Adsortion of Zearalenone by β-D-Glucans in the Cell Wall of Saccharomyces cerevisiae

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ABSTRACT

Cell walls of yeasts and bacteria are able to complex with mycotoxins and limit their bioavailability in the digestive tract when these yeasts and bacteria are given as feed additives to animals. To identify the component(s) of the yeast cell wall and the chemical interaction(s) involved in complex formation with zearalenone, four strains of Saccharomyces cerevisiae differing in their cell wall glucan and mannain content were tested. Laboratory strains wt292, fks1, and mm9 were compared with industrial S. cerevisiae strain sc1026. The complex-forming capacity of the yeast cell walls was determined in vitro by modelling the plots of amount of bound versus amount of toxin added using Hill’s model. A cooperative relationship between toxin and adsorbent was shown, and a correlation between the amount of β-D-glucans in cell walls and complex-forming efficacy was revealed (R² = 0.889). Cell walls of strains wt292 and mm9, which have higher levels of β-D-glucans, were able to complex larger amounts of zearalenon, with higher association constants and higher affinity than those of the fks1 and sc1026 strains. The high chitin content in strains mm9 and fks1 increased the alkali insolubility of β-D-glucans from isolated cell walls and decreased the flexibility of these cell walls, which restricted access of zearalenone to the chemical sites of the β-D-glucans involved in complex formation. The strains with high chitin content thus had a lower complex-forming capacity than expected based on their β-D-glucans content. Cooperativity and the three-dimensional structure of β-D-glucans indicate that weak noncovalent bonds are involved in the complex-forming mechanisms associated with zearalenone. The chemical interactions between β-D-glucans and zearalenone are therefore more of an adsorption type than a binding type.

Mycotoxins are known to be very harmful to animals that ingest contaminated feeds, and they can be recovered in edible animal products as parent entities or metabolites (26). Zearalenone (ZEN), a 6-(10-hydroxy-6-oxo-6b,19,24).zearalenol, is a nonsteroid estrogenic mycotoxin produced by numerous species of Fusarium (2), where it is considered potentially harmful to consumers. ZEN causes alterations in the reproductive tract of laboratory animals and domestic animals. It produces various estrogenic effects (decreased fertility, increased embryothal resorption, reduced litter size, changed weight of adrenal, thyroid, and pituitary glands, and changes in serum levels of progesterone and estradiol), but no teratogenic effects have been found (15).

Empirical work carried out during the last decade has indicated that organic compounds such as clays, bentonites, aluminosilicates, and organic compounds such as yeast or bacterial cell walls have the ability to complex with some mycotoxins (5, 6). Once the complex has been formed, adsorption in the digestive tract of animals is strongly reduced, thus protecting animal health and reducing the risks for consumers.

The purpose of this work was to identify the chemical components in the yeast cell wall that play a major role in the ZEN complex-forming effect and to clarify the nature of the chemical interactions occurring between yeast cell walls and ZEN. The cell wall fraction of Saccharomyces cerevisiae, which is mainly composed of polysaccharides (80 to 90%), makes up 15 to 30% of the dry weight of the whole cell. The mechanical strength of the wall is mainly due to the inner layer, which consists of β-D-glucans, (1,3)- and (1,6)-D-glucans (50 to 60% of the wall dry weight) linked to the plasma membrane through chitin molecules. The outer layer is made of heavily glycosylated mannoproteins, which are involved in cell-cell recognition events and limits wall porosity (14, 18). The β-D-glucans of yeast can adopt helical conformations resulting from single helix polysaccharide chains, giving rise to a fibrillar structure (13). The entire fibrous network of β-D-glucans in the yeast cell wall consists of alternating single- and triple-helix regions. Chitin, which is present in small quantities in S. cerevisiae (approximately 2 to 4%), contributes both to the rigidity and to the alkali insolubility of

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β-D-glucans. Proteins, lipids, and inorganic phosphate are also found in yeast cell walls (12).

To understand the nature of the chemical interaction between the yeast cell wall and ZEN and the mechanisms involved, four strains of *S. cerevisiae* with differing glucan and mannan content in their cell walls were tested for their ability to complex ZEN. Cell walls were extracted and their chemical composition was determined. An original in vitro technique was then used including modelling of adsorption curves (30) to test the ZEN complex-forming abilities of the four yeast cell wall types, and the relationship between complex-forming capacity and the chemical nature of cell walls was evaluated. The equations of Hill’s model accounting for the complex formation between ZEN and whole cell walls were used to highlight the role of each cell wall component in the complex-forming process. Hypotheses were formulated concerning the nature of the chemical bonding involved in the formation of the complex between ZEN and yeast cell wall components.

### MATERIALS AND METHODS

**Production of yeast biomass.** Four strains of *S. cerevisiae* were used: the wild type wt292, the fks1 mutant type, which was deficient in β(1,3)-D-glucan synthase, the mnn9 mutant type, which was deficient in the gene required for N-glycosylation, and strain 1026 (sc1026) supplied by Alltech (Nicholasville, Ky.) originating from a brewery. Yeast cells were grown in flasks at 30°C and shaken at 200 rpm in YPD medium (1% [wt/vol] yeast extract, 2% [wt/vol] bacteriological peptone, and 2% [wt/vol] glucose). Yeast cultures were stopped for cell wall extraction when a concentration of 2 × 10^6 cells per ml was reached (around 3 days).

**Preparation and analysis of yeast cell walls.** Cells were disrupted by shaking with glass beads (micro method), and cell wall carbohydrates were hydrolyzed with 2 N H_2SO_4 at 100°C for 4 h for subsequent quantification of sugars according to a method described elsewhere (3). Cell walls were collected by slow centrifugation to eliminate organelles from the medium. A Bio-LC system (Dionex, Sunnyvale, Calif.) was used for quantitative analysis of sugars. Separation of sugars was carried out on a CarboPac PA1 anion-exchange column (4 by 250 mm) equipped with a CarboPac PA guard column (Dionex). Elution was performed at room temperature at a flow rate of 1 ml/min with 18 mM NaOH.

**Data processing and curve fitting.** Curves representing the amount of bound ZEN as a function of the amount of added ZEN were treated according to Hill’s equation as discussed elsewhere (30). DataFit 7.1 software (Oakdale Engineering, Oakdale, Pa.) was used to plot the experimental data, set up the regression curve (curve fitting), and calculate the data for the binding capacity tests. Hill’s model with n sites (30) was used to characterize the adsorption of ZEN on β-D-glucans for each strain, resulting in a sigmoid plot of fractional saturation (Y) arising from a cooperative interaction. Hill’s model was used to set up three models (Table 1), each expressing the amount of bound toxin as a function of the total amount of toxin added to the medium (Y = f(T_total)) and allowing the calculation of several parameters characterizing the interaction between yeast cell walls and ZEN (Table 1) (30). The HMN equation was used to evaluate individual adsorption capacities of β-D-glucans from each strain and to discriminate among the strains according to their ZEN adsorption efficacy. Equations HMN-GT and HMN-GT2 (Table 1) took into account the computational role played by the amounts of glucans in cell walls of all strains to establish the interaction between β-D-glucan content and adsorption properties for ZEN. Unlike HMN-GT, HMN-GT2 took into account the amounts of β-D-glucans in the association constant (K_Dtotal). The affinity rates (A_total) relative to

### TABLE 1. Models used to fit plots and evaluate ZEN adsorption on β-D-glucans of yeast cell walls of *S. cerevisiae* according to Hill’s equation

<table>
<thead>
<tr>
<th>Model expression</th>
<th>T_bound = f(T_total)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hill’s model with n sites (HMN)</td>
<td>T_bound = T_bound^n /KD_total + (T_total)^n</td>
</tr>
<tr>
<td>Hill’s model with n sites and factor proportional to the amount of β-D-glucans (HMN-GT)</td>
<td>T_bound = [β-D-glucans] T_bound^n /KD_total + (T_total)^n</td>
</tr>
<tr>
<td>Hill’s model with n sites and factor proportional to the amount of β-D-glucans (KD depends on the β-D-glucan content; HMN GT2)</td>
<td>T_bound = [β-D-glucans] T_bound^n /[β-D-glucans] KD_total + (T_total)^n</td>
</tr>
</tbody>
</table>

a KD_{total} = association constant compared with total toxin added; T_bound = amount of bound toxin; T_total = amount of total toxin added; T_max_{bound} = maximal amount of bound toxin.
the total amount of ZEN toxin added to the medium were evaluated with the equation

\[ A_{total} = \frac{T_{total}^{max}}{2K_{Dtotal}} \]

where \( K_{Dtotal} \) represents the association constant per site relative to the total amount of added toxin.

**Statistical analysis.** Samples used for yeast cell wall composition were analyzed in triplicate to calculate means and standard deviations. Means were compared using the Tukey-Kramer multiple comparisons test and the GraphPad InStat 3.01 software system (Apple Computers, Cupertino, Calif.). The significance threshold was set at \( P < 0.05 \).

**RESULTS**

**Chemical composition of cell walls.** The cell wall fraction represented 25.0, 23.4, 23.1, and 13.3% of the dry weight of the total cell and was composed of various glu- can, mannan, and chitin contents for wt292, fks1, mnn9, and sc1026, respectively (Fig. 1). Mannan/glucon ratios in the cell wall were significantly different (\( P < 0.05 \)), with the exception of sc1026 and fks1, and were 1.25, 2.09, 2.18, and 0.21 for wt292, fks1, sc1026, and mnn9, respectively. Glucosamine, which was used as a marker for chitin, was very high in mnn9 cell walls (9.7%) but was low in wt292 and sc1026 cell walls (1.6 and 2.1%, respectively). Thus, this difference in various components among strains can help identify which components are involved in the complex-forming process of ZEN and yeast cell walls. Microscopic examination revealed that sc1026, with a cell wall dry matter content below the threshold of 15 to 30% commonly found (17), had a thin cell wall due to large cell volume and spherical shape. The larger size of brewery strains (+30% in mean diameter) compared with the laboratory strains is due to their use of ethanol as a carbon source, which increases osmotic pressure inside the cell and thus increases cell size (29).

**Complex formation between ZEN and yeast cell walls estimated by Hill’s model.** The \( R^2 \) values for both HMN and HMN-GT2 models were higher for \( [T_{bound} = f(T_{total})] \) than for \( [T_{bound} = f(T_{free})] \) expression, where \( T_{free} \) is the amount of free ZEN in the medium after complex formation (Table 2). For \( [T_{bound} = f(T_{total})] \), the regression curve of the data calculated according to HMN compared with the experimental data (\( R^2 = 0.93 \) to 0.99) confirmed the consistency of the model and the sigmoid shape of the experimental plot.

Despite these results with \( R^2 \) values close to 1, the maximum amount of bound toxin \( T_{bound}^{max} \) for the wt292 and mnn9 cell walls had high mean values but also high associated standard deviations (Table 3). This finding is explained by the greater linearity of the \( [T_{bound} = f(T_{total})] \) curve with consequently less cooperative behavior at the initial stage of the ZEN complex formation for the two strains and inaccuracy of the plateau value determi- nation (30). \( T_{bound}^{max} \) values for wt292 and mnn9 cell walls were

**TABLE 2. Regression coefficients (\( R^2 \)) calculated according to HMN and HMN-GT2a**

<table>
<thead>
<tr>
<th>Strains</th>
<th>Model</th>
<th>wt292</th>
<th>sc1026</th>
<th>fks1</th>
<th>mnn9</th>
</tr>
</thead>
<tbody>
<tr>
<td>HMN: ( [T_{bound} = f(T_{free})] )</td>
<td>0.989</td>
<td>0.990</td>
<td>0.881</td>
<td>0.989</td>
<td></td>
</tr>
<tr>
<td>HMN: ( [T_{bound} = f(T_{total})] )</td>
<td>0.996</td>
<td>0.993</td>
<td>0.934</td>
<td>0.995</td>
<td></td>
</tr>
<tr>
<td>HMN-GT2b: ( [T_{bound} = f(T_{free})] )</td>
<td></td>
<td></td>
<td>0.819</td>
<td></td>
<td></td>
</tr>
<tr>
<td>HMN-GT2: ( [T_{bound} = f(T_{total})] )</td>
<td></td>
<td></td>
<td>0.889</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

\( a \) Each adsorption test for ZEN was done in aqueous medium with 100 \( \mu g \) of \( S. cerevisiae \) cell walls per ml in tubes at 37°C for 1.5 h under orbital agitation.

\( b \) HMN-GT2 equation accounts for the role of the \( \beta \)-D-glucan fraction computed from the four yeast strains tested for their ZEN adsorption properties.

**TABLE 3. Adsorption parameters calculated with HMN equationa**

<table>
<thead>
<tr>
<th>Strains</th>
<th>wt292</th>
<th>sc1026</th>
<th>fks1</th>
<th>mnn9</th>
</tr>
</thead>
<tbody>
<tr>
<td>( n )</td>
<td>1.3 ± 0.2</td>
<td>2.8 ± 0.4</td>
<td>2.1 ± 0.8</td>
<td>1.9 ± 0.4</td>
</tr>
<tr>
<td>( T_{bound}^{max} ) (ppm)</td>
<td>15.0 ± 7.1</td>
<td>3.8 ± 0.2</td>
<td>3.3 ± 0.7</td>
<td>9.3 ± 2.5</td>
</tr>
<tr>
<td>( K_{Dtotal} ) (ppm)</td>
<td>25.6</td>
<td>8.1</td>
<td>6.4</td>
<td>15.7</td>
</tr>
<tr>
<td>( K_{int} ) (ppm)</td>
<td>65.3</td>
<td>14.0</td>
<td>12.6</td>
<td>32.0</td>
</tr>
<tr>
<td>( A_{total} ) (%)</td>
<td>29.2</td>
<td>23.4</td>
<td>25.6</td>
<td>29.6</td>
</tr>
</tbody>
</table>

\( a \) Each adsorption test for ZEN was done in aqueous medium with 100 \( \mu g \) of \( S. cerevisiae \) cell walls per ml in tubes at 37°C for 1.5 h under orbital agitation. Means in the same row with different letters are significantly different (\( P < 0.05 \)).
higher than those for sc1026 and fks1 cell walls (15.0 and 9.3 ppm versus 3.8 and 3.3 ppm). The association constant per site (KD\textsubscript{total}) and saturation point (K\textsubscript{sat}) \cite{30} estimated for the wt292 and mnn9 cell walls were higher (KD\textsubscript{total} = 25.6 and 15.7 ppm, K\textsubscript{sat} = 65.3 and 32.0 ppm, respectively) than those for fks1 and sc1026 cell walls (KD\textsubscript{total} = 6.4 and 8.1 ppm, K\textsubscript{sat} = 12.6 and 14.0 ppm, respectively). Consequently, higher affinity rates were found for wt292 and mnn9 (A\textsubscript{total} = 29.2 and 29.6%, respectively) than for fks1 and sc1026 (A\textsubscript{total} = 25.6 and 23.4%, respectively).

The value of $n$ characterizes both the significance of the number of binding sites involved in the chemical interactions between the adsorbent and the toxin and the intensity of the cooperative effect at the beginning of the complex-forming process. All the tested strains had $n > 1$, indicating that there are several binding sites and a cooperative mechanism \cite{30}. Comparisons between strains revealed that cell walls of sc1026 had significantly higher $n$ ($P < 0.05$) than did cell walls of wt292 and mnn9 (Table 3).

**Role of β-D-glucans in ZEN complex formation.** The HMN-GT2 model indicated a close relationship between the amount of β-D-glucans from yeast cell walls and the ability of the cell walls to complex ZEN (Fig. 2), with the model explaining 96.2% of the experimental data ($R^2 = 0.889$, Fig. 3). Differences observed in β-D-glucan content at 25.6 and 15.7 ppm, $b_{\text{FIGURE 2.}}$

![Graphic representation of the relationship between β-D-glucan content and the rate of adsorption of ZEN using the HMN-GT2 model. $X_1$ = amount of total ZEN added to the medium (ppm); $X_2$ = β-D-glucan content of the cell walls of the four yeast strains tested (%); $Z$ = amount of ZEN adsorbed by yeast cell walls (ppm).]

As above for the HMN model, the expression $[T_{\text{bound}} = f(T_{\text{total}})]$ was preferable to $[T_{\text{bound}} = f(T_{\text{free}})]$, which explained only 93.9% of the experimental data ($R^2 = 0.819$) against 96.2% ($R^2 = 0.889$, Table 4).

**DISCUSSION**

Hill’s model and simulated derivatives HMN-GT and HMN-GT2 gave reliable equations for estimating the complex-forming properties of a constant concentration of β-D-glucans mixed with increasing concentrations of ZEN in an aqueous medium. For the first time, Hill’s model was used to study the properties of the cell walls of four yeast strains and their ability to complex mycotoxins. As concluded in previous work on the HMN model \cite{30}, better modelling of this interaction was obtained when the relation $[T_{\text{bound}} = f(T_{\text{total}})]$ (usual isothermal representation) was used instead of $[T_{\text{bound}} = f(T_{\text{free}})]$ (usual enzymatic relation) for the HMN-GT2 model (Table 2).

Few data have been published related to the use of natural organic binders with mycotoxins. Mixtures of aflatoxins \cite{8, 21–23} and rarely of ZEN \cite{9} have been reported to interact with bacteria such as Lactobacilli, Bifidobacteria, and Propionibacteria more than with yeast. Few investigations on the in vitro capacity of yeast cell walls to adsorb mycotoxins \cite{5, 7, 20} have been carried out; most of these studies have been performed in vivo \cite{27, 28}. Prevention of mycotoxin poisoning at the digestive level has been extensively investigated with various clay-based adsorbents (hydrated sodium calcium aluminosilicate, zeolites, Bentonites, aluminosilicates) using isotherm models such as the Langmuir and Freundlich model. Such simple models can address the mechanisms involved in the adsorption of only a single binder to a single type of site on

**TABLE 4. Contribution of β-D-glucan content in the in vitro test for ZEN adsorption estimated according to HMN-GT2**

<table>
<thead>
<tr>
<th>R$^2$</th>
<th>$n$</th>
<th>$T_{\text{max}}^{\text{bound}}$ (ppm)</th>
<th>KD$^{\text{total}}$ (ppm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.889</td>
<td>1.46 ± 0.21</td>
<td>0.20 ± 0.05</td>
<td>1.50 ± 0.51</td>
</tr>
</tbody>
</table>

$^a$ $T_{\text{max}}^{\text{bound}}$, KD$^{\text{total}}$, and $n$ are expressed per unit of β-D-glucans.
a particular adsorbent. The isothermal curves allowed the computation of a maximum capacity of mycotoxin binding and a distribution constant during the binding process (10). However, chemical interactions between ZEN and β-D-glucans from sc1026 (30) and from wt292, fks1, and mnn9 were not properly assessed by the use of isothermal models. Values for R² calculated with the isothermal models and with Hill’s model were 0.927, 0.900, 0.910, and 0.856 and 0.993, 0.996, 0.934, and 0.995 for the sc1026, wt292, fks1, and mnn9 strains, respectively (Table 2). Thus, Hill’s model gives a better fit than isothermal models for ZEN adsorption by yeast cell walls.

This work clearly confirms the major role played by β-D-glucans in yeast cell walls in the ZEN complex-forming process. The coefficients K<sub>D<sub>total</sub>, T<sub>max<sub>_bound</sub></sub>, and to a lesser extent A<sub>total</sub> increased with the β-D-glucan content in the yeast cell walls. However, because A<sub>total</sub> accounts for the global cooperative effect (first step: low rate of adsorption for low amount of ZEN; second step: increase in adsorption after a little toxin has been attached; third step: saturation of the adsorption sites), the differences among strains were smaller than those indicated by the usual published affinity rates, where adsorption values originated only from the second step, which exhibits only the maximal adsorption A<sub>max</sub> factor (30). Thus, the wt292 and mnn9 strains were more efficient for ZEN adsorption than were strains fks1 and sc1026. HMSN-GT2 confirmed the relation between β-D-glucan content and ZEN adsorption (R² = 0.889) when cell walls were added at 0.1% (wt/wt) to an aqueous medium and for ZEN concentrations higher than 8 ppm. The role of β-D-glucans was also indicated by the closer correlation with HMSN-GT2 than with HMSN-GT (Table 1), emphasizing the links between β-D-glucan content and the affinity coefficient for ZEN adsorption.

With both HMSN and HMSN-GT2, ZEN had n values >1 (Tables 3 and 4) with no significant differences among the four tested strains, indicating a strong cooperative effect but also emphasizing the adsorptive nature of the complex formation between β-D-glucans and ZEN. Thus, the hypothesis of strong specific binding is supported because the number of sites per molecule is not equal to 1. Such n values could also result from the heterogeneity of adsorption sites. Thus, adsorption of ZEN by β-D-glucans probably involves weak hydrogen-bonding reactions.

Chitin, which occurred as linear chains, is glycosidically linked to nonreducing branches of the β(1,3)-d-glucans and β(1,6)-d-glucans (1, 17). Rigidity of the three-dimensional structure of the cell walls is closely linked to the level of chitin encrustation in the β-glucan network, impairing cell wall plasticity (14). Thus, because of the large chitin content and despite high glucan levels in the mnn9 cell wall, K<sub>D<sub>total</sub>, and T<sub>max<sub>_bound</sub></sub> values remained below the values found for wt292 (9.7 versus 1.6%). The sigmoid shape of the adsorption curves tended to be more linear for mnn9 than for the other strains. Similarly, the chemical composition of the sc1026 cell wall differed from that of fks1 only in lower chitin content, leading to highest adsorptive capacity for ZEN when n, K<sub>D<sub>total</sub>, and T<sub>max<sub>_bound</sub></sub> values are considered (Table 3). High chitin content is mainly due to a defence response by cells induced by environmental conditions or genetic modifications (4, 11, 14, 25). In addition, as a part of this rescue mechanism, mutations occurring in mnn9 and fks1 strains could also induce alterations in the β(1,3)-, β(1,6)-d-glucan cross-links to chitin with attendant changes in the organization of the yeast cell wall (14, 25). Thus, fks1 and mnn9 mutants had increased proportions of insoluble components, which decrease their flexibility compared with natural strains wt292 and sc1026.

The mannann content among strains was inversely related to the β-glucan proportions in cell walls and thus to the adsorption capacities of each strain. This highly branched carbohydrate side chain constitutes the outer layer of the yeast cell wall and is associated with phosphodiester bridges, making some regions of the polypeptide backbone relatively rigid. Thus, mannans and mannoproteins are much less permeable to macromolecules (12) such as mycotoxins than is the internal fibrillar layer. However, the results reported here did not indicate any effect of the stereocombination crowning of mannans and mannoproteins on the adsorption of ZEN by β-D-glucans. No significant role for mannans in the complex formation was found; various mannan concentrations in the four strains tested did not affect the adsorption capacity of β-D-glucans. These findings can be explained as follows. First, high levels of mannans in the sc1026 and fks1 strains did not enhance adsorption of ZEN, thus no positive role could be assigned to mannan in complex formation with ZEN. Second, if mannans and mannoproteins had a negative effect on ZEN adsorption, then strain wt292, which has more mannans and less β-D-glucans than does mnn9, would have been less able to form ZEN complexes than was strain mnn9. However, strain wt292 had a higher K<sub>D<sub>total</sub></sub> and T<sub>max<sub>_bound</sub></sub> for ZEN than did strain mnn9. Thus, the adsorption abilities of each strain tested were clearly related to the amount of β-D-glucans in yeast cell walls. Only chitin had a negative effect on the adsorption of ZEN.

Because structural conformation seemed to play a major role in the adsorption mechanisms, further hypotheses were developed regarding the three-dimensional microfilbrillar structure of both β(1,3)- and their branched β(1,6)-d-glucans (14, 18). The β-D-glucans can exist in a random coil in solution or in an ordered structure in the solid state. When β-D-glucans are in both random coil and single- or triple-helix forms (13), adsorption sites on β-D-glucans are accessible to varying degrees. Also, the ratio of soluble to insoluble β-D-glucans could change the three-dimensional network organization (25) and so favor particular β-D-glucan conformations, resulting in modifications of chemical interactions with ZEN and adsorption of toxins. However, cooperative mechanisms remained difficult to explain. Owing to the polarity of some chemical groups with ZEN, a structural change in β-D-glucan conformation that would enhance the adsorption process could occur when the two molecules come into contact.

We reassert that the use of organic material derived from yeast cell walls (5, 7, 20) helps to mitigate the harmful impact of mycotoxins on animals and overcomes the reduction of bioavailability of some nutrients and their non-
biodegradation in the environment. Among the cell wall components, β-D-glucans were the main molecules responsible for ZEN adsorption. Further research will be devoted to elucidating the mechanisms responsible for adsorption by isolating β-D-glucans and studying the influence of abiotic factors on adsorption.

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