,39,40]. Resultant of increased gluconeogenesis, hydrolysis of polysaccharides especially starch, decreased activity and accumulation of sugars and organic acids are the main reasons for the sweetness of banana. A textural alteration during fruit ripening is due to the enzymatic degradation of structural polysaccharides[33,34,35]. During ripening at the pre-climacteric stage of banana, the rate of respiration and ethylene production are low followed by an abrupt increase at the climacteric and at the post climacteric stage again it declines because it is a climacteric fruit. Banana fruit has a high amount of starch which is rapidly degraded into sugars during ripening and due to which a large number of fruits get harmed or degraded during large distance of transportation. Thus the present study was undertaken to project a specific hypothesis to increase the availability of banana through the delay ripening using *in silico* studies. Initially, we have screened potential inhibitors against fruit ripening, using docking studies. Further, selection filters were applied for the identification of a novel inhibitor against PME. Inhibitors have been used to inhibit either the activity of any cell wall degrading enzymes i.e., PME or any substrate which play an important role during the process of ripening.

## MATERIALS AND METHODS

**Sequence retrieval:** Amino acid sequence of PME of *Musa acuminata* (Acc_no: ACQ85264.1) was retrieved from the entrez protein database (http://www.ncbi.nlm.nih.gov/protein) and it has 565 amino acid residues. The 3-D structure of enzyme was designed to inhibit the activity of pectin methylesterase which ultimately responsible for delay the ripening of *Musa acuminate.*

**Cross species analysis:** The cross species analysis across ten plants proteins was performed with ClustalW (http://www.ebi.ac.uk/Tools/msa/clustalw2/) software using default parameters to find a suitable template for predicting the 3D structure of PME of *Musa acuminate.* It also helped in depicting the conserved regions of the proteins. The accession numbers of the plant species were retrieved from NCBI (http://www.ncbi.nlm.nih.gov/) as summarized in Table 1.

**Selection and designing of macromolecule:** The 3D structure of PME of *Musa acuminate* was designed by using carrot (PDB ID: 1GQ8) protein as a template with the help of Modeller9v8. The template was selected by performing protein BLAST[38] using PDB databas[39].

Table 1: Plants selected for cross species analysis

| Plant name | Accession number |
|---|---|
| *Solanum tuberosum* | CBY44654.1 |
| *Nicotiana benthamiana* | AAO85706.1 |
| *Mendicago truncatula* | CAB65290.2 |
| *Arabidopsis thaliana* | CAB87932.1 |
| *Silene latifolia* | CAA69348.1 |
| *Vitis vinifera* | AAD45347.1 |
| *Lycoris amea* | ABJ99595.1 |
| *Oryza sativa* | AAQ20039.2 |
| *Daucous carota* | 1GQ8_A |

Modeller is based on the spatial restraints satisfaction approach[40]. The optimal designed model for PME protein was selected on the basis of DOPE, GA341 scores and lowest Root Mean Square Deviation (RMSD) by superimposing on template. Further, loop, side chain modeling and energy minimization were performed using Swiss PDB Viewer (spdbv) software (http://spdbv.vital-it.ch/).

**Model validation and submission:** Structural Analysis and Verification Server (SAVES) (http://nihserver.mbi.ucla.edu/SAVES/) was used to ensure the quality of the generated 3D structure of enzyme PME. Evaluation and validation of the model were executed with PROCHECK (http://scripts.iucr.org/cgi-bin/paper?gl0276), ERRAT[41], WHAT-IF[42], PROSA[43] and VERIFY3D[44]. Procheck result shows the residues in the allowed region of the Ramachandran plot and Errat depicts the quality of highly refined structures. VERIFY3D postulates the compatibility of the 3D atomic structure of the molecule with its own amino acid sequence. Prove calculates a statistical Z-score deviation for the model from highly resolved (2.0 Å or better) and refined (R-factor of 0.2 or better) crystal structures. Chimera[45], PyMolv0.99 (http://www.pymol.org) and Discovery Studio Visualizer2.5 (http://accelrys.com) software were used for visualizing the 3D structure of proteins.

**Model submission:** The model of the PME1 protein of banana has been submitted to Protein Model Database (http://mi.caspur.it/PMDB).

**Retrieval and preparation of ligand database:** Twelve inhibitors were retrieved from Pubchem database (http://pubchem.ncbi.nlm.nih.gov/) along with their qualitative and quantitative properties such as physiochemical properties were analyzed. The Open Babel software was used to convert the file format (http://openbabel.org). The details of the inhibitors and their properties were summarized in the Table 2.

**Molecular docking:** Molecular docking of screened compounds has been done using Lamarckian genetic

Table 2: Physiochemical properties of selected inhibitors

| Inhibitors | CID | M/W (g moL$^{-1}$) | HBD | HBA | RB | Complexity |
|---|---|---|---|---|---|---|
| 1-MCP | 151080 | 54.090440 | 0 | 0 | 0 | 51.1 |
| Salicylic acid | 338 | 138.120740 | 2 | 3 | 1 | 133.0 |
| Gallic acid | 370 | 170.119540 | 4 | 5 | - | - |
| NAA | 6862 | 186.206600 | 1 | 2 | 2 | 212.0 |
| Vanillic acid | 8468 | 168.146720 | 2 | 4 | 2 | 168.0 |
| PMEI | 451928 | 274.170501 | 3 | 5 | 5 | 397.0 |
| Salicin | 439503 | 286.277820 | 5 | 7 | 4 | 300.0 |
| Ferulic acid | 445858 | 194.184000 | 2 | 4 | 3 | 224.0 |
| Cinnamic acid | 444539 | 148.158620 | 1 | 2 | 2 | 155.0 |
| Green tea catechin extract | 65064 | 458.371720 | 8 | 11 | 4 | 667.0 |
| IAA | 21589 | 189.213840 | 3 | 2 | 2 | 219.0 |
| Ethanol | 702 | 46.068440 | 1 | 1 | 0 | 2.8 |

algorithm inbuilt in Autodock4.2 tools with default parameters. Macromolecule was prepared for the binding energy calculations. The polar hydrogens are added to neutralize the protein whereas non-polar hydrogens are removed from the protein coordinates. Gasteiger partial charges were added to the carbon that held the hydrogen. Aromatic carbon grid map handled the aromatic carbons by reassigning the atom types[21, 22]. Grid map has been generated using a grid of $46 \times 40 \times 52$ spanning the binding pocket of the protein with the grid point spacing of 0.375 Å. The grid map has covered the active site along with significant portions of the surrounding surface[46]. Grid parameter files were built and atom-specific 3D affinity maps was constructed using Autogrid4. The three docking steps (vander Waals interactions, hydrogen bonds and the electrostatic potential) were considered for calculating the binding energy. The relationship between the protein and the ligand has been described by Lamarckian algorithm through orientation, conformation and translation of the ligand. Each docking experiment was derived from 10 different runs that are set to terminate after a maximum of 2,500,000 energy evaluations or 27,000 generations, with a population size of 150 yielding 10 docked conformations. The elitism number, the rate of gene mutation and the rate of gene crossover were 1, 0.02 and 0.8, respectively. After multiple runs, autodock has performed cluster analysis. The docking solutions in complex with inhibitors having all atom RMSDs within 2.0 Å of each other has been clustered together ranking them in descending order of energies. The solution with minimum energy was accepted as the best docked conformation for the binding energy calculations.

**Interaction studies and binding pattern detection:** For the determination of binding energies of complexes, estimated inhibition constant (Ki) was used and ranking was done in the order to their binding scores. The value of Ki was calculated by using Autodock 4.2. For the estimation of binding energy in kcal mol$^{-1}$ the electrostatic energy, vander waals, hydrogen bond, desolvation energy, total intermolecular and torsional energy of binding were used. Chimera, Discovery Studio 3.1 and PyMol software's were used for the visualization of interactions.

## RESULTS

**Cross species analysis:** The cross species analysis of PME enzyme has been performed with the quest to find conserved region as well as to search a suitable template for predicting the 3D structure of PME1.The sequences of ten plant varieties were used for study as mentioned in Table 1. The multiple sequence and cross species analysis of PME sequences (including banana) depicts the presence of all the three conserved regions containing motif three (YQDTL), motif four (DFIFG) and motif five (LGRPW) in different PMEs as given in Fig. 1. These conserved regions are involved in the binding of inhibitors to the protein at their active site. Additionally, Phylogenetic analysis of PMEs of different plants depicts that the *Musa acuminata* has maximum homology with *Daucous carota* (Carrot).

**Model generation and quality assessment:** PME of *Daucous carota* (PDB ID: 1GQ8, R-value 0.181 (obs) and R-Free-0.193) was chosen as a template on the basis of high sequence similarity i.e., 63%, residue and crystal resolution (1.75 Å) (PMID: 11943159). Five models were generated and the model showing the least RMSD with respect to trace (Cα atoms) of the crystal structure of the template, optimal DOPE and GA314 score was selected for further validation. After evaluation, we found that the generated model has high quality both in terms of geometry and stereochemistry. The analysis showed that residues of PMEI in the most favorable region were 70.4%, in allowed region 26.4% and in disallowed region were 0.5% in all the structures. RMSD between the Cα atom of the template carrot and banana is 0.476 Å which indicates the high structural homology (Fig. 2). The G factor of the PMEI is -0.82 which shows that the designed model fits well within the array of a high quality model. Prosa -3.62, Errat 78.228 and Verify_3D 66.74% suggested that the geometric quality, the residue interaction and the energy profile of the structures were well within the limits of reliable structures. Generated model of PME1 shows that the enzyme has seven alpha helices and twenty four beta sheets. The high quality model of *Musa acuminata* has been submitted to pmdb database and has pmdb_id PM0078247.

**Molecular docking studies:** To examine the interaction of inhibitors with the protein PMEI in *Musa acuminate*. Twelve inhibitors were used for this study. The dataset of inhibitors was given in Table 2.

Table 3: Calculated various energies of the different inhibitors

| Ligand name | $BE^e$ | $V_{dw}$-$H_b$-$D_s$ | $I^e$ | $T^d$ | $E^b$ | Calculated $K_i$ |
|---|---|---|---|---|---|---|
| 1Methyl cyclo propane | -2.27 | -2.27 | -2.27 | 0.0 | 0.0 | 21.62 mM |
| Salicylic acid | -4.69 | -3.59 | -0.35 | +0.82 | -1.57 | 363.50 µM |
| Gallic acid | -6.40 | -5.71 | -0.46 | +1.37 | -1.60 | 20.39 µM |
| NAA | -2.26 | -2.26 | +0.00 | +0.00 | +0.00 | 22.08 mM |
| Vanillic acid | -2.25 | -2.87 | -0.34 | +1.10 | -0.14 | 22.27 mM |
| PME1 | -1.46 | -1.61 | -0.84 | +1.92 | -0.93 | 84.92 mM |
| Salicin | -9.29e+06 | +4.59e+05 | +1.12e+05 | +2.47 | -9.86e+06 | 0.00e+00 M |
| Ferulic acid | -1.45 | -2.62 | -0.37 | +1.37 | +0.17 | 87.22 mM |
| Cinnamic acid | -5.23 | -4.41 | -0.04 | +0.82 | -1.59 | 147.78 µM |
| Green tea catechin extract | -9.05e+06 | +0.00 | +3.45e+05 | +3.29 | -9.40e+06 | 0.00e+00 M |
| Indole-3 acetic acid | -5.20 | -5.40 | -0.21 | +0.82 | -0.41 | 155.51 µM |
| Ethanol | -2.21 | -2.33 | +0.00 | +0.27 | -0.15 | 24.10 mM |

$E^b$: The electrostatic component of binding free energy in kcal $mol^{-1}$, $V_{dw}$-$H_b$-$D_s$: Vander waals, hydrogen bond-desolvation energy component of binding free energy in kcal $mol^{-1}$, $I^e$: Total internal energy of binding in kcal $mol^{-1}$, $T^d$: Torsional energy of binding in kcal $mol^{-1}$, $K^i$: Estimated inhibition constant in molar, $BE^e$: Estimated binding free energy in kcal $mol^{-1}$

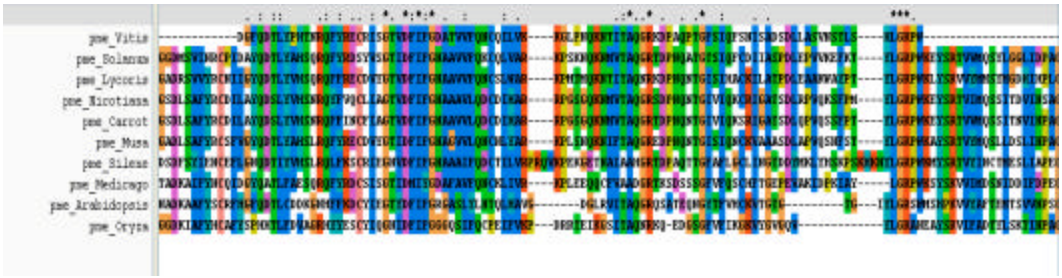


Fig. 1: Cross species analysis shows the three conserved motifs present in the PME of different plants

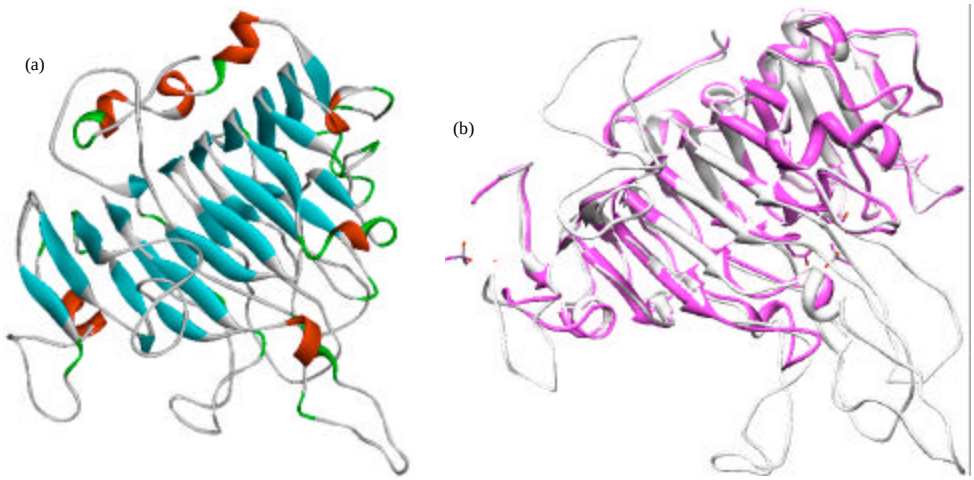


Fig. 2(a-b): (a) The generated model of PME with 78.228% quality factor in cartoon representation and (b) In this superimpose model the gray color is representing the protein model (ACQ85264) and magenta color representing the template (1GQ8). The deviation between both the models is found to be 0.476 Å

**Docking studies and energy calculations:** Docking has been performed between twelve inhibitors to the PME protein in Musa acuminate by using Auto Dock 4.2 tool. After docking the binding energies and inhibitory constant of each ligand has been calculated (Table 3). The results of binding energies have been used to determine the optimal docking energy conformation. Previously, it has shown that there are five conserved regions of the

catalytic C-terminal domain. Asp381 was found in motif three (YQDTL) and has an important role in active site of bacterial and plant PMEs but not found to be conserved in all PMEs. Asp402 residue of motif four DFIFG) and Arg470 and Trp472 of motif V (LGRPW) are also involved in the active sites, whereas Phe403 is found to be involved in the substrate binding[47].

Our study depicts that Asp381 residue was mainly interacting residue that is involved in binding of substrate with enzyme. The electrostatic component of binding free energy, Vander waals, hydrogen bond, desolvation energy, total intermolecular energy, torsional energy and gibbs free energy of binding were estimated for each inhibitors. The value of Ki (inhibitory constant) was calculated. The lower the value of Ki is directly proportional to the docking energy and inversely proportional to the binding affinity.

**Protein ligand interaction:** On the basis of energy values for different protein inhibitor complexes, two out of twelve inhibitors have low binding energy and thus they were selected as good inhibitors for PME1 in *Musa acuminata*. Initially the binding energy of Salicylic acid, Gallic acid, IAA, Salicin and Green tea catechin were very low and could be considered as good inhibitors but some of them have no interacting capacity with the active site pocket. The binding energy of 1-MCP and ethanol were high so they were not included. The binding energies of salicylic acid, gallic acid, IAA were good but they do not show any interaction with the active region. The complex formed by salicylic acid, Cinnamic acid, IAA, gallic acid and ethanol also shows the interaction but it was not present at the active site region. They showed the interaction at Ser387 residue. Although, it has also low energy, due to the absence of any interaction at the active site, it was also discarded. The complex formed by PMEI, Ferullic acid and Vanillic acid showed the pi interaction at active site region i.e., at Arg470 V motif of LGRPW but having very high energies so they were also discarded. The docking energies of Salicin and Green tea catechin extract have very low energy and they were showing the interaction at the active site regions. Both of Salicin and Green tea catechin extract showed the binding on 3rd residue (YQDTL) at position Asp381 i.e., active site of PME1 in *Musa acuminate*. According to our results the binding energy of Salicin and Green tea catechins are very low and are considered as good inhibitors.

## DISCUSSION

The main finding of the present study is to detect an inhibitor (Green tea catechin) against PME for *Musa acuminate*. At the time of transportation, a large number of fruits gets harmed or destroyed. We are trying to increase the availability of fruits mainly banana through delayed ripening by inhibiting the activity of PME. Our *in silico* study resulted in the detection of inhibitors having high specificity and binding affinity. It was already emphasized that there may be five conserved regions of the catalytic C-terminal domain in banana PME. Asp136 amino acid is found in the third motif (YQDTL) of PME which has also proposed function in the activity of bacterial and plant. The Asp of motif IV (DFIFG), Arg225 and Trp227 of motif V (LGRPW) might be involved in the active site of PME[47]. On the basis of cross species analysis of PME in different plants, it was observed that PME *of Musa acuminate* have maximum identity with Carrot (PDB id. 1GQ8/A), we used the PME of carrot as template to design the model. The Quality factor, residue interactions and energy profile of generated model is appropriate within the limits established for reliable structure. Further the structural information of our developed model of PME in *Musa acuminata* was used for molecular docking studies of several compounds *in spite* of identifying the appropriate inhibitor of PME.

Previously it has been seen that, Salicylic acid and Gallic acid were the most effective inhibitors of ripening of tomato fruit. The inhibition of ripening increased as the concentration of both increased above 25 uM[48]. The activity of Peroxidase (E.C.1.11.1.7) was inhibited by Ferulic acid more effectively at high concentration[49]. This enzyme plays an important role in a variety of physiological process such as ethylene biogenesis, cell development, membrane integrity etc.[49]. Vanilic acid inhibits ripening at an appropriate concentration in tomato plant. It showed low extent of inhibition at both low and high concentration and promote ripening whereas it inhibits ripening at some specific concentration (13 µM)[48]. It has been seen that at low concentration (10 and 25 µM) salicin was found to be less effective in delaying tomato ripening but it was more effective at high concentration (250-500 µM). A green tea component namely epigallocatechin gallate (EGCG) act as a natural inhibitor for PME. EGCG blocked the esterase activity of pure PME as well as PME extracts from citrus and from parasitic plant[50]. PMEI was a good inhibitor of PME and also showed the interaction at active site whereas the interaction energy is found to be poor. On the basis of our results and previous studies it was concluded that most of the inhibitors do not showed the interactions at active site pocket of PME. Some of them showed interaction but have poor docking energies. So, we are not considered them as good inhibitors for delayed ripening. Green tea catechin inhibits the activity

of PME and also has low energy due to which it could be considered as best inhibitor for dealayed ripening in *Musa acuminata*. We have proposed that the activity of PME is greatly inhibited by Green tea catechin which plays an important role in delayed ripening of Banana.

We used twelve inhibitors of ripening and on the basis of our docking results; it was found that only five inhibitors (such as Green tea catechins, Salicin, Ferulic acid, PMEI and Vanilic acid) showed the binding at the active site pocket of PME in banana. Remaining inhibitors has been discarded due to not showing interactions with the active site of the protein. Ferullic acid, vanillic acid and PMEI inhibitors showed the interaction at Arg470 (V motif LGRPW) whereas green tea catechin and salicin showed the interaction at Asp381 (IV motif DFIFG) of active site of PME. Among all these five, Green tea catechin and Salicin are the more appropriate inhibitors because they both have very low binding energies i.e., -9.05e+06 and -9.29e+06 Kcal $mol^{-1}$, respectively. PMEI, ferulic and vanilic acid showed the interactions at the active site but do not have good interaction energies.

In Summary, we emphasized that green tea catechin serves as an inhibitor of PME in *Musa acuminate*. On the basis of these studies, we proposed that the compound could efficiently inhibit the functional activity of PME, The new findings are shaping our impression on possibilities, challenges and opportunities for delayed ripening of *Musa acuminate*. The present strategy of screening inhibitors based on their active site interaction through molecular docking against PME that plays important role in fruit ripening. This strategy can possibly be used for *in silico* inhibitor screening of compounds against other enzymes which participate in fruit ripening. The usefulness of the selected inhibitor for PME will eventually be determined by their abilities to inhibit or delayed ripening of banana to make their more availability and less harmed during transportation.

## ACKNOWLEDGMENT

The present study is a part of Ph.D. program of author (CV) who is registered at Uttarakhand Technical University, Dehradun, UK, India.

## ABBREVIATIONS

PME : Pectin methylesterase
PMEI : Pectin methylesterase inhibitor
PDB : Protein database
RMSD : Root mean square deviation

## REFERENCES

1. Perez, S., K. Mazeau and C. Herve du Penhoat, 2000. The three-dimensional structures of the pectic polysaccharides. Plant Physiol. Biochem., 38: 37-55.
2. Markovie, O. and R. Kohn, 1984. Mode of pectin deesterification by *Trichoderma reesei* pectinesterase. Experientia, 40: 842-843.
3. Moustacas, A.M., J. Nari, M. Borel, G. Noat and J. Ricard, 1991. Pectin methylesterase, metal ions and plant cell-wall extension. The role of metal ions in plant cell-wall extension. Biochem, 279: 351-354.
4. Goldberg, R., C. Morvan, A. Jauneau and M.C. Jarvis, 1996. Methyl-esterification, de-esterification and gelation of pectins in the primary cell wall. Progress Biotechnol., 14: 151-172.
5. Giovane, A., L. Quagliuolo, L. Servillo, C. Balestrieri, B. Laratta, R. Loiudice and D. Castaldo, 1993. Purification and characterization of threeisozymes of pectin methylesterase from tomato fruit. J. Food Biochem., 17: 339-349.
6. Markovic, O., K. Heinrichova and B. Lenkey, 1975. Pectolytic enzymes from banana. Collect. Czech. Chem. Commun., 40: 769-774.
7. Brady, C.J., 1976. The pectinesterase of the pulp of the banana fruit. Austral J. Plant Physiol., 3: 163-172.
8. Delingee, H. and B.J. Radola, 1970. Some size and change properties of tomato pectin methylesterase. Biochem. Biophys. Acta., 214: 178-189.
9. Gaffe, J., D.M Tieman and A.K. Handa, 1994. Pectin methylesterase isoforms in tomato (*Lycopersicon esculentum*) tissues. Plant Physiol., 105: 199-203.
10. Markovic, O. and H. Jornvall, 1986. The primary structure of the tomato enzyme. Eur. J. Biochem., 158: 455-462.
11. Nakagawa, H., Y. Yanagawa and H. Takehana, 1970. Studies on the pectolytic enzyme part IV. Purification of tomato fruit pectinesterase. Agric. Biol. Chem., 34: 991-997.
12. Pressey, R. and J.K. Avants, 1972. Multiple forms of pectinesterase in tomatoes. Phytochemistry, 11: 3139-3142.
13. Tucker, G.A., N.G. Robertson and D. Grierson, 1982. Purification and changes in activities of tomato pectinesterase isoenzymes. J. Sci. Food Agric., 33: 396-400.
14. Warrilow, A.G.S., R.J. Turner and M.G. Jones, 1994. A novel form of pectinesterase in tomato. Phytochemistry, 35: 863-868.
15. Warrilow, A.G.S. and M.G. Jones, 1995. Different forms of tomato pectinesterase have different kinetic properties. Phytochemistry, 39: 277-282.
16. Baron, A., A. Benguedouar and C. Prioult, 1991. Purification et proprieties des pectinmethylesterase de mandarine (*Citrus nobilis* Lour.). Lebensm Wiss Technol., 24: 459-465.

17. Cameron, R.G. and K. Grohmann, 1996. Purification and characterization of a thermally tolerant pectin methylesterase from a commercial Valencia fresh frozen orange juice. J. Agric. Food Chem., 44: 458-462.
18. Evans, R and D. Machale, 1978. Multiple forms of pectinesterase in limes and oranges. Phytochemistry, 17: 1073-1075.
19. Hou, W.N., Y. Jeong, B.L. Walker, C.I. Wei and M.R. Marshall, 1997. Isolation and characterization of pectinesterase from Valencia orange. J. Food Biochem., 21: 309-333.
20. Korner, B., G. Zimmermann and Z. Berk, 1980. Orange pectinesterase: Purification, properties and effect on cloud stability. J. Food Sci., 45: 1203-1206.
21. Manabe, M., 1973. Purification and properties of citrus natsu daidai pectinesterase. Agric. Biol. Chem., 37: 1489-1491.
22. Rillo, L., D. Castaldo, A. Giovane, L. Servillo, C. Balestrieri and L. Quagliuolo, 1992. Purification and properties of pectin methylesterase from mandarin orange fruit. J. Agric. Food Chem., 40: 591-593.
23. Versteeg, C., F.M. Rombouts and W. Pilnik, 1978. Purification and some characteristics of two pectinesterase isoenzymes from orange. Lebensm Wiss Technol., 11: 267-274.
24. Wicker, L., M.R. Vassallo and E.J. Echeyerria, 1988. Solubilization of cell wall bound, thermostable pectinesterase from Valencia orange. J. Food Sci., 53: 1171-1180.
25. Al-Delaimy, K.S. and S.H. Ali, 1969. Pectinesterase extraction from Sharabi apples. J. Sci. Food Agric., 20: 660-661.
26. Castaldo, D., L. Quagliuolo, L. Servillo, C. Balestrieri and A. Giovane, 1989. Isolation and characterization of pectin methylesterase from apple fruit. J. Food Sci., 54: 653-673.
27. Denes, J.M., A. Baron and J.F. Drilleau, 2000. Purification, properties and heat inactivation of pectin methylesterase from apple (cv Golden Delicious). J. Sci. Food Agric., 80: 1503-1509.
28. King, K., 1990. Partial characterization of the *in situ* activity of pectinesterase in Bramley apple. Int. J. Food Sci. Technol., 25: 188-197.
29. King, K., 1991. Characteristics of pectinesterase isolated from Bamley apple waste. J. Sci. Food Agric., 57: 43-48.
30. Lee, Y.S and R.C. Wiley, 1970. Measurement and partial characterization of pectinesterase in apple fruits. J. Am. Soc. Hortic. Sci., 95: 465-468.
31. Macdonald, H.C. and R. Evans, 1996. Purification and properties of apple pectinesterase. J. Sci. Food. Agric., 70: 321-326.
32. Miyairi, K., T. Okunu and K. Sawai, 1975. Purification and physic-chemical properties of pectinesterase of apple fruits (Ralls). Bull. Fac. Agric. Hirosaki. Univ., 24: 22-30.
33. Grohmann, R.G.K., 1995. Partial purification and thermal characterization of pectinesterase from red grapefruit finishes pulp. J. Food Sci., 60: 821-825.
34. Robertson, G.L., 1976. Pectinesterase in New Zealand grapefruit juice. J. Sci. Food Agric., 27: 261-265.
35. Seymour, T.A., J.F. Preston, L. Wicker, J.A. Lindsay and M.R. Marshall, 1991. Purification and properties of pectinesterase of marsh white grapefruit pulp. J. Agric. Food Chem., 39: 1080-1085.
36. Hultin, H.O. and A.S. Levine, 1965. Pectin methyl esterase in the ripening banana. J. Food Sci., 30: 917-921.
37. DeSwardt, G.H. and E.C. Maxie, 1967. Pectin methylesterase in the ripening banana. S. Afr. J. Agric. Sci., 10: 501-506.
38. Altschul, S.F., W. Gish, W. Miller, E.W. Myers and D.J. Lipman, 1990. Basic local alignment search tool. J. Mol. Biol., 215: 403-410.
39. Berman, M.H., J. Westbrook, Z. Feng, G. Gilliland and T.N. Bhat *et al*., 2000. The protein data bank. Nucl. Acids Res., 28: 235-242.
40. Sali, A. and J.P. Overington, 1994. Derivation of rules for comparative protein modeling from a database of protein structure alignments. Protein Sci., 3: 1582-1596.
41. Colovos, C. and T.O. Yeates, 1993. Verification of protein structures: Patterns of nonbonded atomic interactions. Protein Sci., 2: 1511-1519.
42. Vriend, G., 1990. What If: A molecular modeling and drug design program. J. Mol. Graphics, 8: 52-56.
43. Sippl, M.J., 1993. Recognition of errors in three-dimensional structures of proteins. Proteins, 17: 355-362.
44. Bowie, J.U., R. Luthy and D. Eisenberg, 1991. A method to identify protein sequences that fold into a known three-dimensional structure. Science, 253: 164-170.
45. Pettersen, E.F., T.D. Goddard, C.C. Huang, G.S. Couch, D.M. Greenblatt, E.C. Meng and T.E. Ferrin, 2004. UCSF chimera-a visualization system for exploratory research and analysis. J. Comput. Chem., 25: 1605-1612.
46. Morris, G.M., D.S. Goodsell, R.S. Halliday, R. Huey, W.E. Hart, R.K. Belew and A.J. Olson, 1998. Automated docking using a lamarckian genetic algorithm and empirical binding free energy function. J. Computat. Chem., 19: 1639-1662.

47. Mbeguie-A-Mbeguie, D., O. Hubert, F.C. Baurens, T. Matsumoto, M. Chillet, B. Fils-Lycaon and S. Sidibe-Bocs, 2009. Expression patterns of cell wall modifying genes from banana during fruit ripening and in relationship with finger drop. J. Exp. Bot., 60: 2021-2034.
48. Obasi, M.O., 2005. Effects of Benzoyl and Salicyl compounds on ripening of physiologically mature green tomato fruit in storage. J. Plant Prod. Res., 9: 19-22.
49. Valdir, A.N., 2002. Ionically bound peroxidase from peach fruit. J. Brazilian Arch. Bio. Technol., 45: 7-16.
50. Lewis, K.C., T. Selzer, C. Shahar, Y. Udi, D. Tworowski and I. Sagi, 2008. Inhibition of PME activity by Green Tea Catechin. J. Phytochem., 69: 2586-2592.