Computational analysis of difenoconazole interaction with soil chitinases

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Computational analysis of difenoconazole interaction with soil chitinases

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Abstract. This study focusses on the investigation of the potential binding of the fungicide difenoconazole to soil chitinases using a computational approach. Computational characterization of the substrate binding sites of Serratia marcescens and Bacillus cereus chitinases using Fpocket tool reflects the role of hydrophobic residues for the substrate binding and the high local hydrophobic density of both sites. Molecular docking study reveals that difenoconazole is able to bind to Serratia marcescens and Bacillus cereus chitinases active sites, the binding energies being comparable.

1. Introduction

Soil fertility is usually described in terms of the chemical, physical and biological properties such as: pH, cation exchange capacity, texture, structure, water holding capacity, diversity of soil microorganism, etc. All these properties are correlated and impact the crop yields.

The researches of the last years proved the involvement of the soil microorganisms in soil fertility and there are divergent opinions in the specific literature concerning the beneficial and/or detrimental impact of fertilizers and pesticides to soil biology. There are numerous available products and their effects on the soil biology are dependent on the types of products used and also on the application rates adopted [1-4]. There also are human health and environmental impact concerns caused by the fertilizers and pesticides use in agriculture.

Fungicides enter is soil, they are the subject of degradation processes and they also are indicated to have a greater impact on soil microorganisms than other pesticides [5]. Within the present study we focus on the assessment of the effect of the fungicide difenoconazole on soil chitinolytic activity using a molecular docking approach.

Difenoconazole (DFC) is a fungicide belonging to the triazole family and managing to control a comprehensive range of fungi causing diseases of field crops [6]. It usually comes into contact with
soil, where it may undergo a variety of transformations and/or causes the loss of soil fertility or environmental damages [7].

Chitinases belong to the family 18 and 19 of the enzymes glycosyl hydrolases being present in a wide range of organisms (fungi, bacteria, plants, humans) that require the reshaping of their own chitin or dissolve and digest the chitin of fungi or animals. [8]. Soil bacteria are usually known to possess chitinolytic activity. Soil chitinases are mainly used for degradation of chitinous fungal cell walls (the main source of the soil chitin) and its utilization as an energy source [8]. They are also used for antagonistic interactions with living fungi, this function being particularly important in the rhizosphere where bacteria and fungi compete for root exudates [9]. Among species of soil bacteria that produce chitinolytic enzymes are *Serratia sp* [10] and *Bacillus sp* [11]. Consequently, we use the molecular docking approach to investigate the possible interaction of DFC with chitinase A from *Serratia marcescens* and from *Bacillus cereus* respectively.

2. Methodology

There are 13 entries in the Protein Data Bank (PDB) [12] concerning crystallographic structures of the native, mutants and complexes with substrates of the *Serratia marcescens* [13] and *Bacillus cereus* chitinases A [14], the corresponding codes entry being: 1CTN, 1EDQ, 1EIB, 1EHN, 1FFR and 1FFQ for the *Serratia marcescens* chitinase A [13] and 3N11, 3N12, 3N13, 3N15, 3N17, 3N18 and 3N1A for the *Bacillus cereus* chitinase A [14], respectively. The structural files of the *Bacillus cereus* chitinase A do not contain the chitin binding and insertion domains [14] but the structural files of *Serratia marcescens* chitinase A contain these domains [13].

The comparison of the considered structures is performed using structures superposition tool under Chimera package [15] and expressed as the root mean square deviation (RMSD) between the equivalent alpha carbon (CA) atoms of the superposed structures [16].

Identification and characterization of the active site cavities of the two enzymes have been performed using Fpocket tool [17].

The computation of the interactions of DFC with *Serratia marcescens* and *Bacillus cereus* chitinases A is performed using SwissDock tool [18], a web-based interface that outcome the most favourable binding modes of the ligand on the protein surface ranked upon the interaction energy expressed as FullFitness score. Preparation of both the protein and ligand for docking in addition to visualization of the binding modes acquired using the SwissDock web-server have been also performed using Chimera package. Selected docking type was „accurate” and we have performed flexible docking.

3. Results and discussions

We have considered all the structural files mentioned above, except the 3N1A structural file because it corresponds to the same mutation of the enzyme as the 3N18 file. For every considered structural file the ligand (if present) has been removed for molecular docking studies.

There are very small differences between the considered structures for every enzyme: the RMSD values are in the interval (0.365÷0.460)Å for the superposition of 538 equivalent CA atoms of *Serratia marcescens* chitinases A and in the interval (0.216÷0.278)Å for the superposition of 320 equivalent CA atoms of *Bacillus cereus* chitinases A, respectively. The superposition of *Serratia marcescens* and *Bacillus cereus* chitinases A gives RMSD values in the interval (1.118÷1.163)Å for 73 equivalent CA atoms.

The active sites cavities of the two enzymes are identified and characterized using the Fpocket tool. These cavities are highly hydrophobic, the local hydrophobic density being 59.76 for the *Serratia marcescens* chitinase A and 59.67 for the *Bacillus cereus* chitinase A respectively. The high local hydrophobic character of these cavities correlates to the hydrophobic character of DFC with the partition coefficient logP=4.92 (Pesticide Properties DataBase, http://sitem.herts.ac.uk/aeru/ppdb/en/).
SwissDock outcomes for the interactions of the DFC with *Seratia marcescens* and *Bacillus cereus* chitinases A as well as with their mutants, expressed as the active site binding modes over the total binding modes of the DFC to the protein, the FullFitness scores and the solvation energies are presented in the table 1. All these data reflect that DFC is able to bind to the active site of *Seratia marcescens* and *Bacillus cereus* chitinases A, the interaction with chitinase A from *Seratia marcescens* being stronger. Also, zinc ions presence in the structural file favorites the interaction of DFC with *Bacillus cereus* chitinase A.

| Enzyme                  | PDB code | active site binding poses / total binding poses | FullFitness score [kcal/mol] | ΔG [kcal/mol] |
|-------------------------|----------|-----------------------------------------------|------------------------------|--------------|
| *Seratia marcescens* chitinase A | 1CTN     | 16/37                                        | -1965.38                     | -8.09        |
|                         | 1EDQ     | 3/42                                         | -1961.94                     | -7.72        |
|                         | 1FFQ     | 4/42                                         | -1928.21                     | -7.16        |
|                         | 1FFR     | 3/35                                         | -1950.75                     | -7.15        |
|                         | 1EIB     | 3/35                                         | -1924.21                     | -7.91        |
|                         | 1EHN     | 17/40                                        | -1925.46                     | -8.10        |
| *Bacillus cereus* chitinase A | 3N11     | 21/34                                        | -1263.84                     | -7.13        |
|                         | 3N12     | 6/34                                         | -1863.48                     | -6.86        |
|                         | 3N13     | 43/44                                        | -1222.91                     | -7.63        |
|                         | 3N15     | 26/34                                        | -1235.51                     | -7.88        |
|                         | 3N17     | 18/36                                        | -1228.74                     | -7.69        |
|                         | 3N18     | 46/49                                        | -1180.76                     | -7.98        |

In addition, the punctual mutations of a few amino acids belonging to the active site of considered chitinases (ASP143, GLU145 and TYR227 for the *Bacillus cereus* chitinase A and ASP311, GLU315 and TYR390 for the *Seratia marcescens* chitinase A) negatively affect their interactions with DFC. Experimental data prove that these mutations also have a negative effect for the substrates binding to these enzymes [13, 14].

Previous computational studies reflected that DFC is also able to bind to the active site of the *Bacillus pasteurii* urease, another important enzyme in maintaining the biological properties of soil [19]. Dose dependent response to the DFC application has been also observed for soil dehydrogenase activity, high doses of DFC having inhibitory effect [20].

4. Conclusions
The fungicide difenoconazole provides the ability to bind to the active sites of the *Seratia marcescens* and *Bacillus cereus* chitinases A suggesting its possible inhibitor effect on the chitinolytic activity of soil bacteria. Zinc ions are known to have an inhibitor effect on the GH18 family of glycosyl hydrolases [21] and their presence in the structure of the *Bacillus cereus* chitinase A results in a stronger interaction of the DFC with the enzyme and consequently the inhibitory effect of the chitinolytic activity is stronger.

The results of this study provide insight in the possible inhibitory effect of the fungicide difenoconazole on the chitinolytic activity of soil bacteria affecting the different ecological functions of bacterial chitinase in soil.

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