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EFFECTS OF HERBICIDES AND FUNGICIDES ON THE SOIL CHITINOLYTIC ACTIVITY. A MOLECULAR DOCKING APPROACH

WPŁYW HERBICYDÓW I FUNGICYDÓW NA AKTYWNOŚĆ CHITYNOLITYCZNĄ GLEB. PODEJŚCIE DOKOWANIA MOLEKULARNEGO

Abstract: A molecular docking study was undertaken using the programs SwissDock and PatchDock to assess the interactions of the bacterial chitinases belonging to the GH18 and GH19 families with two herbicides (chlorsulfuron and nicosulfuron) and two fungicides (difenoconazole and drazoxolon). Both molecular docking programs predict that all considered pesticides bind to the active sites of chitinases produced by soil microorganisms. There are correlations for predicted binding energy values for receptor-ligand complexes obtained using the two programs consolidating the prediction of the chitinases-pesticides interactions. The interactions of chitinases with pesticides involve the same residues as their interactions with known inhibitors suggesting the inhibitory potential of pesticides. Pesticides interact stronger with chitinases belonging to the GH18 family, their active sites reflecting higher polarity than those of the GH19 chitinases. Also, herbicides reveal a higher inhibitory potential to bacterial chitinases than fungicides.

Keywords: molecular docking, herbicides, fungicides, chitinolytic activity, inhibitory potential

Introduction

Herbicides and fungicides are widely used against of pests infecting agricultural crops. From the entire quantity of used pesticides, only a small percent reaches the target and the remaining amount contaminates the soil and aquatic environments [1]. Pesticides may have a harmful effect on soil microorganisms by affecting the soil microbial diversity, soil biochemical processes and enzymatic reactions [2]. There are a few reports revealing the degradation of pesticides by soil microorganisms [3, 4] and even increased soil enzymatic activity due to some pesticides [5]. The effects of pesticides on soil enzymatic activity depend on many factors due to the complexity and intercorrelation of biochemical processes taking place in soil. Soil bacteria are able to produce extracellular enzymes decomposing

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the organic matter at different degrees and facilitating the nutrient mineralization and cycling by increasing the reaction rate of the plant residues decomposition [6]. During these processes, the products of some enzymatic reactions serve as substrates for other enzymes and it illustrates the interrelations and complexity of the enzyme activity in soil [7].

One of the most abundant carbohydrates in nature is chitin. The enzymes hydrolysing chitin are the chitinases, classified using the Enzyme Commission number [8] as the EC 3.2.1 enzyme sub-sub-class. Chitinases hydrolyse the N-acetyl-beta-D-glucosaminide (1→4) - beta-linkages in chitin and chitodextrins [9] and they belong to the families 18, 19 and 20 of the glycosyl hydrolases (GHs) [10]. Considering the depolymerization activity of chitinases, they are classified in two classes [11]: endochitinases, randomly cleaving chitin molecules and exochitinases, splitting the chitin molecules from non-reduced ends. Complete degradation of the chitin polymer, *ie* the chitinoclastic process, usually involves three stages: (1) cleavage of the chitin polymer into oligomers (the chitynolytic process), (2) splitting of the oligomers into dimers, and (3) cleavage of the dimers into monomers, the first two stages being regularly catalysed by chitinases [12].

In soil, the chitin and its chitooligomers hydrolysis is performed by soil bacteria [13]. Bacterial chitinases belong to the families 18 and 19 of GHs. Chitinases from the family 18 use the retaining catalytic mechanism and those from the family 19 use the inverting mechanism for hydrolysing the glycosydic bond [14]. Chitin degradation by soil bacteria implicates firstly the cleavage of the α-(1→4) bond by exochitinases and this is called a chitinolytic process [12]. Taking into account their amino acid sequences, exochitinases are subdivided in groups A, B, and C, the group A producing ecologically significant chitin derivatives and being the most abundant in the environment [15].

Bacterial communities are greatly diverse in soil, *Bacillus*, *Serratia*, and *Streptomyces* spp. being among soil species revealing chitinolytic activity [13, 15]. Among these species, *Serratia marscenses* is capable to secret four chitinases: chitinase A (SmChiA), chitinase B (SmChiB), chitinases C1 and C2 (SmChiC1 and SmChiC2) [16] and *Bacillus cereus* produces two chitinases, chitinase A (BcChiA) and chitinase B (BcChiB) [17], all of them belonging to the family 18 of GHs. *Streptomyces griseus* produces chitinase C (SgChiC) and *Streptomyces coelicolor* produces chitinase G (ScChiG), both enzymes belonging to the family 19 of GHs [18].

The chitinases belonging to the family 18 of GHs present a $(beta/alpha)_8$-barrel catalytic domain with a signature peptide DGXDXDXE occurring in the fourth beta-strand in the barrel [14]. Amino acids belonging to this signature peptide have catalytic roles in SmChiA, SmChiB and BcChiA: The amino acids D313 and E315 in SmChiA, D142 and E144 in SmChiB, D143 and E145 in BcChiA serve as catalytic residues [19-21]. It is also known that chitin substrates are bound within a long cleft across the (beta/alpha)8-barrel, and there are aromatic residues that hydrophobically interacts with the substrate [19].

The bacterial chitinases belonging to the family 19 of GHs have a catalytic domain presenting an alpha-helix-rich fold with a deep cleft as catalytic site, the catalytic residues containing two glutamates: E147 and E159 for the SgChiC [22] and E68 and E77 for the ScChiG [23] respectively.

There are some known inhibitors of bacterial and plant chitinases. Some of them are polysaccharides, such as allosamidin and its derivatives [24] and the others are peptides, such as argadin and argifin [25], psammaplin A and cyclic peptides [26].

We consider chitinases from *Bacillus*, *Serratia* and *Streptomyces spp.* in our study as they are commonly found in soil and have three dimensional solved structures deposited in the Protein Data Bank (PDB) [27]. We assess their interactions with two herbicides (chlorsulfuron - CLS and nicosulfuron - NCS) and two fungicides (difenoconazole - DFC and drazoxolon - DRX) using a molecular docking approach.

Materials and methods

There are 38 entries in PDB [27] concerning crystallographic structures of the: *Serratia marcescens* (15) [21, 28] and *Bacillus cereus* (7) [19] ChiA, *Serratia marcescens* chiB (12) [20, 29-33], *Streptomyces griseus* ChiC (3) [22] and *Streptomyces coelicolor* ChiG (1) [23]. When more than one crystallographic structure is obtainable for a class of chitinases and one organism, we consider the structural file having the highest resolution and containing the complex of the native protein with the substrate or inhibitor (when available). When more than one protein chain is present in the structural file, we consider only chain A in the molecular docking calculations. The structural files considered in this study are given in the Table 1.

Table 1

Protein Data Bank codes entry and structures short description for the chitinases considered in this study

Chitinase	**Organism**	**GH family**	**PDB code entry**	**Resolution [Å]**	**Structural file short description**
Chitinase A	*Serratia marcescens*	GH-18	2WLZ	1.82	residues 2-560 of the protein in complex with the inhibitor chitobio-thiazoline
Chitinase B	*Serratia marcescens*		1E6R chain A	2.5	the protein in complex with the inhibitor allosamidine
Chitinase A	*Bacillus cereus*		3N11	1.35	residues 28-360 of the protein
Chitinase C	*Streptomyces griseus*	GH-19	1WVU chain A	2,50	residues 30-294 of the protein in complex with Cl ions
Chitinase G	*Streptomyces coelicolor*		2CJL chain A	1.50	residues 41-444 of the protein in complex with Zn ions

The considered structural files are cleaned by removing water molecules and heteroatoms (except ions when present). Also, they were prepared for molecular docking studies by adding hydrogens and charges using the DockPrep facility under UCSF Chimera package [34].

Considered enzymes belong to distinct GHs families, every family sharing a distinctive catalytic mechanism, meaning that they are not related by sequence and do not share the same structural fold. We compare the sequences of the SmChiA, SmChiB, BcChiA, SgChiC ScChiG using ClustalW tool [35]. The sequence alignment confirms the low sequence similarity of these enzymes: chitinases belonging to the GH18 family share between 5% and 16% sequence identity, chitinases belonging to the GH19 family reflect 68% sequence identity and the highest sequence identity of the Gh18 and Gh19 families of chitinases is 10%. The crystal structures are superposed using UCSF Chimera package [34] and the structural alignment is quantitatively described by the root mean square deviation (RMSD) between the equivalent alpha carbon (CA) atoms [36]. The expected structural differences between the catalytic domains of the chitinases belonging to the families 18 and 19 of GHs are confirmed by the obtained high RMSD values ranging from 0.880 Å between 85 carbon

alpha (CA) atoms pairs in the case of SmChiB compared to BcChiB to 1.461 Å between only 4 CA atoms pairs in the case of SmChiB compared to ScChiC.

As there are strong sequence and structural differences between the catalytic domains of the chitinases belonging to families 18 and 19, we also expect distinct properties of their binding site cavities. The binding site cavities of the enzymes were identified and characterized using the Fpocket tool [37].

The pesticides considered in this study are the herbicides chlorsulfuron (CLS) and nicosulfuron (NCS) and the fungicides difenoconazole (DFC) and drazoxolon (DRX). They were retrieved from ZINC database [38] and prepared for docking using *DockPrep* facility under UCSF Chimera package [34].

Molecular docking calculations were undertaken using SwissDock [39] and PatchDock [40] web based interfaces. SwissDock interface is built on the docking software EADock DSS [41]. SwissDock tool optimizes the orientation and conformation of a ligand interacting with a protein and outcomes the most favourable binding modes of the ligand on the protein surface that are ranked upon the interaction energy expressed as FullFitness score. It also outcomes the estimated free energy for the binding modes. Selected docking type was accurate and rigid.

PatchDock webserver performs structure prediction for protein-small ligand complexes using a geometry based molecular docking algorithm [40]. This algorithm is based on finding the transformations that produce a local good geometric shape complementarity by considering wide interface areas and small amounts of steric clashes. Considering wide interface areas guarantees the inclusion of the local individualities of the docked molecules with complementary features. The transformations are classified using a scoring function that takes into account both the geometric fit and atomic desolvation energy and the redundant solutions are rejected by applying a root mean square deviation (RMSD) clustering. In our calculations we have used blind docking with default options, a clustering RMSD value of 4 Å and *enzyme-inhibitor* as complex type. The output of PatchDock tool is a list of candidate complexes between a receptor and a ligand molecule, both specified by user. The complexes are sorted according to the geometric shape complementarity score. For every complex the output contains the approximate interface area, the atomic contact energy (ACE), and the 3D transformations (3 rotational angles and 3 translational parameters) applied on the ligand molecule. FireDock webserver [42] has been used to refine the PatchDock predictions and it delivers the global energy of each enzyme-inhibitor complex predicted by PatchDock software. Data obtained through molecular docking are visualized and analyzed using UCSF Chimera package [34].

The interacting residues of the SmChiA and SmChiB with their known inhibitors chitobio-thiazoline and allosamidine respectively are computed using ContPro web tool [43] with a distance cuttoff of 3.5 Å. The same tool is used to compute the interacting residues of considered chitinases with the pesticides for the complexes obtained using PatchDock webserver [40].

Results and discussions

Identification and characterization of the active site binding cavities of the considered chitinases, have been carried out using Fpocket tool [37] and resulting data are presented in Table 2.

Table 2

The physicochemical characteristics of the active site cavities of considered chitinases

Enzyme	Volume [Å^3]	Hydrophobicity score	Polarity score	Charge score
SmChiA	1633.84	10.77	21	–6
SmChiB	1383.66	24.46	14	–1
BcChiA	1284.97	30.55	15	0
SgChiC	882.17	31.89	8	0
ScChiG	603.32	33.33	9	1

The active site cavities of the investigated enzymes reveal distinct properties concerning the volume and the hydrophobicity, the GH18 chitinases (SmChiA, SmChiB and BcChiA) exposing bigger and more polar active site cavities than GH19 chitinases.

ZINC database [38] has been used to extract the physical properties of pesticides considered in this study and known inhibitors of bacterial chitinases: the partition coefficient (logP) for the neutral pH, the topological polar surface area (tPSA), the electric charge and the molecular weight (MW). These properties are presented in Table 3.

Table 3 reveals that, at neutral pH, the known inhibitors of bacterial chitinases are usually polar, charged and bigger than the pesticides. Literature data [26] reveal that argadin inhibits plant and bacterial chitinases stronger than allosamidin, argifin and psammaplin A, respectively that allosamidin inhibits chitinases stronger than argifin and psammaplin A.

Table 3

The physical properties of considered pesticides and known inhibitors of bacterial chitinases: logP - partition coefficient, tPSA - topological polar surface area, MW - molecular weight

Molecule	Role	logP	tPSA [Å^2]	charge	MW [g · mol^{-1}]
difenoconazole	pesticide	4.33	58	0	406.269
drazoxolon	pesticide	2.11	67	0	237.646
chlorsulfuron	pesticide	1.59	129	–1	356.771
nicosulfuron	pesticide	–0.44	160	–1	409.404
allosamidine	inhibitor	–5.14	263	1	623.633
argifin	inhibitor	–3.97	296	–1	674.692
argadin	inhibitor	–4.51	284	–1	673.708
psammaplin A	inhibitor	3.43	167	0	663.398

Also, psammaplin A binds near the active site of chitinases, its inhibitory potential being lower than that of allosamidin, argifin and argadin [26]. Argadin, argafin and allosamidin are polar in comparison to psammaplin A, that is not. These data, correlated to those presented in Table 2, illustrate that polarity seems to be an important property responsible for tight interactions between inhibitors and chitinases.

From the molecular docking studies, we were able to select the best solutions based on lowest binding energies and higher complementarity shape scores for each receptor-ligand complex. Table 4 illustrates the results obtained using SwissDock [39], PatchDock [40] and FireDock [42] webservers for the molecular docking calculations. SwissDock tool outcomes the interaction energy expressed as FullFitness score and the estimated free energy for the binding modes of pesticides to the active sites of chitinases.

PatchDock webserver delivers the geometric shape complementarity score, the approximate interface area and the atomic contact energy (ACE) between the considered pesticides and the enzymes. Refinement of PatchDock results using FireDock webserver estimates the global energy of the protein-ligand complex. As a control, we performed molecular docking studies for the interactions of bacterial chitinases with their known inhibitors allosamidin and psammaplin A. Allosamidin binds to the active site and psammaplin A binds near the catalytic site, having a lower inhibitory potential [26].

Table 4

Outcomes of SwissDock, PatchDock and FireDock webservers for the assessment of the interactions of pesticides with chitinases

Pesticide	Enzyme	SwissDock results		PatchDock results			FireDock
		FullFitness score [$kcal\cdot mol^{-1}$]	ΔG [$kcal\cdot mol^{-1}$]	Score	Interface area [$Å^2$]	ACE [$kcal\cdot mol^{-1}$]	Global energy [$kcal\cdot mol^{-1}$]
psammaplin A	SmChiA	–1936.12	–9.65	5184	639.40	–206.75	–35.37
	SmChiB	–1564.65	–9.53	5664	700.00	–353.44	–39.26
	BcChiA	-	-	5358	636.60	–188.67	–30.67
	SgChiC	–718.02	–8.93	4662	631.60	–257.66	–29.71
	ScChiG	–820.61	–9.07	4630	504.30	–177.85	–26.94
allosamidin	SmChiA	–1936.31	–9.27	6326	731.30	–231.06	–32.53
	SmChiB	–1582.51	–11.77	6956	795.10	–160.88	–32.95
	BcChiA	–1212.80	–7.41	5372	635.70	–191.11	–21.13
	SgChiC	–728.67	–10.11	5996	705.50	–175.16	–28.17
	ScChiG	–826.34	–9.27	5612	680.90	–156.11	–28.52
difenoconazole	SmChiA [44]	–1960.93	–7.42	5110	592.00	–152.20	–45.84
	SmChiB	–1599.07	–7.36	4764	509.10	–98.51	–36.70
	BcChiA [44]	–1263.84	–7.13	4208	463.40	–45.84	–20.11
	SgChiC	–739.90	–7.46	3936	442.10	–83.00	–25.99
	ScChiG	–843.63	–7.66	4396	491.30	–104.62	–38.96
drazoxolon	SmChiA	–1523.42	–5.39	3612	388.70	–106.43	–30.97
	SmChiB	–1545.74	–6.22	3346	370.80	–103.60	–29.16
	BcChiA	–1220.27	–7.05	3184	336.30	–69.24	–25.29
	SgChiC	–690.03	–6.31	2842	312.30	–93.73	–28.62
	ScChiG	–795.79	–7.00	2982	334.80	–114.25	–30.88
chlorsulfuron	SmChiA	–2132.65	–7.39	4404	558.90	–115.55	–41.06
	SmChiB	–1773.80	–8.16	4248	486.10	–180.53	–37.86
	BcChiA	–1431.28	–7.56	3776	427.80	–83.67	–26.02
	SgChiC	–902.37	–5.77	3836	397.30	–153.59	–37.41
	ScChiG	–1012.09	–6.67	3988	453.50	–110.92	–28.25
nicosulfuron	SmChiA	–2179.52	–7,26	5110	606.80	–199.55	–44.84
	SmChiB	–1817.79	–7.21	4764	610.20	–180.43	–45.32
	BcChiA	–1482.47	–7.69	4208	507.10	–117.64	–39.16
	SgChiC	–960.53	–6.92	3936	487.60	–160.11	–37.16
	ScChiG	–1066.81	–8.31	4396	480.10	–217.14	–44.23

Table 4 illustrates the results obtained using the two molecular docking methods for predicted binding energy values of all considered receptor-ligand complexes. The consensus ranking of enzyme-inhibitor complexes obtained for the two molecular docking methods improves the binding energy predictions. Also, Table 4 illustrates that all the considered pesticides show favorable binding to the active sites of chitinases. The binding energies for

the pesticides are comparable to those obtained for the known inhibitors. Allosamidin, provides higher binding energies than psammaplin A and it is in good agreement with published data revealing the lower inhibitory potential of psammaplin A by comparison to allosamidin [26].

The herbicides chlorsulfuron and nicosulfuron interactions with chitinases are stronger than those of the fungicides difenoconazole and drazoxolon, most likely due to the higher polarity of herbicides in comparison with fungicides. Both herbicides are charged, but nicosulfuron has a higher polarity than chlorsulfuron and consequently its interactions with all binhibitors interact stronger with chitinases belonging to the GH18 family, their active sites reflecting a higher polarity than those of the GH19 chitinases.

Figures 1 illustrate the best solutions obtained by using SwissDock (A) and PatchDock refined with FireDock (b) for the DFC binding to the SmChiB (code entry 1E6R) active site. Similarly, Figures 2 exemplify the best solutions obtained by using the two molecular modelling tools for the NCS binding to the SmChiA (code entry 2WLZ) active site. These figures illustrate that pesticides usually bind to the active site of chitinases and it suggests their inhibitory potential.

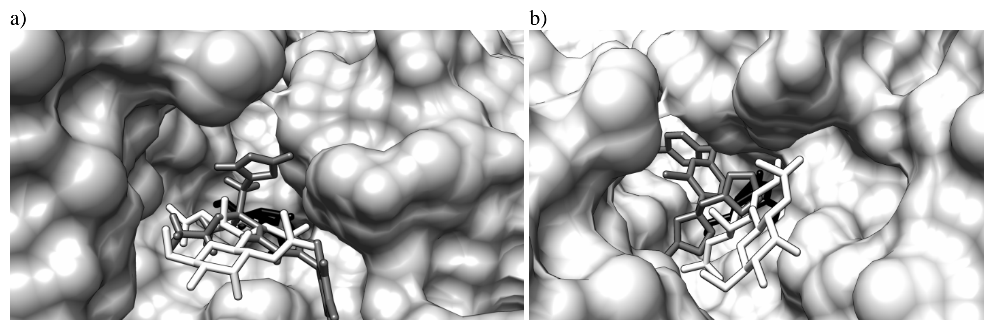

Fig. 1. The best solutions obtained using SwissDock (a) and PatchDock refined with FireDock (b) for the binding of DFC to the SmChiB (code entry 1E6R) active site: the active site of the enzyme is shown as light grey surface, allosamidin inhibitor is presented in dark black sticks, N-acetyl-D-allosamine product is shown in white sticks and DFC is revealed in dim grey sticks

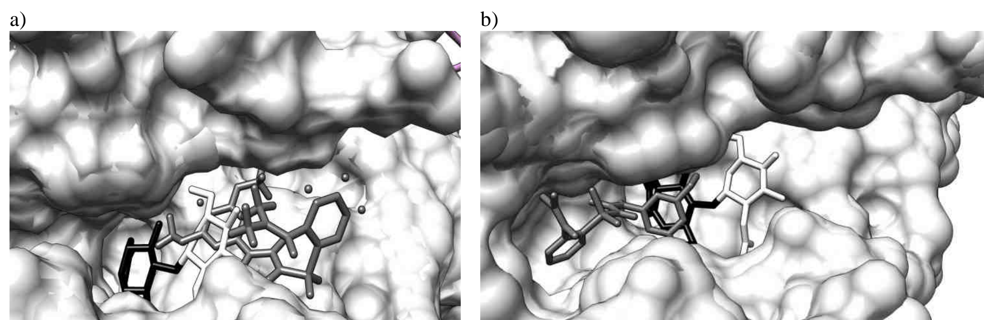

Fig. 2. The best solutions obtained by using SwissDock (a) and PatchDock refined with FireDock (b) for the NCS binding to the SmChiA (code entry 2WLZ) active site: the active site of the enzyme is shown as light grey surface, chitotriobiose inhibitor is presented in black sticks, N-acetyl-D-glucosamine product is shown in white sticks and NCS is revealed in dim grey sticks

For those chitinases having solved three-dimensional structures of complexes with substrate and with known inhibitors (all of them being GH18 chitinases), the interacting residues with the ligands are identified using ContPro software and are presented in the Table 5.

Data presented in Table 5 reflects that the catalytic residues (highlighted using bold letters) of the investigated enzymes are involved in the interactions with the inhibitors. These interactions involve many hydrophobic residues.

Table 5
The interacting residues of the GH18 chitinases with their substrate and/or inhibitors (the catalytic residues are presented in bold)

Enzyme complex	PDB code entry	Interacting residues
SmChiA in complex with the substrate tetra-N-acetyl-D-glucosamine	1K9T	GLY274, TRP275, **ASP313**, **GLU315**, PHE316, LYS369, MET388, TYR390, ALA391, PHE392, PHE396, ASP397, TYR408, TYR444, ARG446, TRP539
SmChiA in complex with the inhibitor chitobio-thiazoline	2WLZ	TYR163, TRP275, **ASP313, GLU315**, ASP391, TRP539
SmChiB in complex with the inhibitor allosamidine	1E6R	TYR10, PHE51, TRP97,**ASP142, GLU144**, MET212, TYR 214, ASP215, TYR292, TRP403, ARG410

The ContPro [43] outputs obtained for the PatchDock [40] predicted complexes of chitinases with pesticides are presented in the Table 6.

Table 6
The interacting residues of the considered chitinases with pesticides (the catalytic residues are presented in bold)

Enzyme	Pesticide	Residues involved in the interaction with pesticide
SmChiA	difenoconazole	PHE191, GLY274, TRP275, **GLU315**, PHE316, MET388, TYR390, ASP391, TYR418, TYR444, ARG446, ILE476, TRP539
	drazoxolone	**GLU315**, SER364, ALA365, GLY366, MET388, ASP391, TYR418
	chlorsulfuron	ARG172, PHE191, TRP275, **GLU315**, TYR390, ASP391, TYR444, ARG446, GLU473, ILE476, TRP 539, GLU540
	nicosulfuron	PHE191, TRP275, PHE316, LYS320, SER364, LYS369, MET388, TYR390, ASP319, PHE392, TYR 418, TYR444, TYR539
SmChiB	difenoconazole	TRP97, **GLU144**, PHE191, MET212, TYR214, ASP215, TYR292, ARG294, ILE339, TRP403
	drazoxolone	TRP97, **GLU144**, TYR145, GLY187, GLY188, PHE191, MET212, TYR214
	chlorsulfuron	TRP97, ***GLU144,*** TYR145, PHE190, PHE191, MET212, TYR214, ASP215, LEU216, TRP220, GLU221, LEU265
	nicosulfuron	TYR10, PHE51, GLY96, TRP97, **ASP142**, **GLU144**, TYR145, PHE191, MET212, TYR214, ASP215, LEU216, TRP220, TYR292, ARG294, TRP403
BcChiA	difenoconazole	PHE43, PHE66, GLN109, ***ASP143, GLU145,*** TYR227, ASN228, ALA287, TRP333
	draxozolone	PHE43, GLY108, GLN109, **GLU145**, GLN225, TYR227, TRP333
	chlorsulfuron	HIS41, PHE43, ASN45, PHE66, **ASP143, GLU145**, GLU190, GLN225, TYR227, ASN228, ALA287, PRO289, TRP333, TRP337
	nicosulfuron	PHE43, PHE66, GLY108, GLN109, ANS110, **ASP143, GLU145**, GLN225, TYR227, ASN228, TRP333

Enzyme	Pesticide	Residues involved in the interaction with pesticide
SgChiC	difenoconazole	ARG105, TYR111, TYR121, PRO122, ALA123, SER145, HIS146, **GLU147**, TYR165, GLN178, ILE187, GLN188, SER190, TRP191, ASP216, LYS222, TYR227, ILE256, ASN257, GLU261, ASN266, ARG273, ASN275
	drazoxolone	ARG105, TYR111, TYR121, PRO122, ALA123, SER145, **GLU147,** GLU156, TYR165, GLN178, ILE187, GLN188, SER190, TRP191, ASN194, ASP216, LYS222, TYR227, ASN266, ASN275
	chlorsulfuron	ARG105, TYR111, TYR121, PRO122, ALA123, SER145, **GLU147**, GLU156, TYR165, GLN178, ILE187, GLN188, SER190, ASN194, ASP216, LYS222, TYR227, ILE256, ASN266, ASN275
	nicosulfuron	ARG105, TYR111, TYR121, PRO122, ALA123, SER145, **GLU147**, GLU156, TYR165, GLN178, ILE187, GLN188, SER190, ASN194, ASP216, LYS222, TYR227, ILE256, ASN257, ASN266, ASN275
ScChiG	difenoconazole	HIS67, **GLU68, GLU77**, TYR86, GLN109, SER111, TYR148, PRO155, ASN178, GLU182, ASP184
	draxozolone	HIS67, **GLU68**, VAL108, GLN109, SER111, TRP112, ASN115, TYR148, ASN178, GLU182, ASP184
	chlorsulfuron	HIS67, **GLU68, GLU77**, TYR86, GLY104, VAL108, GLN109, SER111, TRP112, ASN115, TYR148, ASN178, GLU182, ASP184
	nicosulfuron	HIS67, VAL108, GLN109, LEU110, SER111, TRP112, ASN115, TYR148, PRO155, ILE177, ASN178, SER180, GLU182, ASP184

Table 6 reflects that the pesticides interaction with bacterial chitinases usually implicate beside the two polar catalytic residues many other hydrophobic residues. The same residues are involved in the interactions of chitinases with their substrate and inhibitors (Table 5). It underlines the inhibitory potential of considered pesticides for bacterial chitinases.

The experimental studies have proven that the herbicides and fungicides once entered in contact with soil microorganisms have inhibitory effects on enzymes: nitrogenase [45], hydrolases, oxidoreductases, dehydrogenases [46, 47], catalase [47], urease [44, 47, 48], beta-glucosidase [49] and so on. Our study reveals that the herbicides chlorsulfuron and nicosulfuron and the pesticides difenoconazole and drazoxolon have an inhibitory potential on the soil chitinolytic activity.

Conclusions

Both molecular docking programs used in this study predict that all considered pesticides bind to the active sites of chitinases produced by soil microorganisms, suggesting their inhibitory potential. Furthermore, we observed correlations for predicted binding energy values for receptor-ligand complexes obtained using the two programs, these correlations strengthening the success of the enzyme-ligand interactions predictions. The inhibitory potential of considered pesticides for chitinases produced by soil microorganisms is also sustained by the fact that the interacting residues of chitinases with pesticides are almost the same as the interacting residues with their substrate and with known inhibitors.

Pesticides interact stronger with chitinases belonging to the GH18 family, their active sites reflecting a higher polarity than those of the GH19 chitinases. Herbicides always reveal powerful interactions with bacterial chitinases than fungicides. The herbicide nicosulfuron reveals the strongest interactions with all considered bacterial chitinases. The

herbicides have a higher polarity than fungicides and nicosulfuron is the most polar between the considered pesticides. It seems that polarity is an important property of a molecule to be used as inhibitor for chitinases produced by soil microorganisms.

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WPŁYW HERBICYDÓW I FUNGICYDÓW NA AKTYWNOŚĆ CHITYNOLITYCZNĄ GLEB. PODEJŚCIE DOKOWANIA MOLEKULARNEGO

Abstrakt: W celu oceny oddziaływań chitynaz bakteryjnych należących do rodziny GH18 i GH19 z dwoma herbicydami (chlorosulfuron i nikosulfuron) i dwoma fungicydami (difenokonazol i drazoxolon) przeprowadzono badania dokowania molekularnego za pomocą programów SwissDock i PatchDock. Oba programy dokowania molekularnego przewidują, że wszystkie badane pestycydy wiążą się z miejscami aktywnymi chitynaz, wytwarzanych przez drobnoustroje glebowe. Istnieją korelacje dla przewidywanych wartości energii wiązania kompleksów receptor-ligand uzyskanych za pomocą dwóch programów, potwierdzając przewidywane interakcje między chitynazami a pestycydami. Oddziaływania chitynaz z pestycydami dotyczą tych samych reszt, jak ich interakcje ze znanymi inhibitorami, co sugeruje hamujące zdolności pestycydów. Pestycydy oddziałują silniej z chitynazami należącymi do rodziny GH18 - ich miejsca aktywne wykazują wyższą polarność niż te z chitynaz GH19. Ponadto, herbicydy wykazują wyższe zdolności hamujące wobec chitynaz bakteryjnych w porównaniu do fungicydów.

Słowa kluczowe: dokowanie molekularne, herbicydy, fungicydy, aktywność chitynolityczna, zdolności hamujące