

Molecular Dynamics Simulation and Biological Evaluation of Chitosan–CERK1 (LysM) Interactions in Enhancing Resistance to Fusarium Dry Rot in Potato Tubers

1st Marwa K. El-Din Abdel Salam, 2nd Bassam Yahya Ibrahim,

Department of Plant Protection, College of Agriculture and Forestry, University of Mosul

Email: marwaalkhalifa535@gmail.com; bassamy1966@uomosul.edu.iq

Abstract

Chitosan is a promising biostimulant for inducing plant resistance, yet its molecular mechanisms against post-harvest pathogens remain poorly understood. This study investigated how chitosan (0.5 g L⁻¹) activates potato resistance against dry rot caused by *Fusarium oxysporum* during cold storage (5 ± 1 °C, 30 days), integrating molecular simulation with experimental bioassays. Molecular docking (MOE) and 100 ns molecular dynamics simulations of the AlphaFold2-modeled CERK1 protein revealed a stable chitosan–LysM interface: the whole protein equilibrated at an overall RMSD of ≈ 4 Å, while the chitin-binding LysM domains remained highly stable (RMSD < 1.5 Å). Experimentally, chitosan-treated tubers showed significantly elevated PAL (+79%), PPO (+58%), and POD (+36%) activities and total phenolic content (+15%), with markedly reduced disease severity; principal component analysis explained 71.1% of the variance. These results indicate an associative link between chitosan-induced defence activation and binding to the CERK1 (LysM) receptor; as no functional validation was performed, this mechanism is proposed rather than confirmed, supporting chitosan as an environmentally safe alternative to synthetic fungicides in sustainable post-harvest management.

Key words

Chitosan; Cold storage; Defense enzymes (PAL, PPO, POD); LysM receptor; Molecular docking; Potato tuber

Introduction

Potato *Solanum tuberosum* L. are a major global food crop (Kui *et al.*, 2022), but their production and storage are severely affected by fungal diseases, particularly dry rot caused by *Fusarium* species. (Xue *et al.*, 2023). This poses a significant challenge, especially with increasing restrictions on the use of chemical pesticides and the emergence of resistant strains. (Christian, 2023). In this context, plants rely on a sophisticated innate immune system capable of recognizing pathogens through their associated molecular patterns (PAMPs) via specialized receptors known as pattern recognition receptors (PRRs). These receptors, in turn, activate pattern-triggered immunity (PTI) as a first line of defense (Snoeck *et al.*, 2025). Chitin sensors are among the most important of these receptors because chitin is a fundamental structural component of fungal cell walls. These sensors include highly specialized membrane proteins such as CERK1 and CEBiP, which contain LysM domains capable of binding to chitin fragments. (Muhr, 2022; Wang *et al.*, 2024). Upon binding, receptor complexes are formed, triggering a cascade of cellular signaling pathways that include calcium ion influx, activation of protein kinases (MAPKs), and the generation of reactive oxygen species (ROS) in what is known as an oxidative burst. (Averill-Bates, 2024). This ultimately leads to the activation of transcription factors and the stimulation of defense gene expression, particularly those associated with the phenolic pathway and the synthesis of antipathogen secondary compounds. (Azhar *et al.*, 2025). In this context, chitosan—a deacetylated polymer derived from chitin—is a prominent natural biostimulator capable of

activating these receptors or mimicking their signaling. (Stasińska-Jakubas *et al.* 2022; Suwanchaikasem *et al.*, 2024). Chitosan can bind to chitin receptors or interact with cell wall components, thereby amplifying the plant's defense response through a phenomenon known as "defense priming. (Pongprayoon *et al.* 2022; Rayanoothala *et al.*, 2024). This results in increased activity of defense enzymes associated with the phenolic pathway, such as PAL, which contributes to phenol synthesis; POD, which promotes lignin deposition and strengthens the cell wall; and PPO, which converts phenols into fungitoxic quinones. (Rayanoothala, *et al.* 2024; Tiwari *et al.*, 2025). Structurally, chitosan is a linear, naturally abundant polysaccharide (poly- β -(1 $\rightarrow$ 4)-N-acetyl-D-glucosamine) obtained by partial deacetylation of chitin; its degree of deacetylation, molecular weight, and density of protonatable amino groups govern its solubility and biological activity. Being biodegradable, biocompatible, non-phytotoxic, and generally recognized as safe (GRAS), chitosan exhibits a dual mode of action—direct antimicrobial activity against fungal pathogens through electrostatic disruption of the cell membrane, together with the elicitation of plant innate immunity—which makes it an attractive eco-friendly alternative to synthetic fungicides (Liu *et al.*, 2019; Ren *et al.*, 2021). In potato *Solanum tuberosum* L. specifically, chitosan has repeatedly been shown to limit Fusarium dry rot: it inhibited mycelial growth and spore germination of *F.sulphureum* and reduced tuber dry rot (Li *et al.*, 2009), suppressed *F.oxysporum* while enhancing tuber resistance through transcriptome-level reprogramming of stress- and defense-related genes (Ren *et al.*, 2021), and induced defense-related enzymes and secondary metabolites that controlled Alternaria tuber rot in stored tubers (Liu *et al.*, 2019). Despite this promising role, the efficiency of chitosan under cold storage conditions (5°C) still requires further investigation (Raigond *et al.*, 2019 ;Mejdoub-Trabelsi *et al.*, 2020) These conditions lead to decreased metabolic activity and slowed enzymatic reactions, which may affect the sensitivity and responsiveness of chitin receptors to stimulation. (Raigond *et al.*, 2019; Liang *et al.*, 2020) Cold temperatures may also affect the stability and positioning of these receptors within cell membranes, impacting the efficiency of defense signal transmission. (Liang *et al.*, 2020). Therefore, understanding the interaction between chitosan and chitin receptors under storage conditions is a crucial step in developing effective and sustainable strategies to reduce dry rot and enhance potato tuber resistance during storage. However, the mechanistic link between chitosan and the CERK1 (LysM) receptor in post-harvest potato has not yet been verified, and this constitutes the gap addressed here. We therefore hypothesized that chitosan engages the CERK1 LysM domain and is associated with activation of phenylpropanoid and oxidative defence responses that reduce Fusarium dry rot, and we tested this by integrating AlphaFold2 modelling, molecular docking and dynamics, and post-harvest bioassays. (Miya *et al.*, 2007; Mejdoub-Trabelsi *et al.*, 2020).

Materials and methods

The complete sequence of the CERK1 protein was obtained from the NCBI database (Arabidopsis thaliana CERK1, accession NP_566689.2), the best-characterized LysM receptor kinase mediating perception of chitin and its deacetylated derivative chitosan, and was used as the reference receptor for structural modelling. It consists of 623 amino acids, belongs to the receptor-like kinase (RLK) family, and contains the LysM domain responsible for chitin/chitosan binding. The three-dimensional structure was predicted using AlphaFold2, and the highest-ranked model was selected for subsequent analyses. Reliability was assessed using per-residue pLDDT scores, the predicted TM-score (pTM), the predicted aligned error (PAE), the fraction of disordered residues, and consistency across the five predicted models; the chitin-binding LysM domains showed high local confidence (mean pLDDT $\approx$ 93–94). Due to low confidence in the intercellular

domain-membrane region, the extracellular domain (LysM) has been the focus of molecular docking studies as it is the primary site for chitin-chitosan interaction. (Muhr, M. 2022)

Transmembrane protein structure

The transmembrane topology of the CERK1 protein was determined using TMHMM v2.0 software. (Krogh *et al.*, 2001)

Preparation of Chitosan Ligands

A chitosan hydrochloride ligand was prepared, with its three-dimensional structure obtained from the PubChem database. For the active site, a six-unit glucosamine hexamer was selected, and geometric optimization and energy reduction were performed using the MMFF94 force field to achieve the most stable form. Due to the cationic nature of chitosan, the ionization states were set at physiological pH (~7.4), with the amine groups in the protonated state (NH₃⁺), which enhances electrostatic interactions. The charge distribution and spatial structure were also thoroughly verified to ensure accurate representation of hydrogen bonds and potential interactions with the LysM domains of the CERK1 protein before molecular docking. (Halgren, 1996 ; Kim *et al.*, 2023)

Molecular Docking

A molecular docking study was performed using MOE 2024 software to analyze the interaction of chitosan with the CERK1 protein, focusing on the extracellular domain containing LysM domains as the primary binding site. The active pocket was identified based on evolutionary analysis, previous studies, and electrostatic properties. Docking was performed on several chitosan models (pentamer, hexamer, heptamer), and the optimal binding position was selected based on the lowest binding energy, complex stability, and conserved amino acid participation. (Naïm *et al.*, 2007)

Construction of a Membrane System for CERK1

A membrane system for chitin receptor protein kinase 1 (CERK1) was constructed using the CHARMM-GUI Membrane Builder. The protein was embedded in a POPC phospholipid bilayer with the outer (LysM) domain oriented outwards and the kinase domain towards the cytoplasm, according to structural predictions. The CHARMM36 force field was used for both the protein and lipids, and chitosan parameters were generated using CGenFF to ensure accurate molecular conformation. The system was placed inside a 90×90×120 Å aqueous simulation box (TIP3P) and balanced with 0.15 M Na⁺ and Cl⁻ ions to simulate physiological conditions (Hudek, 2024)

Molecular Dynamics Simulation

Molecular dynamics simulations were performed using NAMD 2.14 software. The simulation involved 10,000 energy reduction steps to eliminate interferences, followed by progressive equilibrium (NVT at 310 K, then NPT at 1 bar, and finally free equilibrium) to reach a stable state. The simulation was then run for 100 ns with standard processors such as SHAKE for hydrogen bonds and PME for electrostatic interactions.

The simulations were performed on two systems: the free protein (Apo-CERK1) and the protein–chitosan complex (Holo-CERK1). The simulation trajectories were analyzed using VMD and NAMD by evaluating structural stability and conformational changes through root mean square deviation (RMSD), root mean square fluctuation (RMSF), and radius of gyration (Rg). In addition, hydrogen bonds, electrostatic interactions, and key intermolecular distances were analyzed to assess the stability of the chitosan–CERK1 complex throughout the simulation (Bitencourt-Ferreira *et al.*, 2019; Alzain *et al.*, 2025).

Plant Samples

Arizona potato tubers were obtained from local markets and inspected for disease. They were surface-sterilized with 1% sodium hypochlorite for 3 minutes, then washed three times with sterile distilled water, and left to dry under sterile conditions for one hour before use.

Activation of *F. oxysporum* Isolation

F.oxysporum was activated on PDA medium at 25±2°C for 7–10 days. The conidia suspension was then prepared by adding distilled water with Tween-80, scraping and filtering the conidia, and adjusting the concentration to 1×10^6 conidia/ml using a hemocytometer slide.

Preparation of Chitosan

Chitosan powder was obtained from Nanochemazone (Canada), derived from chitin extracted from shrimp and crab shells. The chitosan had an average molecular weight of approximately 380 kDa and a degree of deacetylation of 85%. It was supplied as a white to off-white amorphous powder with a viscosity range of 50–800 mPa·s (1% solution) and was stored in a cool, dry place until use. Chitosan solutions were freshly prepared before application by dissolving the powder in 1% acetic acid and adjusting the pH to 5.5, at a concentration of 0.5 g L⁻¹.

Application of Treatments and Infection

Tubers were immersed in the chitosan solution for 10 minutes and then dried 24 hours post-treatment. Sterile superficial wounds (5 mm deep, 3 mm in diameter) were made in the eye region using a sterile cork drill, and 50 µL of the conidia suspension was injected into each wound. The wounds were covered with sterile parafilm tape, and the tubers were placed in plastic boxes lined with moist filter paper to maintain relative humidity.

Storage and Preparation Conditions

After a 30-day cold storage period at 5±1°C and 85-90% relative humidity, the tubers were removed from the storage units

Measurement of dry rot severity

The severity of dry rot infection was assessed using (Yıkılmazsoy & Tosun, 2021)from which the severity of the infection was calculated. Each tuber was cut longitudinally and rated on a 0–4 scale according to the proportion of the cut surface showing dry-rot symptoms (0 = healthy; 1 = 1–25%; 2 = 26–50%; 3 = 51–75%; 4 = >75%). A disease severity index (DSI, %) was then calculated as $DSI = [\Sigma(\text{class} \times \text{number of tubers in that class}) / (\text{highest class} \times \text{total number of tubers})] \times 100$

Estimation of Enzymes and Defense Compounds

Tissue samples (2 g) were collected from the affected area and surrounding tissue and used to assess plant defense responses.(Ismaiel & Ibrahim 2023).

Polyphenol Oxidase (PPO) enzyme activity estimating.

Estimation of Polyphenol Oxidase activity has been done according to (Shi *et al.*, 2002)

Peroxidase (POD) enzyme activity estimating.

Estimation of Peroxidase (POD) activity has been done according to (Howell *et al.*, 2003)

Phenylalanine ammonia-lyase. (PAL) enzyme activity estimating.

Phenylalanine ammonia-lyase. (PAL) activity has been done according to (El-Beltagi *et al.*, 2011)

Total Phenols (TP) Determination.

Total phenol in tuber samples were determined using Folin–Ciocalteu reagent described by Jain *et al* (2017) The samples were measured at 750 nm Gallic acid (0–250mg.L⁻¹) was used as standard.

Statistical Analysis

The experiment was conducted using a completely randomized design (CRD) with six biological replicates per treatment; each replicate comprised 5 tubers, giving a total of 120 tubers across the four treatments. Data on infection severity, defense enzyme activity (PAL, PPO, and POD), and total phenol content were subjected to one-way ANOVA (with F-values, degrees of freedom, and

exact p-values reported), and treatment means (expressed as mean $\pm$ standard error) were compared using the least significant difference (LSD) test, at a probability level of 0.05. All analyses were performed using SAS version 9.4 software.

Principal Component Analysis (PCA)

Principal component analysis was performed on the measured response variables (PAL, PPO, and POD activities, total phenolic content, and disease severity); the observations were the replicate measurements obtained across the four treatments (untreated control, chitosan, *F. oxysporum*, and chitosan + *F. oxysporum*). Data were statistically analyzed using the Shapiro–Wilk test, abnormal values were transformed, and normalization was performed using the Z-score. PCA analysis was performed using R software, retaining components with eigenvalues >1 that explained more than 70% of the total variance, and using loads $>|0.5|$ to identify influential variables.

Results

The 3D model of the CERK1 protein generated by AlphaFold2 (Fig 1) shows the receptor's characteristic architecture with the three LysM domains (yellow beta leaves and white and red loops) in the left-hand side as potential chitosan binding sites, followed by the red helical membrane domain in the middle, and the kinase intrinsic domain (red alpha helices and yellow beta leaves) in the lower right-hand side, ending with a disorganized white flexible terminal tail, reflecting the graded functional organization from extrinsic exposure to intrinsic response **characteristic of plant kinase receptors**

TMHMM analysis of the CERK1 protein revealed a single, distinct transmembrane domain at approximately 230–250 amino acids, confirming its status as a single-crossing transmembrane receptor. Prediction also indicated that the amino terminus containing the LysM domains is located extracellularly, while the carboxyl terminus containing the kinase domain is located intracellularly. This structural arrangement reflects the typical functional arrangement of chitin recognition receptors, where the extracellular domain is responsible for ligand binding, while the intracellular domain is responsible for signal transduction and activation of the immune response.

Comparative analysis of five AlphaFold2 models of the CERK1 protein reveals exceptional consistency in structural confidence ($\mu = 78.5\text{--}78.9$), with the three LysM domains (residues 24–164) exhibiting very high confidence (pLDDT >90) and remarkable concordance across all models, confirming their reliability as chitosan binding sites (Fig 2). The core kinase domain (251–505) shows good confidence ($\sim 75\text{--}85$), except for the activation loop (280–320), which shows a dramatic decrease ($\sim 20\text{--}30$), consistent with its functional flexibility. (Table 1, Fig 3)

The five models showed consistent global confidence (mean pLDDT 78.5–78.9) and uniformly high confidence in the chitin-binding LysM region (mean pLDDT 93.4–93.8), with pTM = 0.53, a ranking score of 0.62–0.63, a minimum chain-pair predicted aligned error (PAE) of 0.76 Å, and no steric clashes (has_clash = 0). Approximately 18–19% of the structure was predicted as disordered, consistent with the flexible terminal and inter-domain regions that account for the higher whole-protein RMSD, while the functional LysM binding interface was predicted with high confidence. In contrast, the terminal and transition regions show low confidence and greater variability, as expected for structurally flexible regions. This assessment thus guides molecular modeling strategies toward the confident use of LysM domains in chitosan interaction studies with specialized handling of low-confidence regions. Figure (4) shows the results of RMSD analysis of the CERK1-Chitosan complex during a 100 ns molecular dynamics simulation. In the top left, we observe that the whole protein (blue line) achieves good structural stability with RMSD values of around 4 angstroms with an initial equilibrium period during the first 10 ns. In the top right, the three LysM domains responsible for chitosan binding show exceptional stability with RMSD values of less than 1.5 angstroms, especially the LysM2 domain (light blue line), which shows the

best stability with a clear chitosan binding event (dashed points) around 10 ns, followed by remarkable stability. The lower bar charts emphasize the clear variation in stability between the different domains, with the LysM domains (blue and green bars) achieving the highest stability compared to flexible regions such as the terminal tail.

Per-domain RMSD analysis (Table 2) confirmed this pattern: the three LysM domains were the most stable (mean RMSD 0.93–1.19 Å; coefficient of variation $\leq 9\%$), with LysM2 the most stable (0.93 ± 0.08 Å), whereas flexible regions were more mobile (C-terminal tail 5.46 ± 1.42 Å; extracellular linker 3.78 ± 0.79 Å). The bound chitosan ligand remained stable (1.69 ± 0.35 Å), supporting a persistent LysM-anchored complex throughout the 100 ns trajectory.

Molecular docking of the glucosamine hexamer into the LysM pocket of CERK1 produced an energetically favourable interaction. The top-ranked pose scored $S = -12.82$ kcal/mol (refinement energy $E_{\text{refine}} = -88.63$ kcal/mol), and the five highest-ranked poses scored between -12.82 and -11.18 kcal/mol (Table 3), indicating a consistent binding mode at the chitin-binding site.

Figure (5) shows a two-dimensional interaction map between the chitosan molecule (ligand) and the chitin kinase 1 (CERK1) receptor. At the center, we see the chitosan structure, composed of two glucosamine units with hydroxyl (-OH) and amino ($\text{NH}_2/\text{NH}_3^+$) groups, characteristic of partially deacetylated chitosan. Surrounding this structure are amino acid residues from the protein, encoded by colored circles according to their chemical properties: green for uncharged polar residues such as serine (Ser) and threonine (Thr), which form hydrogen bonds with the hydroxyl groups in chitosan; blue for positively charged basic residues such as lysine (Lys), arginine (Arg), and histidine (His), which react with negative charges or form hydrogen bonds with oxygen groups; and purple for negatively charged acidic residues such as aspartate (Asp) and glutamate (Glu), which may react with the positively charged amino groups in chitosan. The proton, while the dotted lines indicate hydrogen bonds and polar interactions between the ligand and the binding site residues. This pattern of interactions supports the binding mechanism in the "Slipped Sandwich" model, where glucosamine units are positioned in a binding groove made up of multiple LysM domains, leading to specific molecular recognition and subsequent activation of plant defense signaling pathways by stimulating kinase activity in the receptor's internal domain.

Effect of Chitosan on Dry Rot Infection Severity and Activation of Biological Defense Responses in Potato Tubers Infected with *F. oxysporum*

The results in Table 4 show that treating potato tubers with chitosan (0.5 g/L^{-1}) before inoculation with *F. oxysporum* significantly reduced dry rot infection severity from 0.70 to 0.21, representing a reduction of approximately 70% compared to the untreated infected control. Chitosan treatment with the fungus also showed an increase in PAL enzyme activity (0.52), although this increase was only at the threshold of significance level. In contrast, PPO enzyme activity was highest in both the chitosan + fungus treatment (0.68) and the fungus alone treatment (0.62), significantly exceeding the control. This suggests that infection plays a role in stimulating this enzyme and enhancing the defense response. The POD enzyme showed a significant increase in activity across all treatments compared to the control group, reaching its highest value in the fungus-only treatment (21), indicating its association with oxidative stress associated with infection. Similarly, total phenol content increased significantly in all treatments, with the chitosan + fungus treatment recording the highest value (155), surpassing all other treatments.

Principal component analysis (PCA) showed that PC1 and PC2 together explained 71.1% of the total variance in the data (PC1 = 39.0%, PC2 = 32.1%), (Fig 6) a lower percentage than that resulting from the mean analysis of treatments. This reflects the true variance between replicates and provides a more realistic representation of the data.

The trends of the variables indicated that PC1 was associated with defensive activity, as it was linked to increased PPO and POD activity and total phenol accumulation. PC2, on the other hand, separated infection severity and PAL, which moved in opposite directions, indicating an inverse relationship between PAL activation and infection level.

The chitosan-fungus treatment clustered near the PAL and phenol vectors and away from infection severity, reflecting high defensive efficiency and reduced disease. In contrast, the fungus treatment alone was associated with increased infection severity and POD activity, indicating a response to oxidative stress induced by infection. The comparison was located away from all defense markers, while the chitosan-only treatment showed greater dispersion between replicates. In general, the analysis shows that chitosan in the presence of the pathogen enhances the defense pathways associated with PAL and phenols, directing the tuber response towards resistance rather than a response to pathogenic stress.

Discussion

The AlphaFold2 model of the CERK1 protein reproduced the characteristic architecture of lysine-mediated kinase receptors, progressing from a three-band extracellular domain (LysM), to a single transmembrane domain, to an intracellular kinase domain, and finally to a flexible terminal tail. TMHMM analysis further supported this by revealing a single transmembrane domain at residues 230–250, with the amino terminus (LysM) oriented extracellularly and the carboxyl terminus (kinase) oriented intracellularly—a typical functional split for chitin recognition receptors: ligand binding externally and signal transduction internally (Miya *et al.*, 2007).

Comparative evaluation of the five models demonstrated very high confidence (pLDDT > 90) and concordance in the LysM domains (residues 24–164), compared to a sharp decrease in the activation loop (280–320) and terminal regions. Since the pLDDT value is a reliable indicator of positional prediction accuracy and is inversely correlated with structural irregularity (Jumper *et al.*, 2021), this pattern is interpreted as functional flexibility rather than predictive inadequacy, justifying the confident reliance on LysM domains in docking studies with special handling of low-confidence regions.

Molecular dynamics simulations (100 ns) were consistent with this assessment; the complex stabilized at an RMSD $\approx$ 4 angstroms (Fig 4a) after initial equilibration, and the LysM domains exhibited exceptional stability (RMSD < 1.5 angstroms), with LysM2 showing the best stability and a clear correlation event around 10 ns. This central position of LysM2 is consistent with crystallographic data showing that oligosaccharides are associated with a surface cavity on this scale Liu *et al.*, 2012), while the terminal tail oscillation is consistent with the observed pLDDT decrease.

The two-dimensional diagram revealed hydrogen bonds between polar residues (Ser/Thr) and hydroxyl groups, and interactions of basic (Lys/Arg/His) and acidic (Asp/Glu) residues with positive oxygen and amine groups (NH_3^+) in partially deacetylated chitosan. This pattern supports a "sliding sandwich" mechanism in which glucosamine units are arranged in a bonding groove formed by LysM domains, leading to dimerization and activation of endogenous kinase (Liu *et al.*, 2012). It is noteworthy that the outer domain of CERK1 directly binds to partially deacetylates chitosan—not chitin alone—depending on the three LysM domains (Fig 4b), thus supporting the hypothesis that chitosan is recognized via the CERK1 system itself (Petutschnig *et al.*, 2010).

At the physiological level, principal component analysis revealed a separation, with the two components explaining 71.1% of the variance. This involved a convergent positive loading of PPO, POD, and total phenols on PC1 (the integrated defense axis), while PC2 contrasted PAL activity with disease severity. Chitosan treatment was positioned in the high-defense and low-disease zone. These results are consistent with the documented stimulation of the phenylpropanoid

pathway and its enzymes by chitosan, and its increase in the phenolic content of stored potato tubers (Liu *et al.*, 2019), as well as its reduction of *Fusarium* dry rot infection in potatoes during storage (Mejdoub-Trabelsi *et al.*, 2020; Raigond *et al.*, 2019). The simultaneous assembly of all defense variants supports the hypothesis that chitosan is a broad-spectrum biostimulator that coherently activates natural resistance mechanisms, from molecular recognition at the CERK1 level to an integrated metabolic defense response.

Conclusions and Recommendations

This study has several limitations that frame its conclusions. First, the proposed role of CERK1 rests on computational evidence (modelling, docking, and a single 100 ns molecular dynamics trajectory) and was not functionally validated by gene-expression, receptor-knockout, or biochemical assays; the chitosan–CERK1 link is therefore associative rather than causal. Second, direct antifungal activity of chitosan and the involvement of additional signalling pathways cannot be excluded. Third, a single chitosan concentration (0.5 g/L) was tested, preventing a dose–response analysis, and the bioassay did not include an acetic-acid vehicle control or a registered-fungicide positive control. Replicate simulations, a concentration gradient, and these controls are recommended for future work.

The convergence of simulation and bioassay data is consistent with engagement of the CERK1 LysM domain as a plausible initiating event in chitosan-associated systemic resistance. However, because no functional validation (gene-expression, receptor knockout, or biochemical assay) was performed, this mechanism is proposed rather than confirmed, and direct antifungal activity of chitosan or other signalling pathways may also contribute. These findings provide a basis for the application of chitosan as an environmentally safe postharvest treatment and a candidate alternative to synthetic fungicides in sustainable potato storage management. Chitosan at a concentration of 0.5 g/L is a promising environmentally friendly option in post-harvest disease management, and the proposed chitin-receptor mechanism warrants direct functional verification, dose–response testing, and expansion of studies to include different varieties, pathogens, and diverse storage conditions, adopting an approach that combines computational modeling and practical experiments to support sustainable biological control strategies

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Novelty Statement

To our knowledge, this study is among the first to combine 100 ns molecular dynamics simulations of a CERK1 (LysM) reference-receptor model (*Arabidopsis thaliana* CERK1, NP_566689.2; modeled using AlphaFold2) with post-harvest bioassays to propose a mechanism of chitosan resistance against *F. oxysporum* during cold storage. Unlike previous studies that focused solely on bioassays, this study reveals the role of the LysM domain in binding as a molecular catalyst for the activation of defense enzymes, providing a mechanistic basis for the efficacy of chitosan as a post-harvest bioprotective agent.

Authors' Contributions

Marwa Khair El-Din Abdel Salam: Prepared the study, collected data, conducted experiments, performed statistical analysis, and wrote the original draft of the research.

Bassam Yahya Ibrahim: Supervised the research, developed the study's concept and methodology, reviewed the results, and edited and revised the final version of the research.

Ethical approval

No ethical approval is required.

Conflict of interest

The authors declared no conflict of interest.

References

- Alzain, A. A., Almogaddam, M. A., Yousif, R., Alqarni, M. H., Foudah, A. I., Osman, W., ... & Ibrahim, S. R. (2025). Molecular docking, molecular dynamics simulation, and pharmacophore-based virtual screening unveil natural compounds with TIM-3 inhibitory activity. *Journal of Pharmacy and Bioallied Sciences*, 17(Suppl 2), S1882-S1887.
DOI: [10.4103/jpbs.jpbs_402_25](https://doi.org/10.4103/jpbs.jpbs_402_25)
- Averill-Bates, D. (2024). Reactive oxygen species and cell signaling. Review. *Biochimica et Biophysica Acta (BBA)-Molecular Cell Research*, 1871(2), 119573.
DOI: [10.1016/j.bbamcr.2023.119573](https://doi.org/10.1016/j.bbamcr.2023.119573)
- Azhar, M., Khan, A. H., Nadeem, A. Y., Fatima, A., Al-Suhaimi, E. A., & Shehzad, A. (2025). Cellular receptors and cell signaling. In *Cell Signaling* (pp. 46-63). CRC Press.
DOI: [10.1201/9781003594901-3](https://doi.org/10.1201/9781003594901-3)
- Bitencourt-Ferreira, G., & de Azevedo Jr, W. F. (2019). Molecular dynamics simulations with NAMD2. In *Docking screens for drug discovery* (pp. 109-124). New York, NY: Springer New York.
- Christian, C. L. (2023). Characterization of Fusarium Dry Rot Pathogens of Potato and Fusarium Dry Rot Disease Management in the Pacific Northwest of the United States (Doctoral dissertation, University of Idaho).
- Halgren, T.A., 1996. Merck molecular force field. I. Basis, form, scope, parameterization, and performance of MMFF94. *J. Comput. Chem.*, 17(5-6): 490-519. <https://doi.org/10.1002/jcc.540170502>
- Howell, C. R., Hanson, L. E., Stipanovic, R. D., Puckhaber, L. S., & Wheeler, M. H. (2003). Induction of Terpenoid Synthesis in Cotton Roots and Control of Rhizoctonia solani by Seed Treatment with Trichoderma virens (Phytopathology (2000) 90:3 (248-252)). *Phytopathology*, 93(12), 1606. [Doi.org/10.3864/j.issn.0578-1752.2018.13.002](https://doi.org/10.3864/j.issn.0578-1752.2018.13.002)
- Hudek, M. (2024). Molecular dynamics simulations of chitosan oligomer interactions at surfaces and lipid bilayers (Doctoral dissertation, University of Strathclyde). University of Strathclyde Repository.
- Ismail, M. M., & Ibrahim, B. Y. (2023). Testing the efficacy of silicon and glutathione in inducing systemic resistance against *Fusarium solani*, the cause of broad bean root rot. *Mesopotamia Journal of Agriculture*, 51(2), 14–24.
<https://doi.org/10.33899/magrj.2023.138178.1218>
- Jain, R., Rao, B., & Tare, A. B. (2017). Comparative analysis of the spectrophotometry based total phenolic acid estimation methods. *Journal of Analytical Chemistry*, 72(9), 972-976.
<https://doi.org/10.1134/S106193481709009X>
- Jumper, J., Evans, R., Pritzel, A., Green, T., Figurnov, M., Ronneberger, O., Tunyasuvunakool, K., Bates, R., Židek, A., Potapenko, A., Bridgland, A., Meyer, C., Kohl, S.A.A., Ballard, A.J., Cowie, A., Romera-Paredes, B., Nikolov, S., Jain, R., Adler, J., Back, T., Petersen, S., Reiman, D., Clancy, E., Zielinski, M., Steinegger, M., Pacholska, M., Berghammer, T., Bodenstein, S., Silver, D., Vinyals, O., Senior, A.W., Kavukcuoglu, K., Kohli, P. and Hassabis, D., 2021. Highly accurate protein structure prediction with AlphaFold. *Nature*, 596(7873): 583-589. <https://doi.org/10.1038/s41586-021-03819-2>
- Kim, S., Chen, J., Cheng, T., Gindulyte, A., He, J., He, S., Li, Q., Shoemaker, B.A., Thiessen, P.A., Yu, B., Zaslavsky, L., Zhang, J. and Bolton, E.E., 2023. PubChem 2023 update. *Nucleic Acids Res.*, 51(D1): D1373-D1380. <https://doi.org/10.1093/nar/gkac956>

- Kui, L., Majeed, A., Ahmed, S., Khan, M. S. S., Islam, F., Chen, J., & Dong, Y. (2022). *Solanum tuberosum* (potato). *Trends in Genetics*, 38(11), 1193-1195.
- Liang, S.-m. *et al.*, 2020. The membrane lipid metabolism in horticultural products suffering chilling injury. *Food Qual. Saf.*, 4(1): 9-14. <https://doi.org/10.1006/jmbi.2000.4315>
- Liu, T., Liu, Z., Song, C., Hu, Y., Han, Z., She, J., Fan, F., Wang, J., Jin, C., Chang, J., Zhou, J.-M. and Chai, J., 2012. Chitin-induced dimerization activates a plant immune receptor. *Science*, 336(6085): 1160-1164. <https://doi.org/10.1126/science.1218867>
- Liu, J., Zhang, X., Kennedy, J.F., Jiang, M., Cai, Q. and Wu, X., 2019. Chitosan induces resistance to tuber rot in stored potato caused by *Alternaria tenuissima*. *Int. J. Biol. Macromol.*, 140: 851-857. <https://doi.org/10.1016/j.ijbiomac.2019.08.227>
- Mejdoub-Trabelsi, B., Touihri, S., Ammar, N., Riahi, A. and Daami-Remadi, M., 2020. Effect of chitosan for the control of potato diseases caused by *Fusarium* species. *J. Phytopathol.*, 168(1): 18-27. <https://doi.org/10.1111/jph.12847>
- Miya, A., Albert, P., Shinya, T., Desaki, Y., Ichimura, K., Shirasu, K., Narusaka, Y., Kawakami, N., Kaku, H. and Shibuya, N., 2007. CERK1, a LysM receptor kinase, is essential for chitin elicitor signaling in *Arabidopsis*. *Proc. Natl. Acad. Sci. U.S.A.*, 104(49): 19613-19618. . <https://doi.org/10.1073/pnas.0705147104>
- Muhr, M. M. (2022). Characterization of *Populus × canescens* LysM receptor-like kinases CERK1-1 and CERK1-2 and their role in chitin signaling (Doctoral dissertation, Georg-August-Universität Göttingen, Georg-August University School of Science). Göttingen, Germany. . <https://doi.org/10.53846/goediss-9128>
- Naïm, M., Bhat, S., Rankin, K.N., Dennis, S., Chowdhury, S.F., Siddiqi, I., Drabik, P., Sulea, T., Bayly, C.I., Jakalian, A. and Purisima, E.O., 2007. Solvated interaction energy (SIE) for scoring protein–ligand binding affinities. *J. Chem. Inf. Model.*, 47(1): 122-133. <https://doi.org/10.1021/ci600406v>
- Petutschnig, E.K., Jones, A.M.E., Serazetdinova, L., Lipka, U. and Lipka, V., 2010. The lysin motif receptor-like kinase (LysM-RLK) CERK1 is a major chitin-binding protein in *Arabidopsis thaliana* and subject to chitin-induced phosphorylation. *J. Biol. Chem.*, 285(37): 28902-28911. . <https://doi.org/10.1074/jbc.M110.116657>
- Pongprayoon, W., Siringam, T., Panya, A., & Roytrakul, S. (2022). Application of chitosan in plant defense responses to biotic and abiotic stresses. *Applied Science and Engineering Progress*, 15(1).
- Raigond, P., Sagar, V., Mishra, T., Thakur, A., Singh, B., Kumar, V., Gupta, V.K., Dutt, S. and Changan, S.S., 2019. Chitosan: a safe alternative to synthetic fungicides to manage dry rot in stored potatoes. *Potato Res.*, 62: 393-409. <https://doi.org/10.1007/s11540-019-9421-8>
- Rayanoothala, P. S., Dweh, T. J., Mahapatra, S., & Kayastha, S. (2024). Unveiling the protective role of chitosan in plant defense: A comprehensive review with emphasis on abiotic stress management. *Crop Design*, 3(4), 100076.
- Shi, C., Dai, Y., Xu, X., Xie, Y., & Liu, Q. (2002). The purification of polyphenol oxidase from tobacco. *Protein Expression and Purification*, 24(1), 51–55. <https://doi.org/10.1006/prep.2001.1543>
- Snoeck, S., Johannndrees, O., Nürnberger, T., & Zipfel, C. (2025). Plant pattern recognition receptors: from evolutionary insight to engineering. *Nature Reviews Genetics*, 26(4), 268-278. <https://doi.org/10.1038/s41576-024-00793-z>
- Stasińska-Jakubas, M., & Hawrylak-Nowak, B. (2022). Protective, biostimulating, and eliciting effects of chitosan and its derivatives on crop plants. *Molecules*, 27(9), 2801. <https://doi.org/10.3390/molecules27092801>

Suwanchaikasem, P., Idnurm, A., Selby-Pham, J., Walker, R., & Boughton, B. A. (2024). The impacts of chitosan on plant root systems and its potential to be used for controlling fungal diseases in agriculture. *Journal of Plant Growth Regulation*, 1-22.

<https://doi.org/10.1007/s00344-024-11356-1>

Tiwari, R. K., Goswami, S. K., Gujjar, R. S., Kumar, R., Kumar, R., Lal, M. K., & Kumari, M. (2025). Mechanistic insights on lignin-mediated plant defense against pathogen infection. *Plant Physiology and Biochemistry*, 228, 110224.

<https://doi.org/10.1016/j.plaphy.2025.110224>

Wang, L., He, Y., Guo, G., Xia, X., Dong, Y., Zhang, Y., ... & Li, G. (2024). Overexpression of plant chitin receptors in wheat confers broad-spectrum resistance to fungal diseases. *The Plant Journal*, 120(3), 1047-1063. <https://doi.org/10.1111/tpj.17035>

Xue, H., Liu, Q., & Yang, Z. (2023). Pathogenicity, mycotoxin production, and control of potato dry rot caused by *Fusarium* spp.: a review. *Journal of Fungi*, 9(8), 843.

<https://doi.org/10.3390/jof9080843>

Yıkılmazsoy, G., & Tosun, N. (2021). Characterization of *Fusarium sambucinum* isolates associated with potato dry rot and evaluation of cultivar susceptibility and fungicides. *Turkish Journal of Agriculture and Forestry*, 45(2), 222–233. <https://doi.org/10.3906/tar-2006-100>

Figure legends

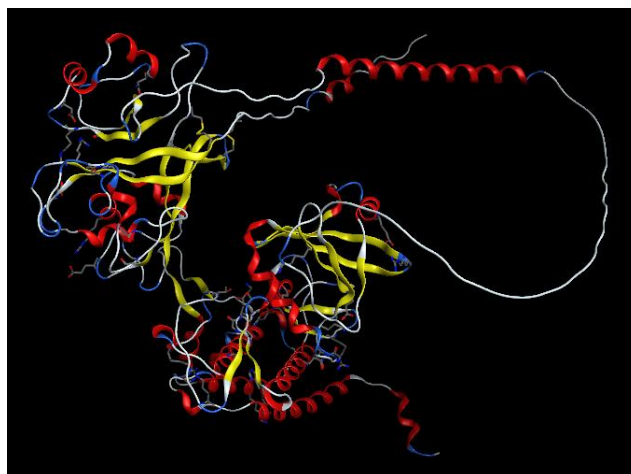


Fig 1 The 3D model of the CERK1 protein generated by AlphaFold2

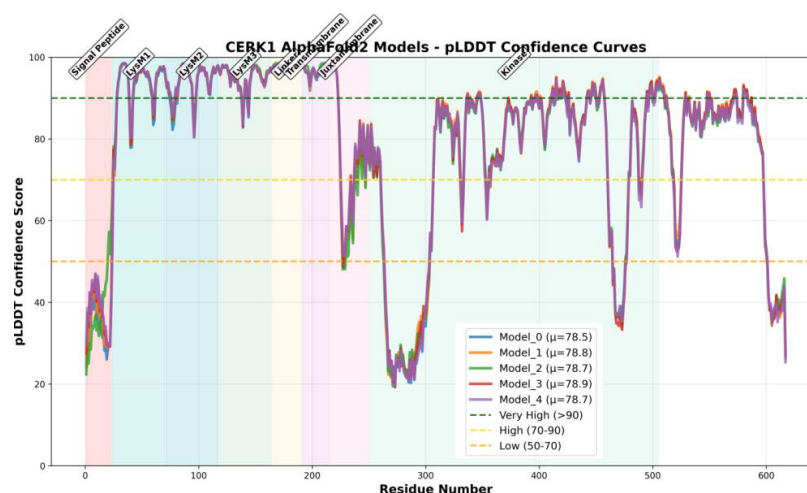


Fig 2 pLDDT confidence curves for each acid residue in the five predicted models of CERK1 protein via AlphaFold2, with the functional domains (signal peptide, three LysM domains, paramembrane region, transmembrane region, and kinase domain) highlighted, and confidence thresholds identified (very high >90, high 70–90, low 50–70).

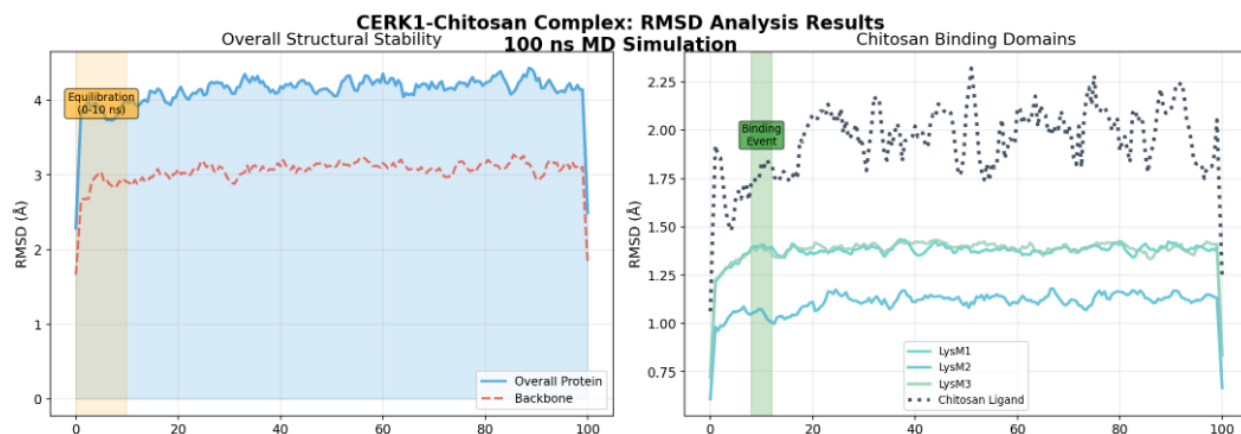


Fig 3. RMSD analysis of the CERK1–chitosan complex during a 100 ns molecular dynamics simulation. (a) Overall structural stability, showing the RMSD of the whole protein and the backbone, with the equilibrium phase (0–10 ns) highlighted. (b) RMSD of the LysM1, LysM2, and LysM3 binding domains and the chitosan ligand, highlighting the binding event

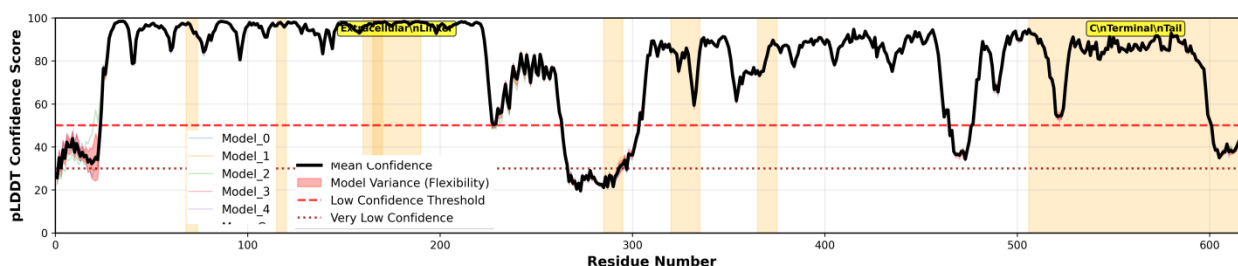


Fig 4. Average confidence curve of pLDDT for each acid residue in the CERK1 protein across the five models predicted by AlphaFold2 (solid line), with the shaded range representing model variance as an indicator of structural flexibility, highlighting the extracellular region and C-terminal tail; the dashed line indicates a low confidence threshold (pLDDT = 50) and the dotted line indicates a very low confidence level.

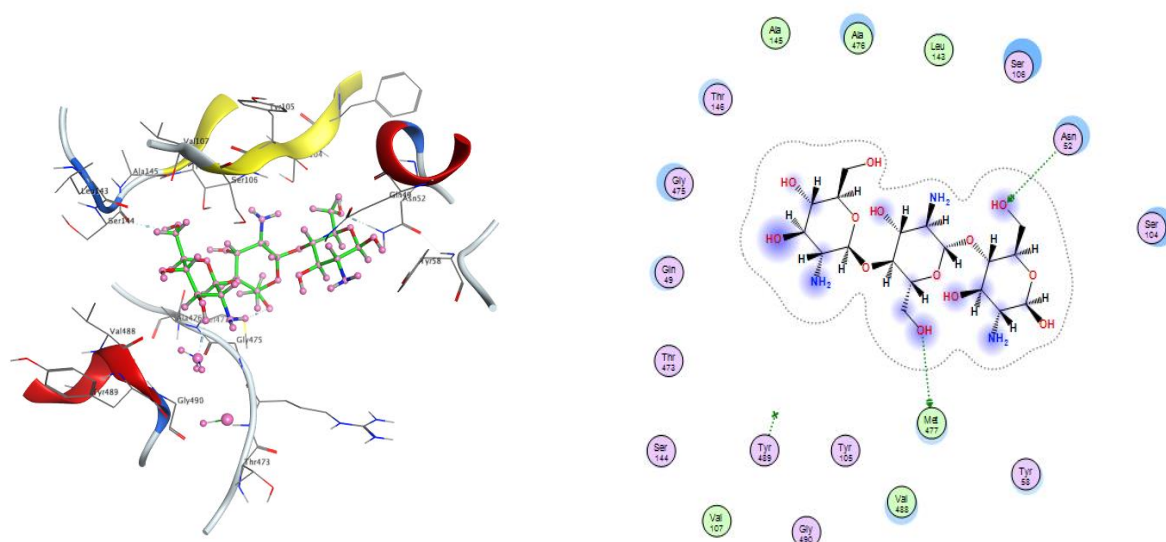


Fig 5. Chitosan ligand binding reactions within the CERK1 active site. (a) Three-dimensional representation of the chitosan–CERK1 complex, with the ligand within the binding pocket surrounded by secondary structural elements (alpha helices and beta sheets). (b) Two-dimensional diagram of the reactions showing the surrounding active site residues and the types of bonds formed with the ligand.

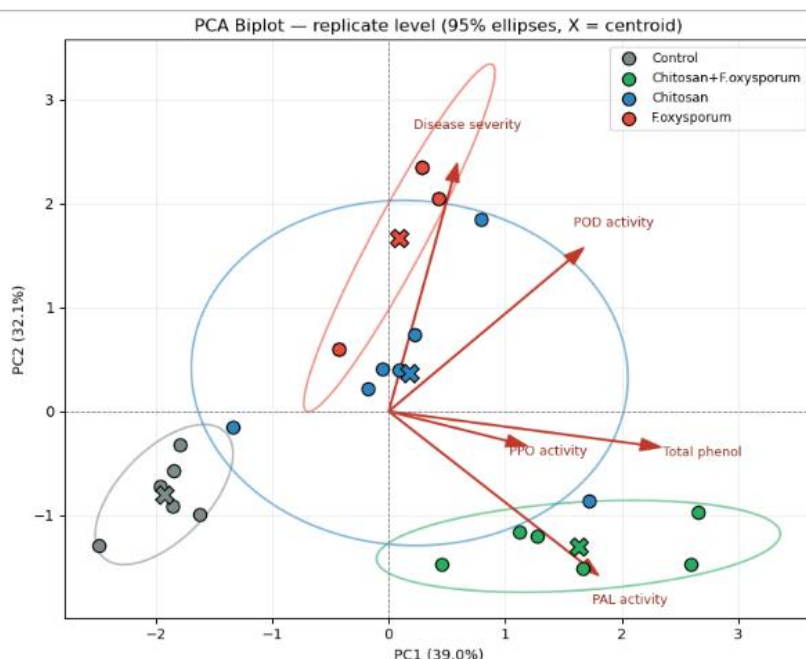


Fig 6. Principal Component Analysis (PCA) Biplot of the integrated defense response in potato tubers under four treatments (chitosan, control, chitosan+ *F. oxysporum* and *F. oxysporum* infection). The first and second components explain 39 % and 32.1% of the total variance, respectively. The vectors represent the loads of the defense variables (PAL, PPO, and POD enzymes, and total phenols) and disease severity, while the symbols represent the replicates of each treatment.

Table 1. Quality comparison of the five AlphaFold2 models of the CERK1 protein.

Model	Mean pLDDT	LysM avg pLDDT	Ranking score	pTM score
Model_0	78.5	93.4	0.630	0.530
Model_1	78.8	93.7	0.630	0.530
Model_2	78.7	93.8	0.620	0.530
Model_3	78.9	93.8	0.620	0.530

Table 2. Per-domain RMSD stability of the CERK1–chitosan complex over the 100 ns simulation (mean $\pm$ SD; CV = coefficient of variation; pLDDT = AlphaFold2 per-domain confidence).

Component	Mean $\pm$ SD (Å)	Min–Max (Å)	CV (%)	pLDDT
Overall protein	3.71 $\pm$ 0.29	2.95–4.52	7.8	–
Backbone	2.60 $\pm$ 0.21	2.12–3.24	8.1	–
LysM1	1.19 $\pm$ 0.06	1.05–1.38	4.8	92.5
LysM2	0.93 $\pm$ 0.08	0.75–1.18	9.0	94.2
LysM3	1.19 $\pm$ 0.06	1.06–1.35	4.8	95.0
Kinase N-lobe	2.02 $\pm$ 0.44	1.32–3.15	21.8	78.5
Kinase C-lobe	2.02 $\pm$ 0.49	1.28–3.42	24.3	79.2
Extracellular linker	3.78 $\pm$ 0.79	2.45–5.85	21.0	65.0
Juxta membrane	2.84 $\pm$ 0.62	1.89–4.52	21.8	55.0
C-terminal tail	5.46 $\pm$ 1.42	3.12–8.95	26.0	35.0
Chitosan ligand	1.69 $\pm$ 0.35	1.15–2.65	20.7	–

Table 3. Top five docking poses of the chitosan hexamer in the CERK1 LysM pocket (MOE; S = GBVI/WSA dG score).

Pose	S (kcal/mol)	RMSD (Å)	E_refine (kcal/mol)
1	-12.82	3.35	-88.63
2	-11.56	2.75	-75.69
3	-11.45	2.10	-82.81
4	-11.26	1.78	-73.86
5	-11.18	2.85	-74.23

TABLE 4 Effect of Chitosan on Dry Rot Infection Severity and Activation of Biological Defense Responses in Potato Tubers Infected with *F. oxysporum*

TRETMENT	Disease severity	PAL activity	PPO activity	POD activity	Total phenol
Control	0 $\pm$ 0	0.29 $\pm$ 0.0024	0.43 $\pm$ 0.047	14 $\pm$ 0.019	135 $\pm$ 1.24
<i>F.oxysporum</i> +Chitosan 0.5 g.L ⁻¹	0.21 $\pm$ 0.027	0.52 $\pm$ 0.036	0.68 $\pm$ 0.039	19 $\pm$ 0.021	155 $\pm$ 2.14
Chitosan 0.5 g.L ⁻¹	0 $\pm$ 0	0.5 $\pm$ 0.037	0.52 $\pm$ 0.074	18 $\pm$ 0.019	149 $\pm$ 2..47
<i>F.oxysporum</i>	0.7 $\pm$ 0.042	0.31 $\pm$ 0.028	0.62 $\pm$ 0.042	21 $\pm$ 0.015	147 $\pm$ 3.15
LSD	0.14	0.23	0.18	2.3	14.7