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The potential of rumen-derived indole-3-acetic acid in enhancing *Capsicum annuum* growth: metagenomic and computational insights

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Abstract: This study investigates the potential of rumen-derived bacteria, specifically *Enterococcus faecalis*, to enhance plant growth through the production of indole-3-acetic acid (IAA), a key plant growth regulator. Using metagenomic 16S rRNA sequencing, we characterized the microbial community in the bovine rumen, revealing a high abundance of *Firmicutes* and *Proteobacteria*, with *Enterococcus faecalis* predominating. These microbes are hypothesized to produce IAA, which could activate the *Transport Inhibitor Response 1* (TIR1) receptor in *Capsicum annuum* (bell pepper), a critical step for promoting plant growth and stress resilience. To explore this hypothesis, we employed an *in silico* approach, combining molecular docking and molecular dynamics simulations. The docking study revealed favorable binding between IAA and TIR1, facilitated by hydrogen bonds and hydrophobic interactions, with a binding affinity of -5.9 kJ/mol. Molecular dynamics simulations further confirmed that IAA binding induces conformational changes in TIR1, enhancing its stability and potentially activating the receptor. These findings suggest that IAA produced by rumen bacteria could serve as a natural bioactivator, offering a promising avenue for sustainable agricultural practices. This study highlights the potential of rumen microbiota as a biofertilizer, contributing to enhanced plant growth and resilience while promoting environmentally friendly agricultural solutions.

Keywords: bioactivator; *Enterococcus faecalis*; indole-3-acetic acid; molecular docking; molecular dynamic.

Abbreviations: ARF_Auxin Response Factor; GMQE_Global Model Quality Estimation; IAA_Indole-3-acetic acid; LAB_Lactic Acid Bacteria; MM/PBSA_Molecular Mechanics Poisson-Boltzmann Surface Area; OTU_Operational Taxonomic Unit; PCA_Principal Component Analysis; PDB_Protein Data Bank; RMSD_Root Mean Square Deviation; RMSF_Root Mean Square Fluctuation; TIR1_Transport Inhibitor Response 1; VFA_Volatile Fatty Acids.

Introduction

The cattle rumen represents one of nature's most dynamic microbial ecosystems, functioning as a specialized biochemical reactor where diverse microorganisms, including bacteria, archaea, fungi, and protozoa, drive nutrient cycling and anaerobic fermentation (Wu et al., 2020; Jami et al., 2013; Kibegwa et al., 2023). Beyond their essential role in ruminant metabolism, these microbes, particularly fiber-degrading bacteria, hold significant potential for sustainable agriculture. They produce volatile fatty acids (VFAs) and facilitate organic matter decomposition, which enriches soil fertility and health when applied as manure (Palevich et al., 2019; Stewart et al., 2019). Notably, specific rumen bacterial strains are also associated with the production of bioactive compounds, such as phytohormones, which can directly enhance plant root development and stress tolerance (Singh et al., 2014).

A critical capability of rumen microbial metabolism is the synthesis of auxins, particularly indole-3-acetic acid (IAA), which modulates physiological processes like root elongation and cell differentiation. Previous studies have confirmed that rumen bacteria, such as *Prevotella* and *Clostridium* species, can produce indolic compounds through tryptophan metabolism (Attwood et al., 2006). Similarly, Arora et al. (2015) highlighted the capacity of rumen microbes to generate IAA via biotransformation, suggesting a largely underutilized role for these organisms in promoting plant growth and soil enrichment.

The functional impact of IAA relies on its interaction with the Transport Inhibitor Response 1 (TIR1) receptor, a key component of the plant auxin signaling pathway. In conjunction with AUX/IAA proteins, TIR1 regulates gene expression governing root architecture and adaptive responses to environmental stimuli (Carranza et al., 2016; Villalobos et al., 2012; Lv et al., 2019). This intricate interplay between IAA and the TIR1-mediated cascade is fundamental to shaping plant development and resilience (Fendrych et al., 2016; Wei et al., 2021).

Despite the established role of plant-associated bacteria as auxin producers, the potential of rumen-derived bacteria to modulate IAA signaling remains largely uncharted. This study aims to bridge this gap by employing an *in silico* approach to investigate the molecular interaction between IAA and the TIR1 protein in *Capsicum annuum*. By integrating metagenomics with molecular docking and dynamics simulations, we provide mechanistic insights into how rumen microbiota can function as biofertilizers. This research establishes a framework for harnessing rumen microbes to enhance crop resilience and promote sustainable, eco-friendly agricultural practices.

Result

Metagenomic analysis of bovine rumen bacteria

Metagenomic analysis (Table 1) identified1,235 operational taxonomic units (OTUs), which were examined to assess alpha diversity. The assessment revealed a Shannon index of 2.353 and a Simpson index of 0.628. These values suggest that microbial diversity within the bovine rumen samples from Tasikmalaya is relatively low, with the Simpson index specifically highlighting a distinct degree of evenness in the microbial community. Conversely, the Chao1 and ACE indices (1985.018 and 1912.930, respectively) indicate high microbial richness. Therefore, while the bacterial diversity is limited, the abundance of microorganisms in the bovine rumen is considerable. Figure 1(d) visually confirms this, showing *Enterococcus faecalis* as the dominant species with a relative abundance of 62%. Analysis of bacterial composition confirmed that *E. faecalis* predominates, comprising over 50% of the total microbial taxa. The bacterial community is dominated by phylum *Bacillota* (Firmicutes), which constitutes >90% of the microbial structure. Other significant phyla include Psudomonadota (Proteobacteria), Actinomycetota (Actinobacteria), and Bacteroidota (Bacteroidetes). Regarding taxonomic order, Lactobacillales was the most abundant (~75%). The remaining dominant orders included Eubacteriales (10%), Enterobacterales (5%), and Bacillales (5%). At the genus level, *Enterococcus* was dominant, with *E. faecalis* representing more than 60% of the relative abundance. Other notable genera included *Lactococcus*, *Paraclostridium*, *Bacillus*, *Romboutsia*, *Macrococcus*, and *Streptococcus*, allpredominantly affiliated with Firmicutes.

Table 1. Alpha diversity in bovine rumen.

Observed	Chao1	ACE	Shannon	Simpson
1235	1985.018	1912.930	2.353	0.628

Figure 1(a) illustrates bacterial composition at the phylum level, showing the prevalence of Firmicutes (Bacillota), followed by Proteobacteria (Psudomonadota), Actinobacteria (Actinomycetota), and Bacteroidetes (Bacteroidota). At the order level (Figure 1b), Lactobacillales was the dominant order. Other prominent orders within Firmicutes included Eubacteriales and Bacillales, which ranked second and fourth in relative abundance. Enterobacterales (Proteobacteria) ranked third, while Micrococcales (Actinomycetota)was the fifth most abundant order. The sixth most abundant order, Flavobacteriales, is derived from Bacteroidetes.

Finally, Figure 1(c) details relative abundance at the genus level. The genera *Enterococcus*, *Lactococcus*, *Paraclostridium*, *Bacillus*, *Romboutsia*, *Macrococcus*, *Streptococcus*, and *Vagococcus* belong to Firmicutes phylum, with *E. faecalis* exhibiting clear dominance. Following these, *Enterobacter* and *Shigella* (Proteobacteria) also contributed significantly to the microbial structure. Among them, *E. faecalis* stood out with a striking 62% relative abundance, highlighting its pivotal role in shaping the bovine rumen ecosystem.

Capsicum annuum TIR1 protein modeling

Homology modeling of the Transport Inhibitor Response 1 (TIR1) protein from *Capsicum annuum* was performed to facilitate subsequent docking and molecular dynamics studies. As shown in the figure 2, the TIR1 model was constructed using a template from the *Arabidopsis thaliana* TIR1 structure (PDB ID: 3C6N.1B), exhibiting a sequence identity of 74.12% and a coverage of 92.57%, values within acceptable thresholds for reliable modeling. The Ramachandran plot indicated that 94.76% of the TIR1 protein residues occupied favorable conformations, signifying a high-quality model. Furthermore, 0% of the residues were located in disallowed regions, confirming the stability and reliability of the generated structure.

Sequence alignment between the model and the template revealed a high degree of similarity, with key residues critical for ligand binding and receptor activation being conserved. This alignment confirms that the model closely resembles the native TIR1 protein, verifying its suitability for docking and molecular dynamics studies.

Model quality was further supported by a MolProbity score of 1.79, indicating a well-refined structure with minimal steric clashes (clash score of 1.53). The QMEAN Z-scores, particularly the high QMEAN score of 2.63, demonstrate that the model's overall geometry and folding align well with known structures of similar proteins.

Collectively, these results demonstrate that the TIR1 structure generated for *C. annuum* is of high quality. Consequently, this model was used in docking simulations to investigate potential interactions with IAA and in molecular dynamics simulations to explore the structural dynamics and stability of the TIR1-IAA complex. The favorable quality metrics, combined with

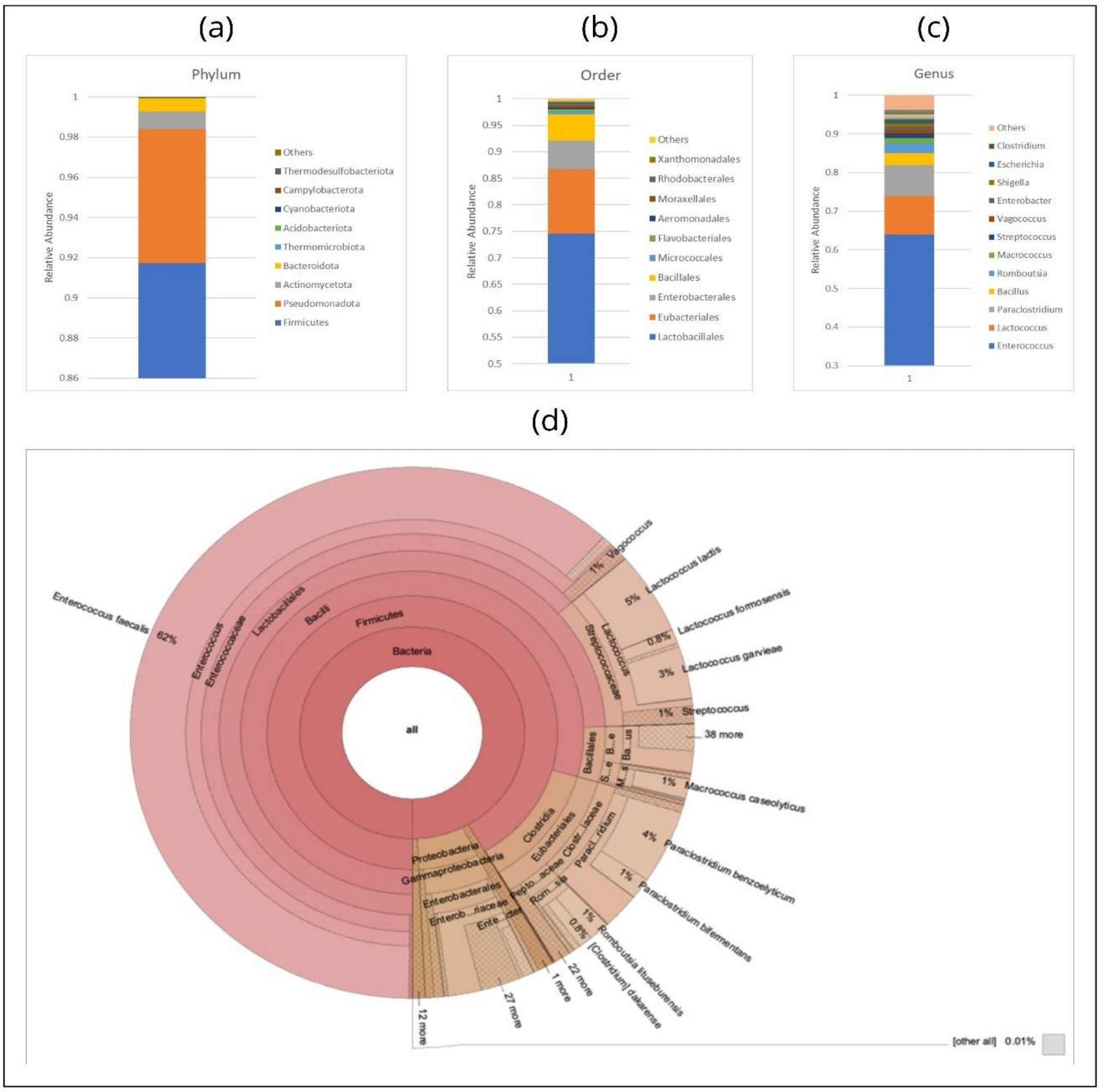


Figure 1. Metagenomic analysis of bovine rumen bacteria: relative abundance on taxa phylum (a), order (b), and genus (c) of bovine rumen bacteria; krona visualization of metagenomic structure of bovine rumen bacteria (d).

accurate sequence alignment and structural validation, confirm that the model is a reliable tool for elucidating the interaction between IAA and TIR1, which is central to our hypothesis of plant growth promotion and resistance induction.

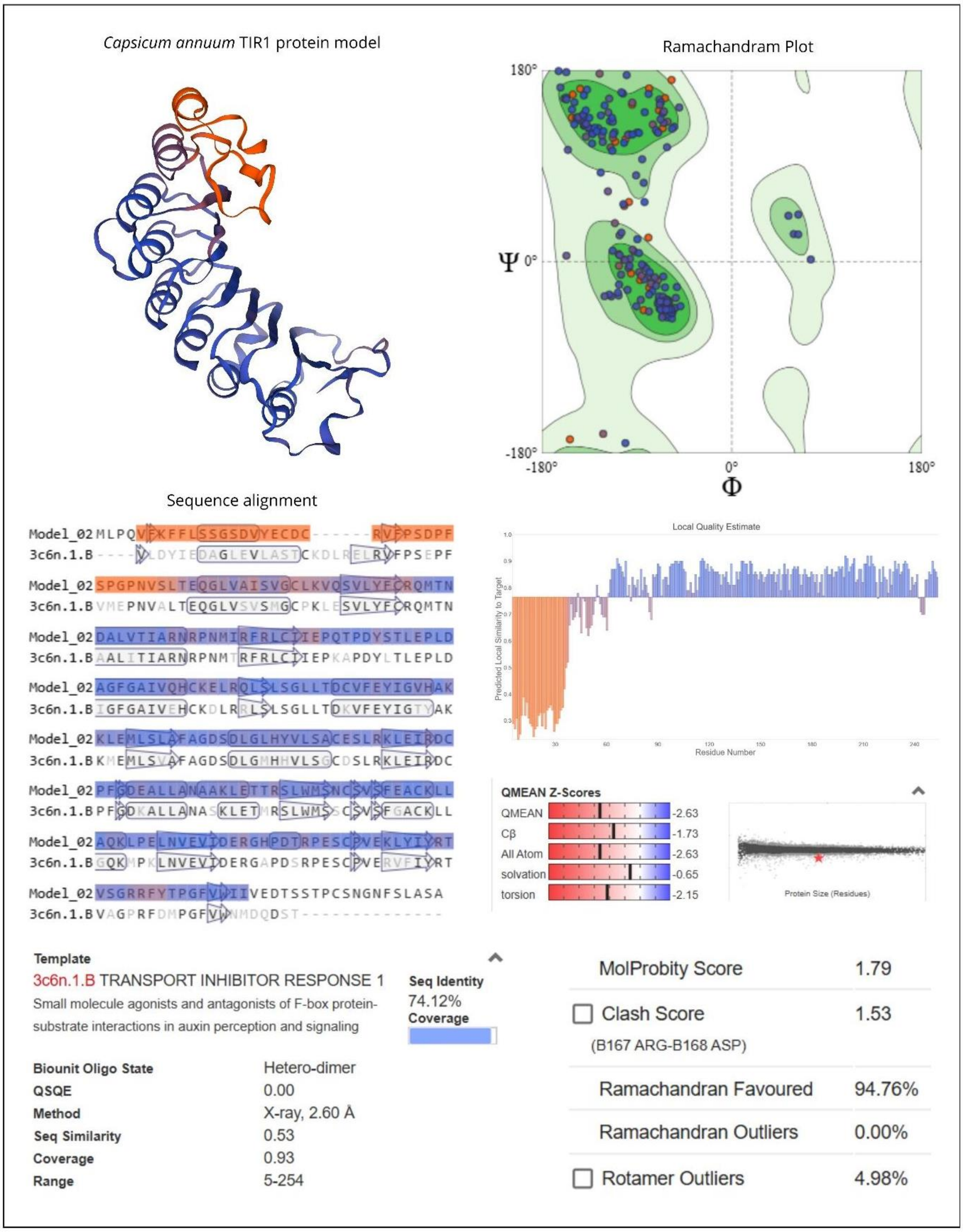
Docking properties of IAA-TIR1

The docking properties between *Capsicum annuum* TIR1 protein and indole-acetic-acid (IAA), as summarized in Table 2, provide valuable insights into the molecular interactions that underpin IAA's ability to activate the TIR1 protein, potentially promoting growth and inducing resistance in *C. annuum*. The binding affinity of IAA with the TIR1 protein was recorded at -5.9 kJ/mol, suggesting a favorable interaction. Analysis of binding sites and types indicates that IAA engages with the TIR1 protein through multiple mechanisms, particularly hydrogen bonds and hydrophobic interactions.

Hydrogen bonds between IAA and the TIR1 protein formed at distances of 3.12 Å, 2.8 Å, and 1.9 Å, interacting with the residues GLN88, GLY119, and ALA142, respectively. These bonds are categorized as conventional hydrogen bonds, facilitating stable binding. Additionally, the hydrophobic interactions at distances of 3.67 Å, 5.45 Å, and 5.47 Å, specifically involving ALA142, CYS83, and PRO87, indicate that IAA binding is driven by both hydrogen bonding and hydrophobic forces, contributing to the overall stability of the complex.

Specific hydrophobic interactions, such as pi-sigma and pi-alkyl interactions, highlight the involvement of non-polar regions within the TIR1 protein in enhancing with IAA. The pi-orbitals and alkyl groups of amino acids such as ALA142 and CYS83stabilize the ligand-protein complex through these hydrophobic contacts. These interactions, alongside the hydrogen bonds, suggest that IAA binds effectively to the TIR1 protein, likely initiating a conformational change that activates the protein and promotes associated biological responses.

Given the favorable binding affinity and diversity of interactions observed, we hypothesize that IAA effectively activates the TIR1 protein in *C. annuum*. The identified binding interactions, particularly the hydrogen bonds and hydrophobic interactions, are critical for the activation of TIR1, which play a significant role in regulating plant growth and resistance.



```
Model_02 MLPQVFKFFLSSGSDVYECDC------RVFPSDPF
3c6n.1.B ----VLDYIEDAGLEVLASTCKDLRELRVFPSEPF

Model_02 SPGPNVSLTEQGLVAISVGCLKVQSVLYFCRQMTN
3c6n.1.B VMEPNVALTEQGLVSVSMGCPKLESVLYFCRQMTN

Model_02 DALVTIARNRPNMIRFRLCIIEPQTPDYSTLEPLD
3c6n.1.B AALITIARNRPNMTRFRLCIIEPKAPDYLTLEPLD

Model_02 AGFGAIVQHCKELRQLSLSGLLTDCVFEYIGVHAK
3c6n.1.B IGFGAIVEHCKDLRRLSLSGLLTDKVFEYIGTYAK

Model_02 KLEMLSLAFAGDSDLGLHYVLSACESLRKLEIRDC
3c6n.1.B KMEMLSVAFAGDSDLGMHHVLSGCDSLRKLEIRDC

Model_02 PFGDEALLANAAKLETTRSLWMSNCSVSFEACKLL
3c6n.1.B PFGDKALLANASKLETMRSLWMSSCSVSFGACKLL

Model_02 AQKLPELNVEVIDERGHPDTRPESCPVEKLYIYRT
3c6n.1.B GQKMPKLNVEVIDERGAPDSRPESCPVERVFIYRT

Model_02 VSGRRFYTPGFVWIIVEDTSSTPCSNGNFSLASA
3c6n.1.B VAGPRFDMPGFVWNMDQDST--------------
```

QMEAN Z-Scores

QMEAN	-2.63
Cβ	-1.73
All Atom	-2.63
solvation	-0.65
torsion	-2.15

Template

3c6n.1.B TRANSPORT INHIBITOR RESPONSE 1

Small molecule agonists and antagonists of F-box protein-substrate interactions in auxin perception and signaling

Seq Identity 74.12%

Coverage

Biounit Oligo State	Hetero-dimer
QSQE	0.00
Method	X-ray, 2.60 Å
Seq Similarity	0.53
Coverage	0.93
Range	5-254

MolProbity Score	1.79
☐ Clash Score (B167 ARG-B168 ASP)	1.53
Ramachandran Favoured	94.76%
Ramachandran Outliers	0.00%
☐ Rotamer Outliers	4.98%

Figure 2. Protein modeling of *Capsicum annuum* TIR1 (Transport Inhibitor Response 1) protein.

Therefore, TIR1 activation by IAA could lead to enhanced growth of *C. annuum* and its increased resistance to environmental stresses, providing a potential pathway for improving crop yields and resilience.

Molecular dynamic of IAA-TIR1

Molecular dynamics simulations of the TIR1 and TIR1-IAA complexes were analyzed across a range of critical parameters, including protein secondary structure, total potential energy, hydrogen bonding interactions, and RMSD values (Figures 4, 5, and 6). The secondary structure content analysis (Figures 4a and 4b) highlights a notable divergence between the TIR1 and TIR1-IAA complexes. While both structures retain a significant proportion of α-helices, the TIR1-IAA complex exhibits slight alterations, suggesting that the binding of IAA induces subtle conformational changes in the receptor. These structural shifts suggest that IAA binding may modulate the receptor's functional state, potentially activating TIR1.

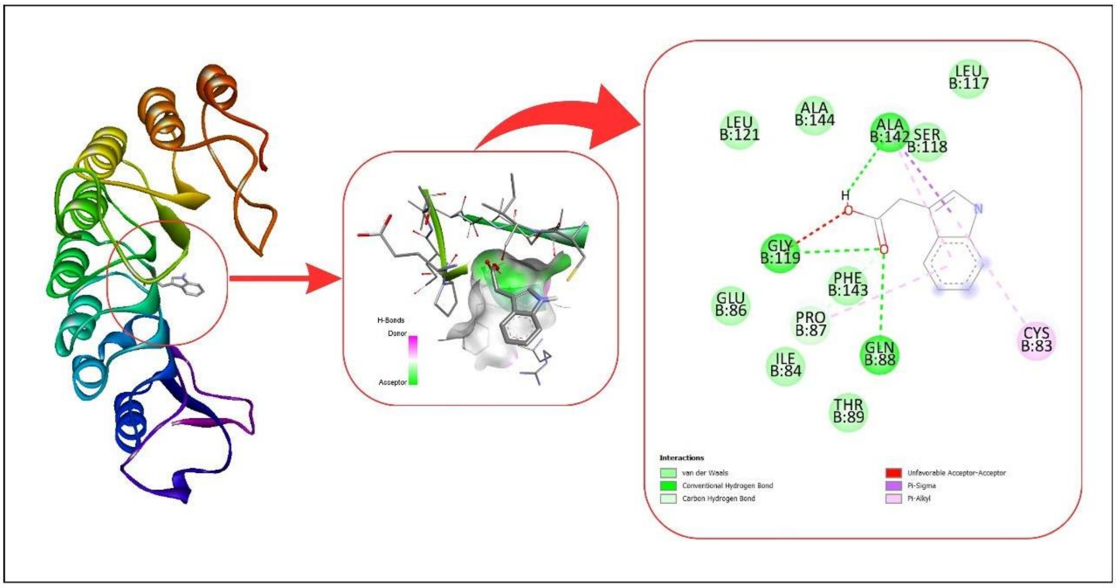


Figure 3. Molecular docking result of indole-acetic-acid (IAA) with *Capsicum annuum* TIR1 protein.

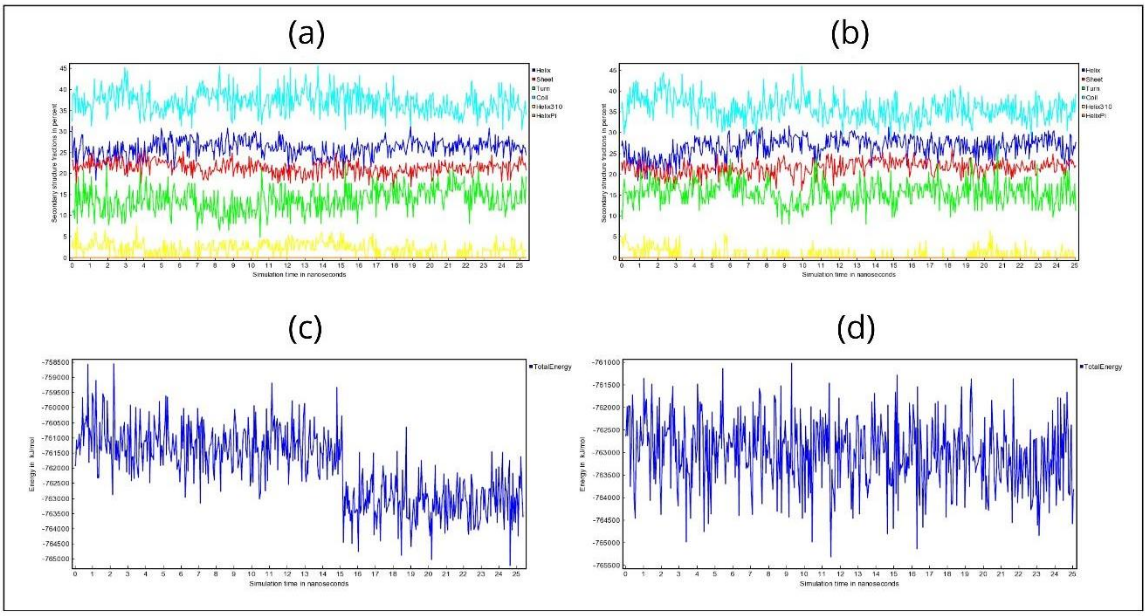


Figure 4. Protein secondary structure content of TIR1 (a) and TIR1-IAA (b) total potential energy of the system of TIR1 (c) and TIR1-IAA (d) according to AMBER14 force field.

Analysis of the total potential energy profiles (Figures 4c and 4d) indicates that the TIR1-IAA system demonstrates greater stability compared to unbound TIR1 system. This enhanced stability supports the hypothesis that IAA binding facilitates receptor activation by inducing a conformational change that strengthens the overall protein structure. The Molecular Mechanics Poisson-Boltzmann Surface Area (MM/PBSA) analysis (Figure 5a) corroborates this interpretation, showing that the binding energy between IAA and TIR1 is energetically favorable. These findings underscore that the interaction between the ligand and the receptor is not only stable but also promotes the receptor's activation.

The radius of gyration analysis (Figure 5b) reveals a slight expansion of the TIR1-IAA complex upon IAA binding, indicating an increase in protein flexibility. This alteration in the protein's structure suggests that IAA binding facilitates the conformational adjustments required for receptor activation. Additionally, the number of hydrogen bonds between the protein and the ligand (Figure 5c) as well as between the protein and the solvent (Figure 5d) increased upon IAA binding. This enhancement strengthens the protein-ligand interaction and stabilize the protein, thereby promoting receptor activation through these additional stabilizing forces.

The Root Mean Square Deviation (RMSD) analysis (Figures 5e, 5f, and 5g) reveals that both TIR1 and TIR1-IAA complexes maintain overall stability during the simulation. However, the TIR1-IAA system exhibits lower RMSD values, suggesting that IAA binding results in a more rigid and stable protein structure. This stability is likely essential for TIR1 activation. The reduced fluctuations observed in the Root Mean Square Fluctuation (RMSF) analysis (Figure 5h) support this notion,

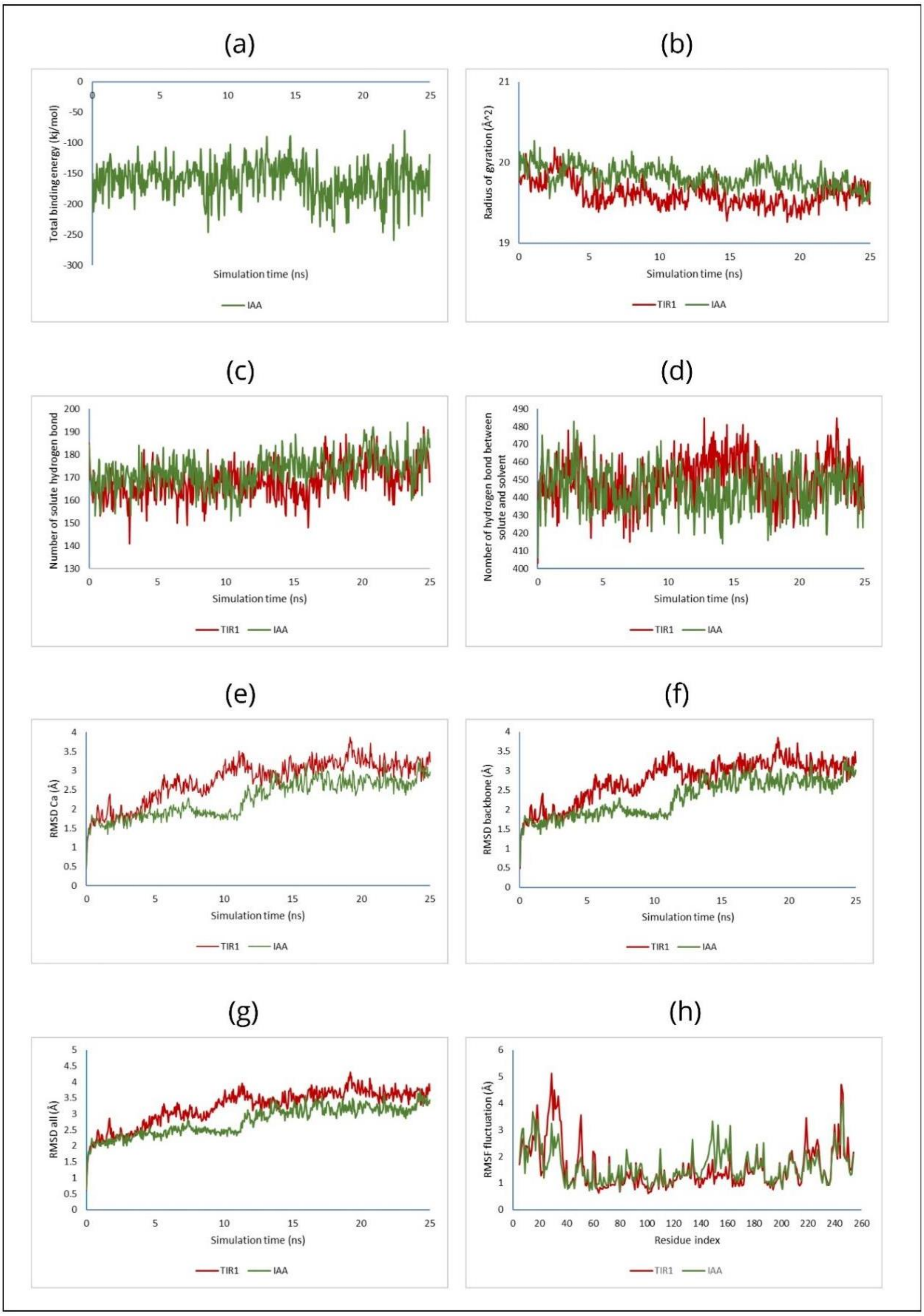


Figure 5. The Molecular Mechanics Poisson-Boltzmann Surface Area (MM/PBSA) of TIR1-IAA (a); radius of gyration of TIR1-IAA (b); number of solute hydrogen bond of TIR1 and TIR1-IAA (c); number of hydrogen bond between solute and solvent of of TIR1 and TIR1-IAA (d); RMSD Ca of TIR1 and TIR1-IAA (e); RMSD backbone of TIR1 and TIR1-IAA (f); RMSD total of of TIR1 and TIR1-IAA (g); RMSF fluctuation (h).

indicating that IAA binding restricts the movement of specific regions of the protein, thus contributing to the overall activation process.

Principal Component Analysis (PCA) (Figures 6a –6j), offers deeper insights into the dynamic behavior of both TIR1 and TIR1-IAA complexes. The PCA results reveal that IAA binding induces significant shifts in the protein's motion trajectory compared to the unbound TIR1 protein. This suggests that the ligand-binding event triggers substantial changes in the global dynamics of the receptor. The PCA plots (Figures 6c–6h) demonstrate that IAA binding prompts a rearrangement of

Table 2. Docking properties between *Capsicum annuum* TIR1 protein and indole-acetic-acid (IAA).

Receptor	Ligand	Binding affinity (kj/mol)	Distance (Å)	Category	Type	Binding site	From chemistry	To chemistry
TIR1	IAA	-5.9	3.12	Hydrogen Bond	Conventional Hydrogen Bond	GLN88	H-Donor	H-Acceptor
			2.8	Hydrogen Bond	Conventional Hydrogen Bond	GLY119	H-Donor	H-Acceptor
			1.9	Hydrogen Bond	Conventional Hydrogen Bond	ALA142	H-Donor	H-Acceptor
			3.24	Hydrogen Bond	Carbon Hydrogen Bond	PRO87	H-Donor	H-Acceptor
			3.67	Hydrophobic	Pi-Sigma	ALA142	C-H	Pi-Orbitals
			5.45	Hydrophobic	Pi-Alkyl	CYS83	Pi-Orbitals	Alkyl
			5.47	Hydrophobic	Pi-Alkyl	PRO87	Pi-Orbitals	Alkyl
			4.72	Hydrophobic	Pi-Alkyl	ALA142	Pi-Orbitals	Alkyl

the protein's internal coordinates, consistent with the hypothesis of a conformational change. Finally, the eigenvalue plots in Figures 6i and 6j confirm that the TIR1-IAA complex exhibits a more stable and well-defined trajectory, compared to the unbound TIR1 system, further supporting the activation mechanism facilitated by IAA binding.

Collectively, these molecular dynamics results provide compelling evidence that IAA binding induces conformational changes that stabilize and activate the TIR1 receptor. The increased stability, enhanced hydrogen bonding, favorable energy profile, and reduced RMSF fluctuations, align with the molecular docking analysis. These findings strongly support the hypothesis that IAA activates the TIR1 receptor in Capsicum annuum, contributing to enhanced growth and resistance.

Discussion

This study integrates 16S rRNA metagenomics and *in silico* analysis to evaluate cattle rumen bacteria as potential bioactivators. Our findings support the hypothesis that rumen-derived indole-3-acetic acid (IAA) enhances *Capsicum annuum* growth and resistance by activating the TIR1 receptor. Metagenomic analysis identified *Enterococcus faecalis* as the dominant species (>50%), highlighting the rumen's role as a nutrient-processing ecosystem. *E. faecalis* is known to improve fermentation quality (Gang et al., 2020) and is integral to anaerobic digestion systems, the byproducts of which, such as slurry, are effective liquid fertilizers (Abanades et al., 2022). However, balancing the nutritional and microbiological composition of these byproducts remains a key area for optimization (Mukhuba et al., 2018).

The identified bacterial community exhibits significant plant growth-promoting traits. *Enterococcus* species are established plant growth-promoting rhizobacteria (PGPR) that improve soil structure and combat pathogens (Jaffar et al., 2023), with *E. faecalis* specifically noted for its tolerance to acidic pH and ability to synthesize IAA (Pruthviraj et al., 2023). Similarly, *Lactococcus lactis* (5% abundance) has been shown to enhance soil quality and help plants adapt to abiotic stress when combined with organic manure (Al-Elwany et al., 2023). It also demonstrates efficacy against agricultural pathogens like *Xanthomonas spp.* (Mačionienė et al., 2022), which infects *C. annuum* (Timilsina et al., 2020). Additionally, *Paraclostridium benzoelyticum* (4% abundance) is capable of degrading hydrocarbons and promoting growth under stress (Habib et al., 2023), likely leveraging its anaerobic metabolism which is also linked to denitrification processes (Yang et al., 2019). Collectively, these microbial consortia offer a sustainable alternative to chemical inputs, enhancing productivity as described by Seenivasagan and Babalola (2021).

To elucidate the molecular mechanism of this growth promotion, we simulated the interaction between bacterial IAA and the *C. annuum* TIR1 receptor. Docking results revealed favorable binding mediated by hydrogen bonds with residues GLN88, GLY119, and ALA142. This interaction is critical, as TIR1 functions within the SCF ubiquitin ligase complex to degrade Aux/IAA repressors and release Auxin Response Factors (ARFs) (Kalve et al., 2020; Yu et al., 2013; Wang & Estelle, 2014). This degradation pathway is essential for activating auxin signaling (Dezfulian et al., 2016; Lakehal et al., 2019). Molecular dynamics simulations further confirmed that IAA binding stabilizes the TIR1 complex, as evidenced by lower RMSD values and reduced RMSF fluctuations. This stabilization restricts unnecessary protein motion, facilitating the conformational changes necessary for receptor activation.

The activation of TIR1 by IAA extends beyond growth regulation to include stress resilience. Upregulation of TIR1 under osmotic stress suggests a role in adaptive responses (Kalve et al., 2020), potentially modulated by nitric oxide signaling pathways (Terrile et al., 2012; Iglesias et al., 2018). Furthermore, our computational data underscores the specificity of IAA; while synthetic auxins like NAA and 2,4-D can interact with TIR1, they typically exhibit lower affinity compared to natural IAA (Wend et al., 2013; Eyer et al., 2016). This specificity is vital for precise developmental regulation, such as root formation (Hao & Yang, 2010). In conclusion, the dominance of IAA-producing *E. faecalis* in the rumen, combined with the stable molecular activation of TIR1 observed *in silico*, confirms the potential of rumen microbiota as a high-value source of biofertilizers.

Materials and method

Metagenomic 16s rRNA sequencing

Brahman Cross (BX) cattle rumen was collected from one of the slaughterhouses in Indihiang Subdistrict, Tasikmalaya City, West Java, Indonesia, approximately as many as 5 grams. In this slaughterhouse, the age of the slaughtered cattle ranges from 2.5 to 3 years old. Then, the sample collection was carried to a third party, Genetika Science, for DNA isolation and metagenomic 16s rRNA sequencing. The genomic DNA was extracted from the bovine rumen using the Quick-DNA Magbead

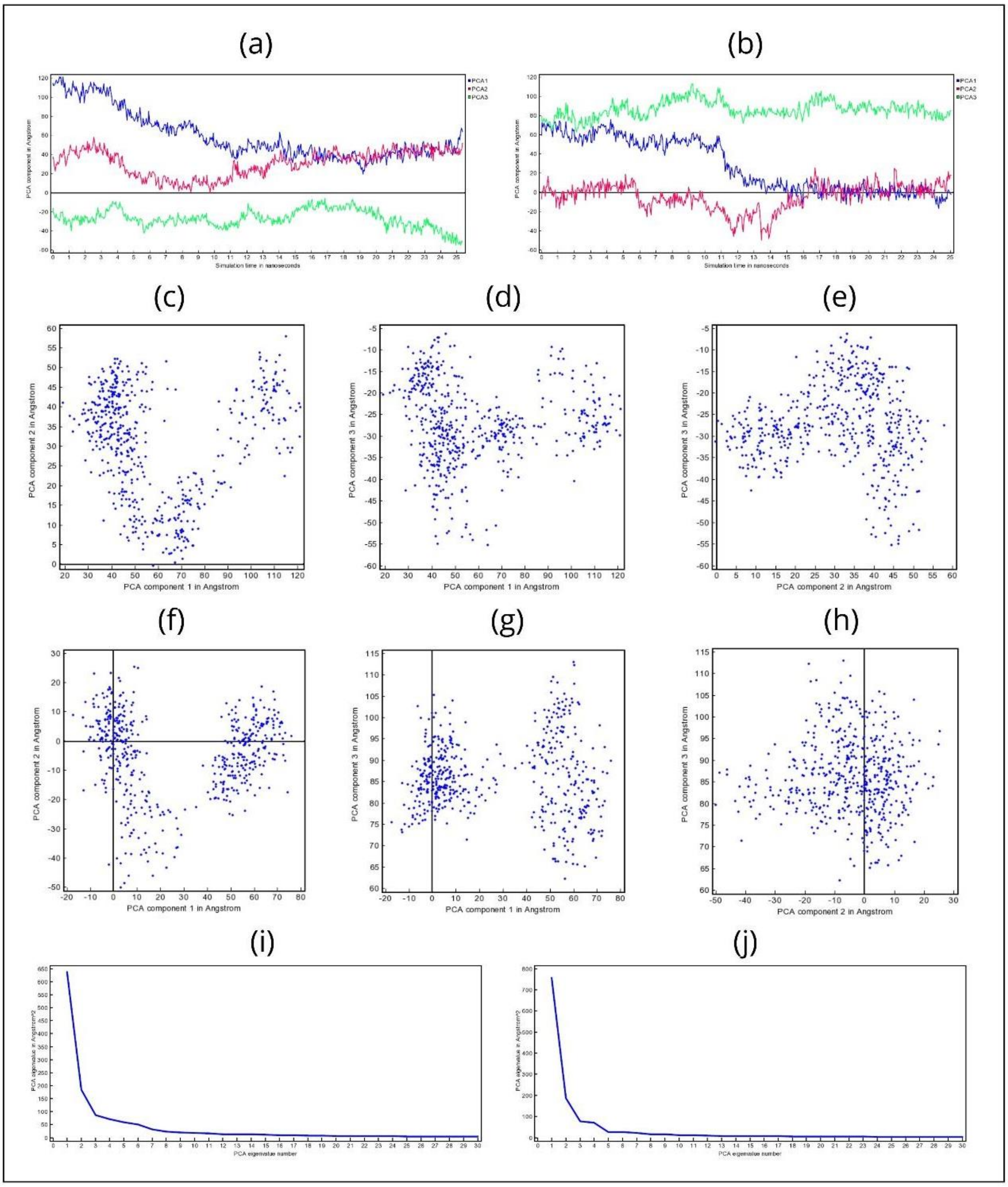


Figure 6. Principal component analysis (PCA) of TIR1 (a) and TIR1-IAA components (b), PCA component 1 vs 2 of TIR1 (c) and TIR1-IAA (f), PCA component 1 vs 3 of TIR1 (d) and TIR1-IAA (g), PCA component 3 vs 2 of TIR1 (e) and TIR1-IAA (h), PCA eigenvalues of TIR1 (c) and TIR1-IAA (f).

Plus Kit (Zymo Research, D4082). Then, DNA concentration was determined using Nanodrop spectrophotometers and Qubit fluorometer to check the quality and quantity of the DNA extraction. The metagenomic 16s rRNA in this study is conducted using the Oxford nanopore platform. The amplified region used was V1-V9, which generates a fragment length of 1500 bp. The primers used were 27F, whose sequence was AGAGTTTGATCMTGGCTCAG, and 1492R, which was GGTTACCTTGTTACGACTT (Lane, 1991; Weisburg et al., 1991; Klindworth et al., 2013). This method could detect bacteria through species. The observed bacterials were analyzed by the Operational Taxonomic Unit (OTU). Then, the OTU conducted an abundance analysis, including alpha diversity and community structure in each taxon.

Protein modeling

Protein modeling using SWISS-MODEL was conducted to generate a three-dimensional (3D) structure of the target protein through homology modeling. The amino acid sequence of Transport Inhibitor Response 1 (TIR1) of *Capsicum annuum* protein (UniProt Entry: A0A2G2XW34) was retrieved from the UniProt (https://www.uniprot.org/) database and uploaded to the SWISS-MODEL web server (https://swissmodel.expasy.org/). The server then identified potential templates from the Protein Data Bank (PDB), selecting the best match based on sequence identity, coverage, and Global Model Quality Estimation (GMQE) scores. After template selection, the target sequence was aligned with the template structure, and a 3D model was built through an iterative modeling process that optimized the backbone conformation and side-chain positioning. Following model generation, the structure was evaluated using various quality parameters, including QMEAN and GMQE scores. The final protein model was used for molecular docking and dynamics simulations (Bordoli & Schwede, 2011; Biasini et al., 2014; Waterhouse et al., 2018).

Molecular docking assessment

Molecular docking simulations were conducted using PyRx 08 and BIOVIA Discovery Studio (Ayodele, 2023; Gartika, 2024; Gebrehiwot, 2024) to analyze interactions between selected protein target and the ligand. The three-dimensional (3D) structure of the *C. annuum* TIR1 protein target was retrieved from the previous modeling. Prior to docking, these structures were preprocessed by adding hydrogen atoms, optimizing the geometry, and removing water molecules and ligands before being saved in .pdb format. The 3D structures of the indole-acetic-cid (IAA) (PubChem CID: 802) ligand, downloaded from PubChem (https://pubchem.ncbi.nlm.nih.gov/), were optimized using OpenBabel within PyRx and loaded alongside the receptor files. A grid box was generated to define the docking region, ensuring comprehensive blind docking simulations via AutoDock Vina. The docking poses were assessed based on binding affinities, with the most favorable configurations selected for further refinement and visualization in BIOVIA Discovery Studio to confirm interaction stability and binding efficiency.

Molecular dynamic simulations

Molecular dynamics simulations were carried out using YASARA Dynamics version 24.10.5 to investigate the dynamic behavior of the protein-ligand system (Biswas et al., 2021; Dey et al., 2022; Darmadi et al., 2023), which included IAA as ligand, along with *C.annuum* TIR11 as protein receptor. The initial 3D structures of the proteins and ligands were imported into YASARA Dynamics, followed by energy minimization to optimize geometry and resolve steric clashes. The system was then solvated in a water box to mimic a biological environment, with counterions added to neutralize the system's charge. Simulations were performed under NPT ensemble conditions at 300 K temperature and 1 bar pressure to reflect physiological conditions. After a 100-picosecond equilibration phase to stabilize the system, production simulations were conducted while collecting trajectory data to analyze system stability and conformational changes in both ligands and proteins. The results were processed and visualized using Microsoft Excel to evaluate ligand-receptor interactions, providing insights into binding stability and flexibility within the protein-ligand complex.

Conclusion

Conclusion This study successfully demonstrates the potential of rumen-derived *Enterococcus faecalis* as a source of the plant growth regulator indole-3-acetic acid (IAA). Metagenomic analysis confirmed the dominance of *E. faecalis* in the bovine rumen, establishing it as a significant candidate for biofertilizer applications. Furthermore, in silico molecular docking and dynamics simulations provided robust evidence that bacterial-produced IAA can spontaneously and stably bind to the *Capsicum annuum* TIR1 receptor. The binding energy of -5.9 kJ/mol, supported by increased hydrogen bonding and reduced conformational fluctuations (RMSD/RMSF) during simulation, indicates that this interaction is capable of activating the auxin signaling pathway. Collectively, these findings bridge the gap between rumen microbial ecology and plant physiology, suggesting that *E. faecalis* from bovine rumen can serve as an effective bioactivator to enhance growth and stress resilience in chili pepper cultivation. Future in vivo studies are recommended to validate these computational models in field conditions.

Acknowledgement

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Statement of contributions

RP and S conceived and designed the experiments; IH, AYR, and EW performed the metagenomic analysis; GVB and NFR performed the molecular docking and dynamics simulations; EW analyzed NGS data; GVB and AHJ wrote the paper. All authors read and approved the final manuscript.

Ethical statement

The rumen samples used in this study were obtained from a commercial slaughterhouse in Tasikmalaya City, Indonesia. No live animals were experimented upon or sacrificed specifically for the purpose of this research. All procedures adhered to the ethical guidelines for the use of animal by-products in research.

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