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ABSTRACTS

ISOLATION AND CHARACTERIZATION OF *PSEUDOMONAS AERUGINOSA* FROM HUMAN CLINICAL SAMPLES WITH FOCUS ON ANTIBIOTIC RESISTANCE

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Pseudomonas aeruginosa is a multi-drug-resistant opportunistic pathogen which is frequently implicated in urinary tract infections and nosocomial infections. This study aimed to isolate and characterise *P. aeruginosa* from human clinical samples, focusing on antibiotic resistance profiles. A total of 264 clinical samples from patients with suspected *P. aeruginosa* infections were screened using culture, standard biochemical tests and conventional PCR. One isolate displayed characteristic morphological and biochemical features consistent with *P. aeruginosa*. Molecular confirmation using PCR targeting the oprL gene and antibiotic susceptibility testing using the Kirby-Bauer method are on-going. Conventional PCR will also be used to detect key resistance genes, including gyrA, bla VIM , bla PDC, aac (6')-Ib, and ampC. This study will highlight the importance of continuous monitoring for resistant *P. aeruginosa* in clinical settings to guide appropriate antimicrobial therapy and infection control strategies.

Key words: *Pseudomonas aeruginosa*; Nosocomial infections, Urinary tract infections, PCR, Multidrug resistance, Resistance genes.

Assessing the Plastic-Degrading Potential of Bacteria Isolated from Industrial Wastewater

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Plastic and microplastic pollution pose a global environmental challenge due to their persistence and accumulation in ecosystems. Conventional disposal methods (e.g., Landfilling, incineration, recycling) are largely ineffective and often cause additional environmental harm. Consequently, biological plastic degradation, particularly microbial degradation, has gained significant attention as a sustainable alternative. This

study aimed to determine the degradation potential of 25 bacterial isolates from industrial wastewater and evaluated their enzyme activity and plastic-degrading genes. Morphological characterisation and Gram staining of bacterial isolates were performed. Preliminary screening for plastic-degrading ability was performed using the agar spotting method, followed by molecular identification of the potential isolates through 16S rRNA sequencing. A plastic biodegradation assay was performed over 21 days in flasks containing mineral salt medium supplemented with 1 g of the respective plastics (polyethylene terephthalate, polypropylene, polystyrene, and polycaprolactone). degradation parameters such as media composition, shaking speed, incubation duration, and inoculum size were optimized to enhance degradation efficiency. Enzyme plate assays were conducted to detect esterase, lipase, laccase, and peroxidase activity. For the Gram staining, 72% of the isolates were Gram-negative and 28% were Gram-positive. Molecular characterisation revealed bacterial isolates are *Bacillus thuringiensis* and *Bacillus cereus*. Initially, *B. cereus* showed the highest degradation efficiency, achieving a 44% weight loss of polycaprolactone after 21 days, however, subsequent degradation results were inconsistent. Lipase activity was observed in both *B. cereus* and *B. thuringiensis*. Overall, this study highlights the potential of wastewater-derived bacteria for plastic biodegradation and their relevance in developing sustainable bioremediation strategies that support a circular economy.

Keywords: Plastics, Biodegradation, Enzymes, Bacillus, Wastewater.

Bioremediation Potential of Indigenous Bacteria in Poultry Wastewater: Enzymatic Dynamics and Nutrient Removal Efficiency

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Excessive nutrient discharge from poultry wastewater accelerates eutrophication and environmental degradation, threatening aquatic ecosystems. This study evaluated the bioremediation potential of indigenous bacterial isolates over a 12-day batch experiment. Three treatments were applied: Treatment A (autoclaved wastewater without microbes, abiotic control), Treatment B (autoclaved wastewater inoculated with functional isolates NA, NB, and PC), and Treatment C (raw unautoclaved wastewater containing native microbial consortia). Selective enrichment from Treatment C yielded nitrite-degrading (NA), nitrate-degrading (NB), and phosphate-degrading (PC) bacteria. NB and PC demonstrated the highest nutrient removal, achieving 96–98% reductions in

nitrate, ammonia, and nitrite, while NA removed 71% phosphate, 28% nitrate, 97% ammonia, and 96% nitrite. Treatment C showed the least removal with 61%, 25%, 23%, and 4%. Physicochemical parameters were highest in Treatment C, with turbidity 2771.56 NTU, conductivity 17.74 mS/cm, TDS 10.33 g/L, salinity 10.77%, and BOD 485.39 mg/L, whereas active treatments exhibited significantly lower values. Optical density increased from 0.06 to 0.67, reflecting exponential microbial growth between days 6 and 9, coinciding with peak enzyme activity. Enzymatic assays revealed significant elevations in nitrate reductase, L-asparaginase, urease, alkaline phosphatase, laccase, catalase, and lignin peroxidase activities in treated systems. Overall, NB and PC demonstrated the strongest bioremediation efficiency, underscoring the metabolic adaptability and enzymatic versatility of indigenous bacteria. These findings highlight their potential for sustainable treatment of nutrient-rich poultry wastewater and mitigation of environmental nutrient pollution.

Keywords: Poultry wastewater, Indigenous bacteria, Nutrient removal, Enzyme activity, Bioremediation

Endophytic Bacteria and Biosurfactants: Elucidating Anti-biofilm and Enzymatic Activities

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The rise of antimicrobial resistance and the persistence of biofilm-associated infections demand innovative biocontrol alternatives. Endophytic bacteria from medicinal plants offer a sustainable source of bioactive compounds capable of disrupting biofilms through biosurfactant enzymatic mechanisms. This study evaluated the enzymatic activity of bacterial endophytes from *Kigelia africana*, and the antibacterial and anti-biofilm activities of their biosurfactants. Enzyme production (amylase, cellulase, lipase, protease, and xylanase) was evaluated using plate-based diffusion methods. Biosurfactants were produced by submerged fermentation using minimal salt media supplemented with olive oil, followed by acid precipitation and solvent extraction. The biosurfactant ability of cell free supernatants and biosurfactants was evaluated qualitatively. Antibacterial activity of biosurfactants was assayed using the disc diffusion method and the microbroth dilution method. Antibiofilm activity of biosurfactants was determined using the crystal violet microtitre assay. Biosurfactants were characterised using GC-MS, LC-MS and FTIR. Enzymatic screening showed that bacterial isolates displayed strong cellulase (20–25 mm) and xylanase (23–25 mm) activity. The drop

collapse test revealed that bacterial isolates exhibited notable biosurfactant activity. Biosurfactants (200-1600 µg/ml) exhibited no inhibitory activity against the clinical bacterial strains tested. The microbroth dilution method showed that the isolates exhibited antibacterial activity at higher concentration, with KA11 inhibiting MRSA and *E. coli* (MIC = 25 mg/mL), KA14 inhibiting MRSA (MIC = 12.5 mg/mL), *A. baumannii* (MIC = 25 mg/mL), and E. coli (MIC = 12.5 mg/mL), while KA15 showed activity against MRSA and E. coli (MIC = 12.5 mg/mL). Biosurfactants did not show good antibiofilm activity at the concentrations tested. This study suggests that biosurfactants contain compounds with potential bioactivities and can be effective at lower concentrations following purification. The great enzymatic activities of endophytic bacteria allude to their capacity to produce biofilm inhibitors and disruptors.

Keywords: Endophytic bacteria, *Kigelia africana*, Biosurfactants, Biofilm disruption, Enzymes

Antimicrobial Resistance in *Pseudomonas* spp. from Amanzimtoti and South Beach: A Public Health Perspective and The Influence of Water Quality

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Pseudomonas spp. are opportunistic pathogens that thrive environmental matrices including beach areas and are responsible for various infections. Thus, exposure to multidrug-resistant (MDR) Pseudomonas poses a risk to beachgoers, primarily through accidental ingestion. However, few studies have investigated the health risks of resistant *Pseudomonas* spp. in beach environments. Thus, this study aimed to assess the antimicrobial resistance of *Pseudomonas* spp. and their potential public health risks. *Pseudomonas* spp. from Amanzimtoti and South Beach waters were isolated by membrane filtration on Pseudomonas isolation agar and identified through Polymerase Chain Reaction (PCR). Susceptibility testing was performed against five antibiotics (ceftazidime, gentamicin, amikacin, imipenem and ciprofloxacin) to identify multidrug-resistant (MDR) isolates. Multiple Antibiotic Resistance Index (MARI) was arithmetically computed. Quantitative Microbial Risk Assessment was used to assess risks of Pseudomonas infection while swimming. Out of 38 isolates from Amanzimtoti and 33 from South Beach, 22 and 24 were confirmed to be *Pseudomonas*. One isolate (4.54%) from Amanzimtoti exhibited MDR, while none (0%) from South Beach did. MARI ranged between 0-0.6 for Amanzimtoti and 0.2-0.4 for South Beach. The risk of infections ranged from $1.604x10^{-6}$ to $6.09x10^{-6}$ for adults and $1.504x10^{-6}$ -$3.958x10^{-6}$ for children in

Amanzimtoti and $1.33x10^{-6}$ to $3.501x10^{-6}$ for adults and $3.281x10^{-6}$ to $8.635x10^{-6}$ in South beach, implying no risk of Pseudomonas infections. The findings confirmed both beach areas to be safe, while underscoring the need for regular monitoring to safeguard beachgoers.

Keywords: *Pseudomonas* spp., Antimicrobial resistance, Beach water, Public

health risk.

Assessment of Vancomycin-Resistant Enterococcus in Durban's Wastewater Treatment Plant: Prevalence, Resistance Patterns, and Quantitative Microbial Risk Assessment

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Vancomycin-resistant *Enterococcus* (VRE) represents a critical antimicrobial resistance threat within aquatic environments, with wastewater treatment plants (WWTPs) serving as potential reservoirs and dissemination points. This study assessed the prevalence, resistance phenotypes, and environmental infection risks of VRE in a Municipal WWTP and its receiving surface water. Physicochemical parameters of influent and effluent were monitored alongside microbial quantification and antibiotic susceptibility testing. Biochemical oxygen demand (BOD) decreased from 2090.91 mg/L in raw wastewater to 69.3 mg/L post-treatment (96.7% removal), while COD and turbidity were reduced from 68,120 mg/L and 353.2 NTU to 720 mg/L and 96.6 NTU, respectively, indicating effective organic load reduction. Despite this, viable VRE persisted in treated effluent and were detected at 500 m upstream (31.02 CFU/100 mL) and downstream (34.21 CFU/100 mL) of the discharge point. All 50 *Enterococcus* isolates exhibited 100% resistance to vancomycin and teicoplanin, with high resistance observed to ciprofloxacin (72%), streptomycin (76%), tetracycline (68%), and amoxicillin (72%). Quantitative Microbial Risk Assessment (QMRA), applying exponential dose–response modelling and recreational exposure scenarios (10–100 mL ingestion per event), estimated annual infection probabilities ranging from 3.0×10^{-4} to 5.6×10^{-1}, surpassing the WHO benchmark of 10^{-4} infections per person per year. These findings reveal incomplete microbial elimination through conventional treatment and underline the need for tertiary disinfection, antimicrobial surveillance, and integration of AMR-specific QMRA in wastewater risk management frameworks.

Keywords: Vancomycin-resistant *Enterococcus*, Municipal wastewater treatment, Antimicrobial resistance, Quantitative microbial risk assessment, Environmental infection risk

Bioprospecting of Marine Actinomycetes for Antibacterial Activity and Exploration of Mechanisms of Action

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The constant increase in multidrug resistance has necessitated bioprospecting of microorganisms such as actinomycetes from untapped habitats including sea sand for potent antibacterial compounds. Thus, this study aimed at exploring antibacterial effect and mode of action of secondary metabolites derived from sea sand actinomycetes. Actinomycetes were isolated from sea sand using actinomycetes isolating agar and identified by 16S rRNA and RpoB gene sequence analysis. The minimum inhibitory concentration (MIC) was assessed using the microdilution assay. Gas chromatography-mass spectrometry (GC-MS) was utilised to identify the secondary metabolites. The antibacterial mode of action was established by a molecular docking and dynamic simulations and in vitro cell constituent release assay. Out of 17 selected actinomyces, isolates A2, B2 and F, which were identified as *Streptomyces koyangenesis*, *Nocardia rhizosphaerae* and *Nocardia* spp., were the most prolific antibiotic producers. Their secondary metabolites exhibited antibacterial activities with MIC values ranging from 19.55 to 625 µg/mL against test pathogens. GC–MS spectra revealed antibacterial compounds such Phenol,2,4-bis(1,1-dimethyethyl), Pentadecane,2,6,10-trimethyl and Tricyclo[4.2.2.1(2,5)] undecane, which had best docking poses ranging between –3.789 and –5.262 kcal/mol against penicillin-binding protein (PBP). They revealed hydrogen and non-covalent interactions with PBP, and the molecular dynamics simulations confirmed their stability with the root mean square deviation (RMSD) ranging between 0 and 1.9 Å. The antibacterial secondary metabolites also inhibited pathogens through cell membrane interference. In conclusion, the identified actinomyces have potential to serve as a source of effective antibacterial compounds to combat the challenge of multidrug resistance.

Keywords: Actinomycetes, Secondary metabolites, Antibacterial activity, Mode

Microbiological insights of different craft and traditional South African beers

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Beer is the oldest and most consumed fermented alcoholic beverage worldwide, ranking as third of well-known drinks after tea and water. The microbiology of beer significantly affects its quality, flavour and safety. There are two main systems of beer: traditional beer that depends on natural fermentation by wild microbial taxa with less hygiene. The second is craft beer, whose fermentation depends on inoculation with *Saccharomyces* strains, namely S. cerevisiae and *S. pastorianus* with a highly controlled process. This study aimed to compare the microbial diversities and detect the presence of spoilage and potential pathogenic microbes in craft and traditional (umqombothi) beer samples using culture-based and metagenomics approaches. Craft and umqombothi beer samples were bought from local stores. Bacterial and fungal isolates were cultured on different media including nutrient agar (NA), plate count agar (PCA), de man rogosa and sharpe (MRS) and Sabouraud dextrose agar (SDA). Results revealed two bacterial and one fungal isolate from craft beer, whereas four different bacterial and one fungal isolate was obtained from umqombothi beer. Genomic DNA was extracted from bacterial and fungal isolates for downstream molecular analysis. Confirmation of isolates to classify them as fungi or bacteria was conducted by PCR amplification of the ITS region and 16S rRNA on all samples then sent for sequencing to identify species. Different craft beer trials were brewed using commercial and the wild inoculum from umqombothi to investigate the fermentation regimes on maintaining desired fermentation microbes. Effects of temperature, hop addition and inoculum sizes were investigated. Results revealed that pH, temperature and hops, affects fermentation profiles regardless of the quantity of fermentable sugars. This study contributes to better understanding of microbial diversity of different types of beers, importance of knowing the fermenting strains used and further reveals high spoilage-related microbes in traditional beers.

Keywords: Spoilage-related, Microbial diversity, Craft beer, Umqombothi, Beer microbiology.

Characterization and classification of bacterial isolates with potential capacities for complete denitrification

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Nitrate (NO^{3-}) pollution has been a growing global concern caused by agricultural runoff, industrial effluents and wastewater treatment plant discharges. This leads to eutrophication, biodiversity loss and human health risks such as colorectal cancer. The traditional NO^{3-} removal methods employed by wastewater treatment plants (WWTPs) and industries, are very costly, energy-consuming and produce secondary contaminants. Thus, biological denitrification, which uses microorganisms to convert NO^{3-} into Nitrogen gas (N_2), is a preferred eco-friendly and sustainable alternative. This study aimed to characterize bacterial isolates from WWTPs for complete denitrification under various environmental conditions. Under anoxic conditions and glucose as the sole carbon source at 500mg/L, isolate 5 showed the highest NO^{3-} rate of 95, 19% at 16 hours, followed by isolate 3 with a removal rate of 92,7% at 20 hours. A commercial denitrifying strain serving as positive control, achieved 96, 09% over 24-hour period. The isolates were subjected to molecularly screening for denitrification key genes (narG, nirS/K, napA , and nosZ) as the presence or absence of these genes provides the insights into whether denitrification is complete or incomplete. Furthermore, trace metals copper and iron, were supplemented as co-factors to enhance denitrification. Copper was observed to be promoting complete denitrification. The results of this study will enhance the understanding of indigenous microbial communities for bioremediation of wastewater treatment processes in South Africa.

Keywords: Bacterial isolates, Denitrification, Molecular screening, Nitrate, Trace metals.

Optimising Microalgal Removal of Nutrients and Biomass Yield in Poultry Wastewater Treatment

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Poultry wastewater contains high concentrations of nitrogen and phosphorus posing severe risks of eutrophication and ecological degradation. This study investigated the optimisation of *Chlorella vulgaris* cultivation for nutrient removal and biomass

enhancement in poultry wastewater using Response Surface Methodology (RSM). Raw wastewater (pH 6.16, temperature 26 °C, total nitrogen ≈ 221 mg/L) was inoculated with 10% algal culture and incubated under varying light intensities, photoperiods and inoculum densities. Growth kinetics showed a steady increase in optical density (OD_{680}) from 0.033 at day 0 to over 1.48 by day 12 corresponding to a maximum specific growth rate of 0.97 day^{-1} and a minimum doubling time of 0.71 days. Nutrient analyses revealed substantial reductions in total nitrogen, phosphorus, and COD confirming efficient phycoremediation. PAM fluorometry demonstrated consistent photosystem II efficiency (Fv/Fm = 0.73–0.77) indicating high photosynthetic stability throughout cultivation. Statistical optimization confirmed that moderate light intensity (75 µmol m^{-2} s^{-1}) and a 12 h:12 h photoperiod maximized biomass productivity and nutrient uptake. The harvested biomass exhibited significant lipid, protein, and carbohydrate fractions suitable for biofuel and feed applications. Optimising environmental parameters significantly enhanced *C. vulgaris* growth and pollutant removal efficiency demonstrating the potential of algal bioremediation as a sustainable alternative to conventional treatment methods.

PHYSIOLOGICAL RESPONSE AND BIOCHEMICAL COMPOSITION OF CHLORELLA SOROKINIANA UNDER OPTIMISED CULTIVATION CONDITIONS

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Optimising cultivation parameters remains critical for enhancing microalgal productivity for biofuel and pigment applications. This study investigated the physiological and biochemical responses of a freshwater microalgal isolate, preliminarily identified as *Chlorella sorokiniana*, cultivated under optimised conditions (250 mg/L salinity, 750 mg/L carbon, and 200 µmol photons m^{-2} s^{-1}). Growth kinetics, chlorophyll content, and lipid accumulation were compared against standard conditions. The optimised cultures achieved significantly higher biomass yields (0.445 g/L) compared to the control (0.154 g/L), with a specific growth rate of 0.394 day^{-1} and a doubling time of 1.76 days. Enhanced chlorophyll accumulation indicated improved photosynthetic performance, supported by higher maximum quantum yield (Fv/Fm) values measured via PAM fluorometry. Microscopic analyses revealed robust cellular morphology and pronounced lipid body accumulation under optimised conditions. Lipid extraction and GC-MS profiling were performed to support further compositional analysis. These findings demonstrate that fine-tuning salinity, carbon availability, and light intensity can substantially improve microalgal photosynthetic efficiency and biochemical productivity. The outcomes of this

work provide a foundation for developing scalable and cost-effective cultivation strategies to enhance microalgae-based pigment and biofuel production, advancing the feasibility of sustainable biorefinery systems.

Keywords: Microalgae, *Chlorella sorokiniana*, Optimised cultivation, Photosynthetic efficiency, Biofuel production, Pigment yield

Genetic Diversity, Virulence and Resistance Pattern of *Mycoplasma* Species from Urines of Symptomatic Patients.

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Mycoplasma genitalium and *Mycoplasma hominis* are emerging sexually transmitted pathogens that are linked to various clinical urogenital and reproduction complications including urethritis in males, pelvic inflammatory disease in females, and infertility. With the increase in antimicrobial resistance of these pathogens and treatment complications; there is an urgent need for molecular surveillance. The genetic diversity of these pathogens is understudied, particularly in developing countries. This study therefore aimed to investigate the genetic diversity, virulence, and resistance patterns of *M. genitalium* and *M. hominis* isolated from urine samples of symptomatic patients. Genotypic characterization was conducted through DNA extraction, sequencing, and BLAST analysis on the NCBI database. While phenotypic analysis was performed through the inoculation of samples into the urogenital *Mycoplasma* broth followed by antimicrobial susceptibility testing using microdilutions assay. Phylogenetic relationships were assessed using NGPhylogeny.fr and visualized on iTOL. The phylogenetic tree showed distinct clustering of *M. genitalium* and *M. hominis* isolates, confirming species separation and genetic variation within each group. Outgroup taxa, including *Ureaplasma* spp., supported their evolutionary divergence from other urogenital pathogens. Mutations within the *M. hominis* 23S rRNA V domain were identified in 78% of the isolates, indicating high macrolide resistance, while phenotypic broth microdilution assay are ongoing to validate genotypic findings with observations of colour changes in the presence of macrolides, fluoroquinolones and tetracyclines. These primary findings enhance understanding of the molecular epidemiology and evolutionary diversity of *Mycoplasma* species in South Africa, supporting future diagnostic and therapeutic strategies.

Keywords: *Mycoplasma genitalium*, *Mycoplasma hominis*, Genetic diversity, Antimicrobial resistance, phylogenetic analysis

A MULTI-COMPONENT BIOFERTILIZER FROM INDUSTRIAL WASTE

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Materials that are often discarded during production, packaging, distribution and consumption can be recovered and recycled for a different yet valuable purpose in other processes. These secondary waste such as vinasse, a liquid that remains following the removal of ethanol during distillation, and aquafarming waste shrimp shell, contain nutrients that are of agricultural value. Several biofertilizers were formulated using the shrimp cell wastes after chitin extraction, the diluted vinasse and a mycorrhizal inoculant. The biofertilizers were evaluated by growing sunflower seedlings and comparing physical and physiological characteristics of the seedlings. A commercial fertilizer was used as a control. The results showed that plant growth using the formulated biofertilizers was comparable to or performed better than the commercial fertilizer. This demonstrates that industrial wastes can serve to enrich soil and enhance plant growth as biofertilizers which can be produced using a circular bioeconomy approach to reduce waste production.

Keywords: Vinasse, Chitin waste, Circular economy.

PHOTOCATALYTIC AND BIOLOGICAL SCREENING OF MORINGA OLEIFERA SYNTHESISED NICKEL-DOPED CERIUM OXIDE NANOPARTICLES

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Nanoparticles have a wide range of applications, with continuous advancements being made. Green synthesis of nanoparticles serves as a clean, cost-effective and biocompatible alternative to current synthesis methods. In this study, nickel-doped cerium oxide nanoparticles (NCNP) were synthesised using a green chemistry approach utilising an aqueous leaf extract of Moringa oleifera at different concentrations of nickel (0%, 0.5%, 1%, 2%, 5% and 10% w/w). Characterisation was done by UV-Vis spectroscopy, Fourier Transform-Infrared spectroscopy, Dynamic light scattering, Scanning Electron Microscopy with Energy Dispersive X-ray Spectroscopy (EDX) and Transmission Electron Microscopy. The incorporation of nickel caused a size reduction of the nanoparticles due to differences in the ionic radius and valency of $Ni^{2+}$and Ce^{4+}.

Antibacterial activity was investigated using the Kirby-Bauer disk diffusion method against Gram-positive and Gram-negative bacteria, with similar results observed due to the multi-mechanism nature of NCNPs and the cell wall composition and surface charge of the bacterial species. DPPH free radical scavenging assay was used to investigate the antioxidant potential of the synthesised nanoparticles, in which the leaf extract demonstrated the highest antioxidant activity; however, 2% NCNP had the highest activity amongst the synthesised nanoparticles. The photocatalytic activity was investigated against crystal violet and 4-nitrophenol dyes, with better degradation of 4-nitrophenol. The findings of this study highlight the potential of *Moringa oleifera* as a viable route for green synthesis of NCNP for enhanced physicochemical properties. The size reduction and enhanced biological properties of NCNPs, notably 2% NCNPs, suggest potential applications in the medical and environmental fields. Further studies should focus on preventing agglomeration and assessing biocompatibility for therapeutic applications.

Keywords: Green synthesis, Nickel-doped cerium oxide nanoparticles, *Moringa oleifera*

Exploring the Antihypertensive Properties of *Mangifera indica* Using *In silico* and *In Vitro* Analyses

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Hypertension is a severe health concern, commonly treated with angiotensin-converting enzyme (ACE) inhibitors like captopril, which may cause adverse side effects. *Mangifera indica* leaves are rich in bioactive compounds with potential ACE inhibitory activity, offering a safer natural alternative. This study aimed to identify these bioactive compounds and evaluate their antihypertensive potential through combined *in silico* and *in vitro* analyses. Solvent extraction was performed on leaves, using varying polarities: hexane, dichloromethane and water; and subjected to Liquid Chromatography – Mass Spectrometry profiling, revealing distinct metabolite distributions, with the aqueous extract showing the highest abundance of phenolics and flavonoids. Multivariate analyses confirmed solvent-dependent extraction, while heatmapping indicated a higher concentration of bioactive metabolites in the aqueous extract. All compounds were docked against ACE and showed stronger binding affinities than captopril. The top 20 compounds were screened using pharmacokinetic analysis and the lead four compounds underwent molecular dynamic (MD) simulation (100 ns): UNP (ΔGbind (kcal/mol) = −49.30 ± 8.51), M6G (−44.04 ± 7.94), IKF (−62.84 ± 10.94), and GPB (−28.34 ±

7.97), relative to captopril (-32.78 ± 3.9). Each exhibited stable interactions with key residues (HIS383, HIS387, TYR523). MD simulations confirmed stability (RMSD < 2.5 Å) and Density Functional Theory found low HOMO-LUMO gaps and high softness for all lead compounds relative to captopril, supporting strong reactivity and bioactivity. *In vitro* ACE inhibition assays validated findings, with the water extract showing 83.2% inhibition with IC_{50} of 0.72 mg/mL, compared to captopril's 71.6% at standard 0.625 mg/mL. Overall, *M. indica* - particularly the aqueous fraction, demonstrates promising natural ACE inhibition.

Keywords: *Mangifera indica*, Hypertension, ACE inhibitor, Molecular docking, *In silico*, *In vitro*, Traditional medicine

Investigating the production of industrially significant enzymes in a South African *Aspergillus niger* isolate

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Enzymes have become indispensable in the juice industry due to their effectiveness in fruit juice processing, significantly improving fruit juice marketability by reducing haziness, turbidity and cloudiness. The clarification step is one of the most important aspects in fruit juice processing and production. Recent studies have shown that the use of multiple enzymes is highly effective due to the complex structure of polysaccharides present in fruit juices. Thus, in the present study an *A. niger* isolate was used to produce a carbohydrase enzyme cocktail, the conditions for enzyme production were optimised via one-factor at a time (OFAT) approach and the cocktail was used for apple juice clarification. Pectinase, xylanase, amylase and cellulase presented clearing zones on agar plates supplemented with different carbon sources. Optimisation using maize straw as a low-cost substrate yielded 2.09-, 1.41-, and 1.28-fold increases in pectinase, xylanase, and amylase production, respectively. The enzyme mixture was partially purified by ammonium sulphate precipitation and concentrated in 50 mM sodium citrate buffer (pH 5.5). Optimum activities were observed at pH 3 and 50 °C for pectinase, pH 4 and 50 °C for xylanase, and pH 5 and 40 °C for amylase. When applied to apple juice, clarification efficiency increased by 53% with the combined use of enzyme and bentonite, compared with 42% and 19% using bentonite or enzyme alone, respectively. These results demonstrate that an *A. niger*-derived carbohydrase cocktail produced from inexpensive agricultural residues can serve as an effective and economical biocatalyst for juice clarification in industrial applications.

Keywords: Juice clarification, Xylanase, Pectinase, Amylase, Optimisation, Agrowaste, Maize straw

UREASE PRODUCTION AND CHARACTERIZATION FROM THERMOPHILIC BACTERIA FOR IMMOBILIZATION ON BIOCHAR NANOPARTICLES

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Urease (EC 3.5.1.5) is a di-nickel containing metalloenzyme belonging to the amidohydrolase family. It catalyses the hydrolysis of urea to ammonia and carbamate, which further decomposes to ammonia (NH_3) and carbon dioxide (CO_2), allowing microbes to use urea as a source of nitrogen. Industrial enzymes such urease tend to have difficulties with stability, rapid denaturation, difficulties with recovery and reuse. This study aimed isolate thermostable urease-producing bacteria from local compost sites, produce and characterise the enzyme, and evaluate the potential of BCNPs for immobilising partially purified urease for enhanced stability and reusability. The bacteria were isolated and preserved on NB supplemented with urea and stored at 4 °C. This was followed by characterization and purification. The stability assay revealed that the enzyme is stable at high temperature and pH. Biochar nanoparticles (BCNPs) were synthesized from chicken manure via pyrolysis. The BCNPs were then coated with chitosan, functionalized using APTES to enhance the adsorption and reactivity of the nanoparticle. Urease was immmobilized on the BCNPs using 5%-10% gluteraldehyde as the cross-linker. FTIR, DLS, TEM / SEM, DSC and TGA was used to characterise the BCNPs after synthesis, functionalization, and immobilization of urease. Immobilization efficiency was calculated, and the free & immobilised enzyme were compared to investigate their temperature and pH stabilities. Overall, this study demonstrates a sustainable and efficient approach for urease immobilization with potential in cost-effective industrial and environmental biotechnology applications.

Keywords: Biochar nanoparticles, Thermophilic, Immobilisation.

Effect of chemical modulators combined with nitrogen stress on the lipid and biomass productivities of *Scenedesmus* sp.

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Microalgae have been investigated as bio-factories for a wide range of high-value metabolites, with polyunsaturated fatty acids (PUFAs) being a major attraction. However, strategies to improve cellular lipid content, such as nutrient starvation, usually suppress biomass, which reduces overall lipid productivity. The aim of the study was to investigate the effects of modulators, in the presence and absence of nitrogen stress, for the enhancement of microalgal biomass and lipid productivities. OD was measured every third day, and biomass concentration and productivity were determined to assess the modulator effect on the biomass. SPV essay was conducted for total lipids. We investigated the effect of certain food preservatives (ascorbic acid and sodium benzoate) on the biomass and lipid productivities of *Scenedesmus* sp., either individually or in combination with nitrogen starvation. Ascorbic acid (AA) and sodium benzoate (SB) (at 50 – 10000 nM) did not have any significant effect on the biomass or the lipid productivities, with the variations being statistically insignificant. The completely nitrogen-starved cultures supplemented with AA (5000 nM, AA5000) had a significantly lower growth (~ 64% ↓). However, the lipid productivities with AA5000 were significantly better (~ 47 – 112% ↑) except for the completely nitrogen-starved culture (~ 41% ↓). The nitrogen starvation coupled to SB supplementation (1000 nM, SB1000) showed progressively lower biomass productivities (~ 17 – 62% ↓).

Keywords: Chemical modulators, Food additives, *Scenedesmus* sp., High-value lipids, Nitrogen stress.

Bioprospection of Fungal Endophytes from *Bridelia micrantha* for Secondary Metabolite Production

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Researchers are investigating endophytes associated with medicinal plants as a potential source of bioactive compounds. Secondary metabolites produced by endophytic fungi improve growth parameters (enhance root length, shoot length and biomass) of the host plant. *Bridelia micrantha*, a medicinal plant indigenous to South Africa, has not been studied for its endophytic microflora, hence the project aims to investigate endophytic fungi from *Bridelia micrantha* and its secondary metabolite production and its application in plant growth parameters. The methodology includes the following steps: standardized surface sterilization of *B. micrantha*, isolation of fungal endophytes, production, extraction and characterization of secondary metabolites, untargeted metabolomics study and application of the potent endophytic fungal extracts on *Vigna radiata* seeds to observe plant growth promotion parameters (root number, root length and shoot length) and seed germination properties (speed of germination, seed vigour index and seed germination rate). Surface sterilization was conducted with five different treatments utilizing different combinations of concentration and exposure periods of sterilization agents (ethanol, sodium hypochlorite, mercuric chloride). The second treatment yielded the highest number of endophytes. Initially, there were 20 isolates, however based on morphological distinction, the study yielded a final total of 11 isolates which were further evaluated to produce phenols, alkaloids, saponins, terpenoids and flavonoids by standard assays. The 11 isolates were sent to INQABA for molecular identification. The screened isolates (for both ethyl acetate and chloroform) produced secondary metabolites, however *Nigrospora sphaerica* and *Neofusicoccum umdonicola* (both chloroform extracts) produced the highest concentration of metabolites. Thereafter, the most potent isolates were subjected to the metabolomics study and applied for the seed germination test. A total of 27 compounds were detected via GC-MS for the chloroform extract of *N. sphaerica* and 97 compounds detected for the chloroform extract of *N. umdonicola*. The study highlights the potential of endophytic fungi associated with medicinal plants as a novel source of bioactive compounds, with broad applications and properties.

Keywords: Endophytic fungi, Secondary metabolites, Plant growth promotion, Metabolomics analysis, Medicinal plants

A Comparative Insight into the Modulatory Role of Phylloquinone and Obolactone on LasR-dependent Virulence Factors in *Pseudomonas aeruginosa*

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The LasR receptor within the quorum-sensing (QS) system of *Pseudomonas aeruginosa* is a key player in its virulence, making it a promising therapeutic target for anti-virulence strategies. Unlike conventional antibiotics, phytochemicals exert less selective pressure for resistance development, offering potential as anti-virulence agents. Through cheminformatics bioprospection, phylloquinone and obolactone emerged as potential LasR modulators, though their mechanisms remain uncharacterized. This study used computational analyses to compare the modulatory mechanisms of phylloquinone and obolactone against LasR. These results were further validated using anti-QS assays (violacein, cell attachment, biofilm, and pyocyanin inhibition). The thermodynamic binding free energy (BFE) calculated from 100 ns molecular dynamics (MD) simulations showed that phylloquinone had a more favourable binding free energy (-44.74 ± 1.91 kcal/mol) than obolactone (-27.63 ± 1.70 kcal/mol), surpassing reference standards [erythromycin (-11.67 ± 7.03 kcal/mol) and cinnamaldehyde (-13.74 ± 0.42 kcal/mol)]. Energy decomposition analysis indicated that van der Waals interactions were the main contributors to the BFE, with phylloquinone exhibiting the strongest interaction profile. Notably, phylloquinone engaged three catalytic amino acid residues (Tyr56, Trp60, and Ser129), whereas obolactone interacted with only Ala127. Biofilm inhibition assays confirmed phylloquinone's effectiveness (70.29%) at sub-minimum inhibitory concentrations compared to cinnamaldehyde (65.28%) and erythromycin (45.18%). Phylloquinone effectively inhibited biofilm formation, thereby enhancing the efficacy of bacterial treatment. These findings suggest that phylloquinone and obolactone are promising candidates for LasR-targeted QS modulation, with phylloquinone showing potential as a new therapeutic agent against *P. aeruginosa* infections. Further studies are underway to monitor their downstream effects on lasR expression.

Keywords: LasR; Molecular dynamics simulation; Obolactone; Phylloquinone; *Pseudomonas aeruginosa*; Quorum sensing.

Co-valorisation of Polyethylene Terephthalate and Dairy Wastewater for Polyhydroxyalkanoate Production: A Sequential Hydrolysis-Fermentation Approach

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This study presents an integrated biorefinery strategy for the simultaneous valorisation of polyethylene terephthalate and dairy wastewater via a sequential hydrolysis-fermentation approach. Polyethylene terephthalate was completely depolymerised

under alkaline hydrothermal conditions (150 °C, 90 min, 3 g sodium hydroxide) using dairy wastewater as a green solvent with ethanol co-solvent, achieving 97% terephthalic acid yield. Following terephthalic acid separation and ethanol removal, the remaining hydrolysate was utilised as carbon source for polyhydroxybutyrate production by *Cupriavidus necator*. Nitrogen source screening identified ammonium sulphate as the most effective among tested sources (ammonium chloride, sodium nitrite, sodium nitrate), and optimisation with 2 g/L ammonium sulphate and 0.5 g/L yeast extract significantly enhanced polyhydroxybutyrate accumulation under nitrogen-limited conditions. The optimised fermentation achieved 6.24 g/L polyhydroxybutyrate, representing a 40-fold improvement over unoptimized conditions (155.4 mg/L with ethanol inhibition). Additionally, glycolic acid was co-produced at 1.34 g/L as a valuable platform chemical, demonstrating efficient carbon utilisation from the hydrolysate. This dual waste valorisation approach provides a sustainable biorefinery platform, converting post-consumer plastic and food industry wastewater into biodegradable bioplastics and platform chemicals while addressing two major environmental challenges simultaneously.

Keywords: Microbial plastic, waste valorisation, PET recycling, Dairy Wastewater, Biorefinery

Functional and Molecular Characterisation of Efflux-Mediated β- lactam Resistance in *Vibrio* species Isolated from Wastewater

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Antimicrobial resistance among environmental *Vibrio* species is a growing concern with significant public health implications. These organisms are abundant in aquatic and wastewater environments and increasingly withstand β-lactam antibiotics, raising concern about environmental reservoirs that sustain resistance. This study investigated the mechanisms underlying β-lactam resistance in *Vibrio* isolates obtained from municipal wastewater treatment plants by examining the relative roles of β-lactamase production, efflux pump activity, and bacterial survival dynamics through time–kill assays. Thirty-three Vibrio isolates were recovered on thiosulfate–citrate–bile salts–sucrose agar and confirmed by oxidase test and V16S rRNA PCR identification. Antibiotic susceptibility profiling revealed high resistance to ampicillin (57.8%), ampicillin–sulbactam (51.5%) and cefoxitin (48.5%), whereas all isolates remained susceptible to

cefotaxime, ceftazidime, and imipenem. Time–kill kinetics demonstrated sustained bacterial viability across 0.5× to 2× MIC, with minimal reduction (<1 $\log_{10}$ CFU/mL) after 24 h, indicating tolerance rather than bactericidal response. Ethidium bromide and Hoechst 33342 fluorescence assays revealed active efflux pump activity in resistant isolates, as CCCP and reserpine reduced ampicillin MICs up to 16-fold. Nitrocefin hydrolysis assay confirmed β-lactamase production in 66.7 % of isolates, while the remaining 33.3 % showed no detectable hydrolysis activity. Their persistence in time–kill assays suggests resistance driven primarily by active efflux, reduced permeability, or tolerance phenotypes rather than classical enzyme- mediated hydrolysis. These findings highlight a dual mechanism of β-lactam resistance involving enzymatic activity and active efflux, emphasizing the role of wastewater as a reservoir for multidrug-resistant *Vibrio*.

Keywords: Antimicrobial resistance, *Vibrio*, Wastewater, β-Lactam, Efflux pumps, β-Lactamases

Extraction, Quantification, and Bioactivity Assessment of Lipopeptides Produced by *Bacillus velezensis* R16

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Lipopeptides are amphiphilic secondary metabolites produced by *Bacillus* species with diverse biological activities and significant potential in biotechnology, agriculture, and medicine. This study aimed to extract, quantify, and evaluate the bioactivity of lipopeptides produced by *Bacillus velezensis* isolates B81, R16, and FZB42, with *B. amyloliquefaciens* DSM 7 and *B. subtilis* DSM 10 serving as controls. Lipopeptides were extracted using an acid precipitation protocol followed by solvent extraction with methanol or phosphate buffer. Methanol demonstrated the highest extraction efficiency. Bioactivity screening at varying dilutions included haemolytic, antifungal against *Rhizoctonia solani* and *Fusarium* spp.), and antibacterial assays against *Escherichia coli*, *Micrococcus luteus*, *Serratia marcescens*, *Staphylococcus aureus*, *Enterococcus faecalis*, and *Salmonella typhimurium*. Isolate R16 exhibited the most potent and broad-spectrum antimicrobial activity and was selected for further investigation. Growth kinetics of R16 in Landy medium were monitored over 72 hours, and gravimetric analysis revealed maximal extract mass between 48 h and 72 h, corresponding to peak lipopeptide biosynthesis. Quantification using the Lowry method confirmed that R16 had the highest measurable lipopeptide yield among isolates. Thin-layer chromatography (TLC) profiles indicated strong surfactin presence across samples harvested at 24 h, 48 h, and 72 h, whereas the log phase showed negligible production. In conclusion, these

findings show *Bacillus velezensis* R16 as a high-yielding lipopeptide producer with potent antimicrobial potential, thus highlighting its value for sustainable biocontrol and industrial applications.

Keywords: Lipopeptides, *Bacillus velezensis*, Surfactin, Antimicrobial activity, Qantification

The Isolation and Characterization of a Lytic Bacteriophage against *Salmonella typhimurium*

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The rise of global foodborne salmonellosis caused by *Salmonella enterica* subsp. *Enterica* serovar *typhimurium* created an urgent need for alternative, effective, and sustainable antimicrobial strategies. Lytic bacteriophages offer a highly specific biocontrol approach for eliminating pathogenic *Salmonella* spp. This study aimed to isolate and characterize a lytic phage active against *Salmonella typhimurium*. The isolation was done using various environmental samples as inoculum and *Salmonella typhimurium* (ATCC14028) as the host. Using the double-layer technique, phage plaques were obtained after enrichment and purified. The selected lytic phage produced clear plaques of 1.0–1.2 mm in diameter. Suppression of bacterial growth was observed at an MOI of 0.1, with substantial reductions in OD_{600} evident relative to untreated host cell controls. Transmission electron microscopy revealed an icosahedral head (~93 nm diameter) and a tail (~79 nm length; ~20 nm width), consistent with a typical head and tail phage morphology found within the class Caudoviricetes. Host range analysis demonstrated specificity towards two Salmonella serovars (*S. typhimurium* and *S. enteritidis*). However, while some lytic activity was detected against two ExPEC strains of *E. coli*, no lytic activity was detected against *Klebsiella pneumoniae* and *Enterobacter hormaechei* subsp. steigerwaltii. The phage retained infectivity across a pH range of 4–8, with optimal activity at pH 7.5. Minimal titer reduction was observed following 45 min exposure at -18 °C, 4 °C (optimum), and 25 °C, with quantitative inactivation at 75 °C. The phage also remained stable in high-salinity conditions of up to 2 M NaCl after 30 minutes of exposure. Exposure to the disinfectant ethanol (2 hours) resulted in reduced PFUs at concentrations of 20–35% ethanol, with complete inactivation observed at ≥50% ethanol. This study demonstrates the potential of a newly isolated lytic bacteriophage as a viable biocontrol strategy for pathogenic *Salmonella* spp. associated with food and the environment.

Keywords: Lytic bacteriophage, *Salmonella typhimurium*, TEM imaging, Caudoviricetes.

Generative AI–Integrated Response Surface Methodology for Medium Optimization in Bacterial Polyhydroxyalkanoate (PHA) Production

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The escalating global environmental crisis caused by extensive plastic pollution demands an urgent transition toward biodegradable alternatives such as polyhydroxyalkanoates (PHAs). This study presents an innovative approach to model and optimize the separate hydrolysis and fermentation (SHF) process for PHA production using potato peels, an abundant agro-industrial waste as a sustainable and low-cost carbon source. A hybrid framework combining Generative Artificial Intelligence (AI) and Response Surface Methodology (RSM) was employed to identify and refine optimal process conditions. Using retrieval-augmented generation (RAG) strategies, the model predicted ideal operating parameters: a temperature of 27–35 °C, a pH range of 6.8–8.2, and a fermentation duration of 48–72 hours. Experimental validation confirmed a maximum PHA yield of 2.482 g/L under optimized conditions (27 °C, pH 8.2, and 48 hours). Kinetic analysis revealed that PHA accumulation peaked at 48 hours, beyond which a decline was observed, likely due to nutrient depletion or product inhibition. The integration of Generative AI with traditional optimization techniques such as RSM provides a novel, data-driven pathway for efficient SHF process design and scale-up of PHA production from lignocellulosic waste.

Keywords: Optimization, Separate Hydrolysis and Fermentation (SHF), Polyhydroxyalkanoate (PHA), Potato Peels, Generative AI, Response Surface Methodology (RSM)

Isolation and Characterization of a Novel Lytic Bacteriophage Effective Against AmpC-type β- Lactamase-producing *Enterobacter hormaechei* subsp. steigerwaltii

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Enterobacter hormaechei subsp. steigerwaltii belongs to the so-called ESKAPE group of bacterial pathogens and is capable of producing AmpC-type β-lactamase. This bacterium is associated with nosocomial infections in humans, particularly in immunocompromised individuals. Due to the growing concerns of antimicrobial

resistance (AMR) in public health settings, alternatives to traditional antibiotics are urgently sought to combat antibiotic-resistant ESKAPE strains, with phage therapy reemerging as a promising approach. This study aimed to isolate and characterize a lytic bacteriophage targeting a representative ESKAPE pathogen. Thus, *E. hormaechei* subsp. Steigerwaltii (ATCC BAA-1143, formerly E. cloacae) was used as the host, and soil as inoculum. The morphology of the purified phage isolate, designated ATLAS22, was characterized by transmission electron microscopy (TEM), and the burst size and latent period were estimated using a one-step growth curve. Additionally, the physicochemical stability (temperature, pH), chemical tolerance (ethanol, sodium lauryl sulfate (SLS)), host range, and biofilm inhibition capability (crystal violet assay) were analysed. TEM analysis demonstrated a typical icosahedral head and short contractile tail, matching a myovirus-like phage morphotype. The phage ATLAS22 had a short latent period and high burst size, was host-specific for the genus *Enterobacter*, and inhibited biofilm formation by its host. Upon exposure for 2 hours, the phage remained stable at temperatures up to 60 ℃ and within a pH range of 4-10. The phage showed stability after 30 minutes of exposure to 70% ethanol and 5% SLS. The lytic bacteriophage ATLAS22 is a promising candidate for controlling *E. hormaechei* subsp. Steigerwaltii and its biofilm formation.

Keywords: *Enterobacter hormaechei* subsp. steigerwaltii, AmpC-type β-lactamase, antimicrobial resistance, lytic bacteriophage, bacteriophage therapy

Large Language Model–Assisted Response Surface Optimization of Napier Grass Saccharification and Co-Fermentation for Efficient Lignocellulosic Bioethanol Production

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This study explored the optimization of Napier grass (*Pennisetum purpureum*) saccharification using an integrated Generative Artificial Intelligence (AI) and Response Surface Methodology (RSM) framework. The approach employed Large Language Models (LLMs) enhanced with Retrieval-Augmented Generation (RAG) to predict and narrow the optimal parameter ranges, which were subsequently refined through statistical modelling via RSM. The resulting hydrolysate was then utilized for co-fermentation of bioethanol using a mixed culture of *Saccharomyces cerevisiae* and *Scheffersomyces stipitis*. Within the hybrid AI–RSM framework, LLM-driven generative modelling was used to synthesize prior knowledge and experimental datasets to propose optimal operating conditions. These AI-derived insights guided the experimental design of the Box–Behnken

RSM model, which systematically evaluated the effects of temperature, nanoparticle dosage, and Tween 80 concentration on reducing sugar release. Statistical modelling produced a robust quadratic fit (R2 = 0.9781; adjusted R2 = 0.9387). ANOVA confirmed that temperature, along with its quadratic term, exerted the most significant influence ($p < 0.0001$), while Tween 80 and nanoparticles had negligible effects. The optimized conditions—50 °C, 1.5% nanoparticles, and 1.5% Tween 80—yielded a predicted reducing sugar output of 0.98 g/g, closely matching the experimental yield of 0.95 g/g. Structural characterization validated the process efficiency: FT-IR spectra revealed intensified cellulose-associated peaks, while SEM micrographs showed extensive disruption of the lignocellulosic matrix compared to untreated biomass. Importantly, the saccharification time was reduced by 50% (from 72 h to 36 h) without compromising yield, reaching sugar recovery comparable to reported literature values (up to 1.7 g/g). The subsequent co-fermentation step efficiently converted both hexose (C6) and pentose (C5) sugars using a microbial consortium of *Saccharomyces cerevisiae* and *Scheffersomyces stipitis*, leading to the successful production of ethanol. Overall, this work establishes a Generative AI-assisted optimization framework that effectively accelerates enzymatic saccharification, sustains high sugar yields, and enhances the economic feasibility of Napier grass–derived bioethanol. The study highlights how integrating computational intelligence with experimental design can streamline process discovery, reduce experimental costs, and promote scalable, sustainable lignocellulosic biofuel production.

Keywords: Napier grass (*Pennisetum purpureum*); Generative Artificial Intelligence (AI); Response Surface Methodology (RSM); Enzymatic saccharification; Co-fermentation; Lignocellulosic bioethanol

Enrichment of ANAMMOX bacteria in a Lab-Scale Up-flow Anaerobic Sludge Blanket Reactor

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Anaerobic ammonium oxidation (ANAMMOX) process is recognized as a sustainable and cost-effective biotechnology for nitrogen removal from wastewater, requiring no external carbon source and minimal aeration. This study aimed to develop and optimize strategies for enhancing the enrichment of ANAMMOX bacteria for nitrogen removal in a lab-scale up-flow anaerobic sludge blanket (UASB) reactor operated under controlled conditions. A 25 L UASB reactor with a working volume of 20.5 L was maintained at a hydraulic

retention time (HRT) of 2.85 days and a temperature of 38 °C for 231 days. The inoculum was obtained from the Kingsburg Wastewater Treatment Plant. The synthetic feed medium consisted of ammonium chloride (NH_4Cl), sodium nitrite ($NaNO_2$), potassium dihydrogen phosphate (KH_2PO_4), dipotassium hydrogen phosphate (K_2HPO_4), sodium bicarbonate ($NaHCO_3$), magnesium sulfate ($MgSO_4$), and calcium chloride ($CaCl_2$), with the pH maintained at 8. The stoichiometric ratio of ammonium nitrogen (NH^{4+}–N) to nitrite nitrogen (NO^{2-}–N) was controlled at 1:1.32, while the dissolved oxygen (DO) concentration was kept below 0.2 mg/L throughout the experiment. The study was conducted in three operational phases. In Phase 1 (without carriers), influent NH^{4+}–N and NO^{2-}–N concentrations were 100 ± 32 mg/L and 132 ± 50 mg/L, respectively, resulting in an average total nitrogen (TN) removal efficiency of 13.3%. In Phase 2 (with carriers, same influent concentrations), TN removal increased to 30.2%. In Phase 3 (with carriers and reduced influent concentrations; NH^{4+}–N = 50 ± 10 mg/L; NO^{2-}–N = 66 ± 10 mg/L), the system achieved an average TN removal efficiency of 80.4%. Quantitative PCR (qPCR) analysis revealed low ANAMMOX gene abundance in Phase 1, followed by a significant increase in Phase 3, while competing microbial populations and declined. Overall, these findings confirm the successful enrichment of ANAMMOX bacteria within a UASB reactor under low-oxygen and well-controlled conditions, demonstrating its potential for stable and sustainable nitrogen removal in wastewater treatment applications.

Keywords: ANAMMOX bacteria; Up-flow anaerobic sludge blanket (UASB) reactor; Nitrogen removal; Wastewater treatment; Microbial community enrichment; Quantitative PCR (qPCR)

Ability of Selected *Pseudomonadota* strains Exhibiting β-lactamase Activity to Cross- Protect Susceptible Bacterial Strains Against β-lactam Antibiotics

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Antibiotics are essential tools in the chemotherapy of bacterial infections. However, the efficacy of antibiotic compounds can be drastically reduced by bacterial resistance mechanisms, such as efflux pumps, uptake limitation, target site modification, or enzymatic transformation. As β-lactam antibiotics are widely used, resistance to these antibiotics is frequently reported and is a cause for concern. This study aimed to verify if isolated bacteria exhibiting phenotypic β-lactam resistance exhibit β-lactamase activity as a means of resistance. Therefore, representative type strains, such as *Escherichia coli* ATCC 29222 (as a β-lactamase-negative control) and *Enterobacter hormaechei* subsp. steigerwaltii (formerly E. cloacae) ATCC BAA-1143 (as a β-lactamase-positive control), as

well as environmental isolates, including an MDR S. marcescens and an MDR E. coli from produce, were analyzed. Furthermore, selected strains exhibiting elevated β-lactamase activity were tested for their ability to protect β-lactam-susceptible bacteria. The presence of β-lactamase activity in crude extracts obtained from harvested cells by ultrasonication and subsequent centrifugation (40000 x g, 30 min) was determined using a chromogenic cephalosporin. While the negative control demonstrated no measurable β-lactamase activity, the environmental isolates exhibiting phenotypic resistance against β-lactam antibiotics showed high specific β-lactamase activity against the chromogenic cephalosporin tested. Furthermore, strains exhibiting elevated β-lactamase activity were able to cross-protect susceptible strains, such as *Escherichia coli* ATCC 29222, against inactivation by β-lactam antibiotics such as ampicillin.

Isolation and molecular identification of Bioflocculant Producing-Microorganism linked to *Encephalartos ferox* plant

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Water pollution from industrial and agricultural processes contributes to the decline of water quality and poses major threats to human health and the environment. Advanced oxidation processes (AOPs), membrane bioreactors (MBRs), and nanotechnology-based treatments are examples of conventional wastewater treatment technologies that have shown promise but are frequently constrained by high operating costs, energy requirements, and the requirement for specialized equipment. Through the isolation and characterization of microorganisms that produce bioflocculants from the indigenous plant *Encephalartos ferox* in South Africa, this study sought to investigate a sustainable and environmentally suitable substitute. After screening the isolates for the generation of bioflocculants, 16S rRNA gene sequencing revealed that *Priestia aryabhattai* NR 115953.1 was the strain with the highest flocculating activity (84%) among them. Inoculum size, carbon and nitrogen sources, temperature, shaking speed, initial pH, fermentation time, and metal ions, on the formation of bioflocculants were investigated. The findings showed that the maximum flocculating activity was obtained with an inoculum size of 0.5 mL, lactose as a carbon source, yeast extract as a nitrogen source, at 45 °C, and initial pH of 9 resulted in 3.09 g/L bioflocculant. The results show that *Priestia aryabhattai* NR 115953.1, which was isolated from *E. ferox* plant extract, with a potential to produce a bioflocculant, is a viable option for environmentally friendly wastewater treatment uses. This study adds to the mounting evidence that

bioflocculants provides a more economical and ecologically friendly option for water filtration than chemical flocculants.

Keywords: Bioflocculants, *Encephalartos ferox*, flocculating activity, *Priestia aryabhattai* NR 115953.1, kaolin podwer

Evaluation of antimicrobial resistance, virulence factors, sequence types, serotypes, and molecular epidemiology characteristics of *Escherichia coli* from beef using whole genome sequence analysis

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The emergence of antimicrobial-resistant bacteria poses a public health challenge as infections become challenging to treat due to ineffective antimicrobials. In South Africa, there is limited research on antimicrobial resistance of *Escherichia coli* from beef using whole genome sequencing. Therefore, this study aimed to use whole genome sequencing to evaluate antimicrobial resistance, virulence factors, sequence types, and serotypes of *E. coli* from beef in KwaZulu-Natal province, South Africa. For this purpose, *E. coli* was isolated using classical microbiological methods and confirmed using MALDI-TOF MS. The confirmed *E. coli* was whole genome sequenced using the ONSO system, followed by bioinformatics analysis alongside 453 *E. coli* sequences from the NCBI. Multilocus sequence typing assigned the *E. coli* to ST7106, which has not been previously reported in South Africa to our knowledge. Various antimicrobial-resistance genes, such as efflux pump genes (acrA, mdfA), ampC, ampC1, ampH, tet(B), aadA1, and blaTEM variants, were detected. Whole-genome sequencing of the beef-derived *E. coli* isolate revealed an extensive virulence repertoire spanning adherence, outer-membrane integrity, secretion systems, and iron acquisition. The *E. coli* belonged to serotype O18ab:H11. The pangenome analysis revealed an open structure of the gene presence or absence of the 454 *E. coli* isolates that consist of a total of 17186 genes. This study revealed *E. coli* O18ab:H11 with resistance genes from different classes, diverse virulence genes, and an uncommon sequence type, which may be a threat to human health. This highlights the necessity for pathogen routine surveillance and monitoring to prevent outbreaks and protect public health.

Keywords: *Escherichia coli*, WGS, Antimicrobial resistance, Virulence genes, pangenome analysis

ISOLATION, SCREENING AND IDENTIFICATION OF BIOFLOCCULANT-PRODUCING MICROORGANISM FROM UMHLATHUZE RIVER WATER

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Bioflocculants are sustainable alternative for treating wastewater due to their eco-friendliness and biodegradability to the environment. Bioflocculants are natural substances produced by living organisms such as bacteria and fungi. For bioflocculant production they used to identify bacteria that show the highest flocculating activity. This study aimed at isolating, screening and identification of bioflocculant producing microorganism from uMhlathuze river water. Optimal medium composition such as inoculum size, metal ions carbon and nitrogen sources and culture condition such as pH, shaking speed and cultivation temperature were assessed by the one factor at a time course assay. The mixture of butanol and chloroform in a ratio of 5:2 (v/v) was used to extract the bioflocculant. A 16S rRNA gene sequence analysis was used to identify the isolates. The bacterial strain was identified as *Klebsiella pneumoniae* with the accession NR_117683.1. Phylogenetic tree based on 16S rRNA confirmed to the isolate's evolutionary relationship other *Klebsiella pneumoniae*. A maximum flocculating activity of 97% and a yield of 3,61 g/L were obtained when a 2% (v/v) inoculum size was used in the presence of sucrose and yeast extract at pH 7, 30 °C, shaking speed 110 rpm and after 72 hours of cultivation temperature. The bacterium showed a great potential to produce a bioflocculant which could be the solution to replace the in use synthetic flocculants in wastewater treatment.

Keywords: Bioflocculant, Optimization, *Klebsiella pneumoniae* NR_117683.1, Flocculating activity, uMhlathuze river

WHOLE GENOME SEQUENCE ANALYSIS OF ANTIMICROBIAL RESISTANCE, VIRULENCE FACTORS AND MOLECULAR EPIDEMIOLOGY CHARACTERISTICS OF COAGULASE NEGATIVE STAPHYLOCOCCUS FROM BEEF

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Coagulase-negative staphylococci (CoNS) are opportunistic pathogens, particularly in patients with indwelling devices. CoNS such as *Staphylococcus ureilyticus* is increasingly implicated in infections of immunocompromised patients, including urinary tract infections raising concerns about antibiotic effectiveness; data from South African beef sources are limited. This study evaluated antimicrobial resistance (AMR), virulence factors, and molecular epidemiology of three beef- derived *S. ureilyticus* strains and compared them to clinical genomes using whole-genome sequencing. AMR genes were identified with AMRFinderPlus, SRST2 and Gamma, while virulence genes were detected with Virulence Finder and ABRicate (virulence factor database). Few AMR genes were detected: fusF and str, indicating possible aminoglycoside resistance. No virulence genes were detected. All the three isolates harbored the plasmid replicon repUS10_1 (Rep initiation protein; pSSP1-like), consistent with a plasmid backbone rather than a named plasmid. SNP phylogeny placed the South African beef-derived isolates within 9–15 SNPs of clinical strains from other countries, indicating closely related lineages and a credible One-Health risk. These findings support WGS-based surveillance and hygiene controls, alongside phenotypic validation to confirm virulence and AMR impact.

Keywords: Coagulase-negative *Staphylococci*, *Staphylococcus ureilyticus*, Whole genome sequence analysis, Beef, Antimicrobial resistance

Phytochemical Profiling, Antimicrobial Activity, and Molecular Docking studies of Methanolic Leaf Extract of *Sclerocroton integerrimus*

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One of the most significant public health issues is antimicrobial resistance (AMR), which poses a threat to human health worldwide. AMR has the potential If left unchecked, to become a major threat to healthcare and perhaps trigger another pandemic. This highlights the need for the creation of considering microdata from all over the world to come up with global health solutions for AMR. It results from bacteria, fungi, parasites, and viruses evolving to survive antimicrobial drugs, worsened by antibiotic overuse in healthcare, agriculture, and food production. Key bacterial resistance mechanisms include altering drug targets, reducing membrane permeability, using efflux pumps, enzymatic degradation, and biofilm formation. Gram-positive and Gram-negative

bacteria differ in their strategies, with pathogens like Staphylococcus aureus resistant to nearly all antibiotics. As new antibiotics become scarce, plant-derived secondary metabolites offer promising alternatives. This study examines the methanolic leaf extract of *Sclerocroton integerrimus*, a southern African medicinal plant rich in triterpenoids, flavonoids, and phenolic acids with antibacterial and anti-inflammatory properties. Using GC-MS and FT-IR for phytochemical profiling, broth dilution for antimicrobial testing against *Staphylococcus aureus* and *Pseudomonas aeruginosa*, molecular docking to explore interactions with bacterial proteins, the research aims to identify bioactive compounds capable of combating AMR. The findings support developing novel, plant-based therapies to address AMR, especially in regions like South Africa facing high drug resistance and limited antibiotic access.

Keywords: Antimicrobial resistance, *S. integerrimus*, Molecular docking, Phytochemical profiling, GC-MS and FT-IR

Isolating, Screening and Identifying a bioflocculant producing microorganism from *Spinacia oleracea* (Spinach)

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Bioflocculants are biological compounds produced by different microorganisms with many applications for wastewater treatment and as such become an important product in biotechnology to be used in industries. The current study aimed to isolate, identify, and screen bioflocculant-producing bacteria from *Spinacia oleracea*. The production of bioflocculants was enhanced by optimization of various cultural conditions such as carbon source, nitrogen supply, initial pH, cations, shaking speed, cultivation time and inoculum sizes which were estimated in terms of flocculating activity test. The identification of the isolated bacterium capable of producing a bioflocculant was performed by 16S rRNA gene sequencing analysis. The bacterium was identified as *Providencia vermicola* MN689842.1 with 98% similarities. Its optimal medium and culture conditions included Xylose, Ammonium sulphate, $MnCl_2$, initial pH 8, 30 °C, inoculation size of 0.5 mL, shaking speed (160 rpm) for 72 h of fermentation. The bioflocculant has the potential for pollutant removal in industrial wastewater.

Keywords: Bioflocculants, *Spinacia oleracea*, *Providencia vermicola* MN689842.1, Flocculating Activity, Wastewater

ISOLATION AND IDENTIFICATION OF YEASTS IN SPONTANEOUSLY FERMENTED BANANA WINE

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Bananas (*Musa* spp.) are highly nutritious yet extremely perishable fruits, leading to considerable postharvest losses. Fermentation is an effective biotechnological strategy to reduce waste and develop value-added products such as banana wine. However, the yeast communities driving spontaneous banana wine fermentation remain poorly characterised. This study aimed to isolate and identify yeasts involved in spontaneous banana wine fermentation. Overripe bananas were chopped and spontaneously fermented under sterile conditions. Samples were collected on days 0, 7, and 14, and analysed using classical microbiological techniques and sequencing of the Internal Transcribed Spacer (ITS) region, followed by phylogenetic analysis. Physicochemical parameters, including pH and titratable acidity, were measured. The pH decreased from 4.82 to 3.58, accompanied by an increase in titratable acidity from 0.13 to 1.17 g/100 mL citric acid equivalent, indicating active microbial metabolism. Phylogenetic analysis revealed yeast succession, with *Pichia kudriavzevii* present on day 0, *Kodamaea ohmeri* detected on day 7, and *P. kudriavzevii*, *Wickerhamomyces anomalus*, *Issatchenkia orientalis*, and *Meyerozyma guilliermondii* detected on day 14. The observed yeast diversity suggests dynamic microbial adaptation, where shifts in dominant species likely reflect changes in substrate availability and fermentation conditions. This diversity highlights the ecological interplay among yeasts contributing to fermentation performance and product quality. Overall, this study provides insights into yeast diversity in spontaneous banana wine fermentation, which may serve as a basis for developing potential starter cultures to enhance product consistency, flavour, and sustainable fruit utilisation.

KEYWORDS: Banana wine, Spontaneous fermentation, Yeasts, PCR, ITS sequencing, Phylogenetic analysis

Evaluation of Antimicrobial Resistance, Virulence factors and Molecular Epidemiology of *Staphylococcus saprophyticus* From Beef using Whole Genome Sequencing

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Staphylococcus saprophyticus causes urinary tract infections (UTIs) including prostatitis, epididymitis and pyelonephritis particularly in young women. Rising antimicrobial resistance (AMR), complicates treatment. Limited genomics data from South African beef restrict understanding of AMR spread and human health risks. This study assessed AMR, virulence factors, and molecular epidemiology of *S. saprophyticus* from South African beef and compared them to clinical and non-clinical African strains using whole-genome sequencing. AMR genes were identified with AMRFinderPlus, SRST2 and Gamma; virulence genes were detected with ABRicate (VFDB) and VirulenceFinder. Phylogenetic analysis was based on single nucleotide polymorphisms (SNPs). SNP-based phylogeny identified two lineages associated with beef: one distantly related (differing by > 2000 SNPs) and a clonal group with no SNP variation. The beef clonal group was genetically distinct from clinical and non-clinical isolates, suggesting historical gene flow or environmental adaptation. AMR detection tools identified resistance genes blaZ, blaTEM-1B, blaACT-9, qnrS1, tet(K) and dfrA1, conferring resistance to beta-lactams, fluoroquinolones, tetracycline and trimethoprim. Plasmid replicon families repUS10, rep7a and rep20 were detected, indicating possible horizontal gene transfer. No virulence genes were detected. These findings reveal genetic diversity and AMR potential of S. saprophyticus from beef, indicating that meat may serve as a reservoir for resistance genes. Continued genomics-based surveillance within the One Health framework is essential.

Keywords: *Staphylococcus saprophyticus*; Antimicrobial Resistance; Beef; Whole Genome Sequencing, Single Nucleotide Polymorphism; Human Health Risk

EVALUATION OF MICROBIAL DIVERSITY IN SPONTANEOUSLY FERMENTED BANANA WINE USING CULTURE-BASED METHODS AND SANGER SEQUENCING

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Bananas (*Musa* spp.) are highly nutritious, but perishable and are often discarded, contributing to food waste. However, fermentation can be used to circumvent the wastage whilst simultaneously making value- added products such as banana wine. Understanding the microbial diversity of banana wine during spontaneous fermentation is essential for improving product quality, safety, and shelf life. There is limited

information microbial diversity of banana wine during spontaneous fermentation in South Africa. Therefore, the aim of the study was to evaluate the bacterial diversity in spontaneously fermented overripe bananas sourced from Kwa-Dlangezwa, KwaZulu-Natal and to determine the physio-chemical parameters. For this purpose, bananas were mixed into a must, which was spontaneously fermented for 14 days at 35 ℃ ±2. The bacterial diversity was evaluated using classical microbiological techniques. Confirmation of the bacterial identity was done using 16S rRNA sequencing, followed by phylogenetic analysis. The pH and total titratable acids were measured during different fermentation stages. The pH decreased from 4.94 ± 0.05 on day 0 to 3.01 ± 0.01 on day 14. The total titratable acids increased from 0.129 ± 0.000g/L on day 0 to 1.172 ± 0.000g/L on day 14, reflecting progressive acid production. Phylogenetic analysis assigned the bacteria to *Staphylococcus warneri*, *Staphylococcus xylosus*, *Enterobacter* spp., *Pantoea agglomerans*, *Micrococcus aloverae*, *Lapilliococcus jejuensis*, *Staphylococcus shinii*, *Staphylococcus xylosus*, and *Micrococcus* spp. Some of the bacteria detected in this study have potential for producing aroma and enhanced shelf-life of the banana wine. This study is not only innovative but also environmentally and socially impactful.

Keywords: Banana wine, Physio-chemical parameters, 16S rRNA Sequencing, Bacterial diversity, Culture-based methods, Spontaneous fermentation

Bioactive Analysis, *In vitro* Antimicrobial Testing and Molecular Docking Studies of Dichloromethane Leaf Extract from *Sclerecroton integerrimus*

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Antimicrobial resistance (AMR) presents a major global health challenge by enabling pathogens to resist previously effective treatments, leading to increased mortality and healthcare costs. This study investigates the antimicrobial potential of bioactive compounds extracted from the threatened Southern African plant *Sclerocroton integerrimus* using dichloromethane (DCM) to isolate lipophilic phytochemicals. Chemical profiling by GC-MS and FT-IR revealed diverse secondary metabolites with known antimicrobial properties. The extracts were tested against drug-resistant *Pseudomonas aeruginosa* and *Staphylococcus aureus*, and antimicrobial activity was quantified by determining minimum inhibitory and bactericidal concentrations via broth microdilution assays. Molecular docking analyses using AutoDock Vina showed that key compounds, notably gamma-Sitosterol and Phytol, strongly bind to essential bacterial proteins involved in cell division and quorum sensing, suggesting mechanisms for their

antimicrobial effects. Antibiotic susceptibility tests confirmed the multidrug resistance of the bacterial isolates. These findings underscore the potential of *S. integerrimus* compounds as promising alternative agents to address antibiotic resistance and support further research toward therapeutic development.

Keywords: *Sclerecroton interrigesum*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, antimicrobial resistance, molecular docking

Isolation, screening and identification of bioflocculant producing microorganism from Richards Bay wastewater treatment plant

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Synthetic flocculants have been employed to cluster colloidal particles in wastewater treatment. However, due to negative impacts and elevated costs of traditional flocculants, natural flocculants like microbial flocculants have gained an interest. This study aimed to isolate, screen, and identify bioflocculant producing microorganisms from the Richards Bay wastewater treatment plant. The wastewater sample was serially diluted in sterile saline water and placed on a nutrient agar plate. Colonies were randomly selected and subcultured on fresh nutrient agar plates and incubated. The isolate with the highest flocculating activity was identified using 16S rRNA sequencing method and morphological analysis. The isolate had 99% similarity to *Klebsiella* spp, it was named *Klebsiella pasteurii* NR180640.1. Culture conditions, such as inoculum size, carbon and nitrogen sources, cultivation temperature, starting pH, fermentation time and shaking speed, were optimised using the one factor time method. The ideal conditions for achieving maximal flocculating activity of 85% were 3% (v/v) inoculum size, sucrose and peptone, Na^+, temperature of 35 °C, initial pH of 9, and shaking speed of 165 rpm for 72 hours of fermentation. The extracted and purified bioflocculant had a concentration of 2.654 g/L. These results demonstrate the potential of the bioflocculant produced by *Klebsiella* species obtained from Richard's Bay wastewater to replace in use harmful chemical flocculants for wastewater treatment.

Keywords: Bioflocculant, *Klebsiella pasteurii* NR180640.1, Wastewater treatment, Optimization, Parameters, Flocculating activity

GC-MS Profiling, Antimicrobial Evaluation and Molecular Docking Studies of *Sclerocroton integerrimus* Leaf Extracts from n-Hexane Fraction

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Antimicrobial resistance (AMR) presents a significant threat to global health, compromising the effectiveness of existing antibiotics and intensifying infection-related morbidity and mortality worldwide. Given the critical need for alternative antimicrobial agents, medicinal plants have gained attention owing to their diverse bioactive secondary metabolites that exhibit multi-targeted antimicrobial activity with potentially fewer side effects than conventional antibiotics. This study investigates the antimicrobial potential of *Sclerocroton integerrimus* a traditional plant native to the coastal regions of KwaZulu-Natal, South Africa, which is currently used as a mouthwash for oral and toothache treatment, through in silico molecular docking analysis against *Pseudomonas aeruginosa* and *Staphylococcus aureus*. The research involves the extraction of bioactive lipophilic compounds from *S. integerrimus* leaves using n-hexane solvent, followed by phytochemical profiling through GC-MS and FT-IR analysis to characterise the chemical constituents. Computational studies employ PyRx with AutoDock Vina for docking, Open Babel for ligand preparation, and Discovery Studio 25 for visualisation and interaction analysis. Antibacterial activity screening was carried out by ten-fold microdilution to obtain MIC value. The results showed that a total number of 100 compounds were detected after being extracted with n-hexane; only twelve compounds were identified which were >2% of the % area peak value. The FT-IR analysis of extract confirmed the presence of bioactive phytochemical constituents, including hydroxyl, aliphatic hydrocarbons, carbonyl, ether, and unsaturated groups and the bioactive compounds identified by GC-MS were analysed in silico molecular docking studies to determine the binding affinity between ligands and proteins (*Pseudomonas* LasR and *S. aureus* gyrase). The leaves n-hexane extracts showed increased antibacterial activity at concentration 0.02 mg/mL. In conclusion, *S. integerrimus* possesses potential as an alternative medicinal plant for therapeutical utilisation, and further research is needed to explore its use in the treatment of chronic diseases.

Keywords: Antimicrobial resistance, *S. aureus*, *P. aeruginosa*, GC-MS, FT-IR, *S. integerrimus*, phytochemicals, molecular docking

Antimicrobial Resistance Factors and Characteristics of *Salmonella* from poultry intestines

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Non-typhoidal *Salmonella* is a major cause of foodborne infections worldwide, which is commonly linked to animal-derived foods, particularly poultry. Antibiotics are used to treat salmonellosis, however the rise in antimicrobial resistance (AMR) threatens treatment effectiveness. Data on intestinal *Salmonella* carriage and resistance in poultry from KwaZulu-Natal, South Africa is limited. Therefore, the aim of this study was to evaluate the prevalence and antimicrobial resistance profiles of *Salmonella* isolated from poultry intestines in KwaZulu-Natal. For this purpose,100 poultry intestinal samples were aseptically collected and analysed following ISO 6579-1:2017 guidelines. Presumptive isolates were biochemically identified and confirmed by 16S rRNA gene sequencing, and their identity verified by phylogenetic analysis. Antimicrobial susceptibility testing was performed using the Kirby–Bauer disk diffusion method. The 16S rRNA sequencing and phylogenetic analysis revealed that *Salmonella* was detected in 6% (n = 6; Confidence Intervals 2.2 - 12.5) of the samples, indicating a low but significant prevalence. All isolates showed resistance to cefuroxime, suggesting possible extended-spectrum β-lactamase (ESBL) production. All isolates were susceptible to ampicillin. These findings indicate that poultry intestines may serve as reservoirs of drug-resistant *Salmonella*. Strengthening hygienic practices during poultry processing, continuous AMR surveillance, responsible antibiotic use, and improved biosecurity measures are essential to prevent the spread of resistant *Salmonella* strains within the poultry value chain.

Keywords: *Salmonella*; Antimicrobial resistance; poultry intestines; 16S rRNA sequencing

Isolation, Screening and Identification of a Bioflocculant-producing Microorganism from Chicken Eggshells Waste

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The global issue of clean water scarcity, and the need for an eco-friendly approach to wastewater treatment have made bioflocculants promising alternatives to chemical flocculants. However, the low yield at high production cost and efficiency of microbial flocculants have limited their industrial applications. Therefore, there is a need to screen and identify novel bacteria with high bioflocculant production capacity at low production

cost. Thus, the study aimed to isolate, screen, and identify a bioflocculant-producing microorganism from chicken eggshells waste. About five isolates from chicken eggshells waste were screened for their bioflocculant-producing potential. The strain with a bioflocculant-producing potential was identified using a 16S rRNA gene sequence and showed 99.45% similarities with *Klebsiella pneumoniae* NR_117686.1. The medium composition and culture conditions were assessed against kaolin solution (4 g/L) using one- factor-at-a-time method. The most effective inoculum size for bioflocccculant production by K. pneumoniae was 1 mL (2% v/v), with 96% flocculating activity. Galactose served as the best carbon source with 96% flocculating activity while ammonium sulfate was the most effective nitrogen source with the highest flocculating activity of 86%. The optimum shaking speed was 165 rmp, with a flocculating activity of 86% and NaCl (97%) was the most suitable stimulating agent. The temperature of 35 °C and pH of 4 were optimal and they both showed 98% flocculating activity. *K. pneumoniae* produced 2.20 g/L of bioflocculant after 72 hours of fermentation. Based on findings, *K. pneumoniae* from chicken eggshells waste has a potential to produce a bioflocculant.

Keywords: Bioflocculants, Chicken eggshells, *Klebsiella pneumoniae* NR_117686.1, Optimization, Flocculating activity

Isolation, Screening and Identification of a Bioflocculant Producing Microorganism from Dead Wood Fungi in Pine Trees

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Water pollution is an issue which has grown globally, and it threatens the accessibility of clean and safe water to be consumed by humans and environmental sustainability. Conventional wastewater treatment methods, which have been effective, usually depends on chemical flocculants that are toxic, not biodegradable, and are not environmentally friendly. This study investigates the ability of a bioflocculant-producing microorganism which was isolated from decaying wood samples as a sustainable substitute to chemical flocculants. Fungal samples were collected from fallen pine trees and were then cultivated on Potato Dextrose Agar (PDA) plates to fungi isolates which can produce a bioflocculant. Screening for flocculating activity utilizing kaolin clay helped to identify isolate with the highest flocculating activity (75%), then identified using 16S rRNA gene sequencing as *Enterobacter sichuanensis* NR 179946. The study optimized multiple culture conditions to improve bioflocculant production, including inoculum size, carbon and nitrogen sources, temperature, initial pH, cations, fermentation time and shaking

speed. Optimal production was inoculum size of 1%(v/v), lactose as a carbon source, urea as a nitrogen source, pH 11, temperature 20 °C, LiCl as the cation, and a shaking speed of 110 rpm. Maximum flocculating activity reached 96% after 72 hrs of incubation under these conditions. The purified bioflocculant yield was 1.9 g/L, which is higher than other yields reported in correlated bacterial studies. These findings show that *Enterobacter sichuanensis* NR 179946 is a potential candidate for an eco-friendly bioflocculant production, which offers a biodegradable and non-toxic substitute to conventional flocculants in wastewater treatment applications.

Keywords: Flocculating activity, Decay wood samples, Kaolin suspension, *Enterobacter sichuanensis* NR179946, Bioflocculant

Isolation, Screening and Identification of a Bioflocculant-Producing Microorganism from Richards Bay Harbour, KwaZulu-Natal

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Water pollution remains a great challenge globally, with a large volume of untreated wastewater deposited into aquatic environments. Traditional chemical flocculants are effective, but they pose major risks in the environment and in human health which raises a need for a safer, biodegradable alternative such as microbial bioflocculants. The aim of this study is to isolate, screen and identify a bioflocculant-producing microorganism from marine environment of Richards Bay harbour, KwaZulu-Natal. Marine water samples were collected, serially diluted and plated on nutrient agar then further sub-cultured to obtain pure colonies. The pure colonies were screened using the kaolin clay suspension assay. Among the 20 isolates, only 3 showed the highest flocculating activity with the highest strain showing 81% of activity. Morphological and molecular identification showed the bacterium to be *Bacillus cereus* JCM 2152 with 99% similarity to reference strains on NCBI database. After optimization it showed that 5% (v/v) inoculum size, starch as carbon source and ammonium sulphate supported the highest production of bioflocculant. The strain showed maximum activity at pH 3 which indicates that it is acid tolerant. Time course assay showed that the production of a bioflocculant was growth-associated, and it showed peak activity of 97% after 72 hours of incubation. The crude bioflocculant after extraction had a yield of 3.2 g/L and the purified bioflocculants had a final yield of 2.8 g/L. The results show that the strain of *Bacillus cereus* JCM 2152 isolated from Richards Bay harbour has high bioflocculant potential and is suitable to be used as an agent for wastewater treatment.

Keywords: *Bacillus cereus* JCM 2152, Bioflocculant, Kaolin solution, Flocculating activity, Optimization, Wastewater

Isolation, Screening and Identification of Bioflocculant-Producing Microorganism from *Azadirachta indica*

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Flocculation is one of the most efficient approaches for the removal of suspended particles in wastewater treatment. Bioflocculants are gaining attention in research due to their environmental friendliness and non-toxicity compared to conventional flocculants. The present study aimed to isolate and identify a high bioflocculant-producing bacterium from *Azadirachta indica* plant. The isolate was identified using 16S rRNA gene sequencing analysis and exhibited 98.90% similarity to *Sporosarcina koreensis* and was named *Sporosarcina koreensis* NR043526.1. A phylogenetic tree based on 16S rRNA further confirmed its evolutionary relationship with other Sporosarcina species. The medium composition (carbon and nitrogen sources) and culture conditions (inoculum size, temperature, shaking speed, pH, and cultivation time) were optimized using the one-factor-at-a-time method. Under optimized culture conditions (2% v/v inoculum size, sucrose as carbon and ammonium nitrate as nitrogen sources, respectively, at 30 °C temperature, with 110 rpm shaking speed and pH 8). The bacterium produced 2.35 g/L of bioflocculant yield with a 98.90% flocculating activity after 72 hours of cultivation. In conclusion, the bacterium *S. koreensis* NR043526.1 isolated from *Azadirachta indica* has the potential to produce bioflocculant to replace synthetic flocculants in future.

Keywords: *Azadirachta indica*, *Sporosarcina koreensis*, Bioflocculant, Flocculating activity, Kaolin powder

Computational Evaluation of Herb-Drug Interactions: Assessing the Impact of LerumoTM and Air ImmuneTM Herbal Immune Boosters on Ethinylestradiol Pharmacokinetics and selected Gut Microbiome Dynamics

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Herbal supplements are being consumed worldwide alongside conventional drugs. However, because of their complex phytochemical, their ability to change the pharmacokinetics of the drugs mostly is unexplored. A synthetic estrogen called Ethinylestradiol is mostly used in oral contraceptives. The pharmacokinetics of EE when simultaneously used with herbal medicine may be altered and how its EE metabolism and transport is influenced by herbal products is still under investigations. This study aimed to investigate how LerumoTM and Air ImmuneTM herbal immune boosters interact with EE transport and how they influence selected gut microbiota (*E. coli*). Compounds from Lerumo and Air Immune were identified using GC- MS and checked for metabolism by CYP450 enzymes. Their drug-like properties were predicted using SwissADME and pkCSM, and docking was done against OATP1B1, OATP1B3, and P-gp using UCSF Chimera and AutoDock Vina. Antimicrobial activity was tested using a disk diffusion assay on *E. coli* ATCC 43888. Most of the compounds were well absorbed in the gut and interacted with P-glycoprotein, which may compete with EE at drug exit points. Molecular Docking showed strong binding to transporters (OATP1B1, OATP1B3, and P-gp), with Sutherlandiosides D and isobutyramides binding even better than EE. Antimicrobial testing showed EE was active against *E. coli* alone, but its effect dropped when mixed with herbal boosters, that highlighted that GUS activity may be promoted, increasing the recycling of EE and its increased systemic exposure. The findings highlighted the complex interaction between EE, herbal immune booster compounds, drug transporters, and selected gut microbiota, necessitating further investigations.

Keywords: Ethinylestradiol (EE), Herbal supplements, Drug transporters, Molecular docking, Gut microbiota

Targeting Host Autophagy Pathways: Computational Evaluation of Bioactive Compounds from Northern KwaZulu-Natal Medicinal Plants

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Host directed therapies (HDTs), which target host autophagy pathways, are one of the innovative therapeutic approaches needed to fight the growing threat of infectious illnesses and antimicrobial resistance (AMR) on a global scale. The phytonutrient profile of the five herbal extracts such as *Ricinus communis*, *Sarcophyte Sanguena*, *Psidium guajava*, *Pyrenacantha roseus* and *Catharanthus roseus*, is evaluated in this study. These herbal plants are native to northern KwaZulu-Natal, a region known for its extensive

ethnobotanical knowledge. The study aims to identify and analyze bioactive compounds from these medicinal plants that can potentially regulate autophagy. The most abundant compounds were chosen from each extract based on phytochemical profiling using GC-MS. These compounds were then assessed by in- silico screening against important autophagy-related proteins, including P62, Beclin-1, mTOR, LC3, ULK1 and AMPK. Some of the compounds such as Cyclododecane, 1,5,9-tris(acetoxy)-, have safety profiles and drug-likeliness characteristics. Molecular docking then revealed strong binding affinities between Cyclododecane, 1,5,9-tris(acetoxy)- compound and a variety of targets, surpassing several of the known drugs including metformin, isoniazid, hydrochlorothiazide and fluorouracil. Structural visualization of interactions using discovery studio software validated the stability of these interactions, demonstrating their potential for therapeutic use. These results demonstrate the potential of northern KwaZulu-Natal medicinal plants as a significant source of multi-target autophagy modulators, which would enable the development of novel HDT medicines to fight AMR and infectious diseases.

Keywords: Autophagy, Herbal extracts, Bioactive compounds, Autophagy-related proteins, Antimicrobial resistance (AMR)

Phytochemical characterization and in-silico screening of indigenous herbal plants from Northern KZN for autophagy modulation

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Autophagy is an intracellular process that recycles unwanted or damaged components, promoting cellular health and balance. Pharmacological activation of autophagy has *shown* potential in targeting various of diseases as host-directed therapeutic (HDT). Previous studies have shown that compounds derived from medicinal plants extract have potential in modulating autophagy. This study aims to investigate five selected indigenous herbal plants from Northen KwaZulu-Natal (*Hypoxis hemerocallidea*, *Senecio serratuloides*, *Musa acuminata*, *Strychnos madagascariensis* and *momordica balsamina*), for their potential to modulate autophagy. Plant materials were collected, prepared and extracted. The GC-MS analysis was performed. The phytochemical compounds were subjected to molecular docking against six autophagy related proteins namely LC3B, beclin-1, mTOR, AMPK, P62 and ULK1, and four known drugs (metformin, isoniazid, fluorouracil and hydrochlorothiazide) were used as controls. The toxicity and drug -likeness of the compounds was performed using ProTox prediction tool and

swissADME tool. The results revealed that viridiflorol and 7-Acetyl-2-hydroxy-2-methyl-5- isopropylbicyclononane demonstrated strong binding affinity across multiple autophagy-related protein with binding scores that were stronger than most of the control drugs. The drug-likeness and toxicity prediction confirm that these compounds have favorable ADME profile and were non-toxic. This suggests that phytochemicals from senecio serratuloides can be a valuable source of new autophagy modulators and further in-vitro and in-vivo studies are needed to explore their therapeutic potential.

Keywords: Autophagy, Indigenous herbal plants, Phytochemicals, GC-MS, Molecular docking

ISOLATION AND IDENTIFICATION OF ENDOPHYTES FROM *HELICHRYSUM ODORATISSIMUM* FOR THEIR ANTIBACTERIAL PROPERTIES

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Antimicrobial resistance is a rising global issue, increasing the demand for novel treatment methods that are cost-effective and efficient in the public health sector. The aim of this study was to identify potential antibacterial properties of endophytes of *Helichrysum odoratissimum*. Endophytes were isolated from the stem and identified using 16S rRNA sequencing as Alternaria spp. Primary screening was used to evaluate antagonistic compounds. Phytochemical screening was conducted using ethyl acetate, acetone, chloroform, distilled water, methanol, and 70% ethanol, followed by DPPH assay for antioxidant activity detection. Antibacterial activity of plant extracts was assessed using minimum inhibitory concentration (MIC). The endophytes of *H. odoratissimum* exhibited no antibacterial activity, while the plant extract showed promising antioxidant activity with ethyl acetate showing the highest IC_{50} (0.0085 mg/mL) compared to ascorbic acid, followed by acetone (0.009 mg/mL). Ethyl acetate, chloroform, and methanol extracts exhibited notable antibacterial activity against *S. aureus* and *P. aeruginosa*. Although endophytes showed no inhibition, the plant extracts demonstrated potential for new antibiotic production.

Keywords: *H. Odoratissimum*, Endophytes, Antibacterial activity, *Alternaria* spp, Plant extracts

BIOPROSPECTING ENDOPHYTES IN *HYPOXIS HEMEROCALLIDEA* FOR THEIR MEDICINAL PROPERTIES

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Medicinal plants are a rich source of natural products used to treat numerous diseases. Endophytes inhabit healthy plant tissues and produce novel bioactive compounds with antimicrobial, antioxidant, anticancer, antiparasitic, and immunosuppressive properties. This study aimed to isolate and characterize endophytes from *H. hemerocallidea* and evaluate their antibacterial and antioxidant properties. Three endophytic bacteria were isolated and identified using 16S rRNA sequencing as *Enterobacter wuhouensis*, *Pantoea cypripedii*, and *Enterobacter sichuanensis*. Antimicrobial activity was tested against *Staphylococcus aureus* and *Pseudomonas aeruginosa*. Secondary metabolites from the endophytes and plant extracts (ethanol, water, methanol, acetone, chloroform) were analyzed phytochemically, and antioxidant activity was measured via DPPH assay. The extracts exhibited antibacterial activity with MIC values ranging from 0.15–2.5 mg/mL. Distilled water extract showed maximum DPPH scavenging (84% at 6.0 mg/mL) with IC_{50} of 0.009 mg/mL, stronger than ascorbic acid. The isolated endophytes showed no antimicrobial properties; however, the plant extracts demonstrated potent antibacterial and antioxidant activity, suggesting pharmacological value.

Keywords: Endophytes, *Hypoxis hemerocallidea*, Antibacterial, Antioxidant, Secondary metabolites

Computational and Experimental Evaluation of the Effects of Herbal Immune Boosters (AirmuneTM and LerumoTM) on the Hepatic Metabolism of Ethinylestradiol-Based Oral Contraceptives

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Oral contraceptives remain one of the most widely used and effective methods of birth control worldwide, with ethinylestradiol (EE) serving as a key component in many formulations. However, the increasing global use of herbal supplements, particularly immune-boosting formulations such as AirmuneTM and LerumoTM, raises concern regarding potential herb-drug interactions that may compromise contraceptive efficacy. While intestinal herb-drug interactions have been more extensively studied, the hepatic metabolism of EE in the context of herbal co-exposure remains poorly understood. This study evaluated the phytochemical components of AirmuneTM and LerumoTM using GC-MS. computational studies, more specifically molecular docking, is used to predict physiological toxicity as well as the potential herb-drug interactions. The results were

then validated using the vivid assay was conducted to identify the inhibitory effects of herbal supplements on CYP3A4, and the HMG-CoA Reductase Assay was conducted to determine its inhibitory effects. The results obtained from Protox, molecular docking and enzymatic based experiments suggests that the collective bioactive constituents present in these herbal products might influence hepatic enzyme activity of Ethinylestradiol (EE). This study revealed that herbal immune boosters, AirmmuneTM and LerumoTM consists of bioactive constituents which can modulate hepatic enzymes responsible for the metabolism of EE. I*n-Silico* and *in-vitro* results shown that these formulations may influence CYP3A4 activity.

Keywords: Oral contraceptives, Herbal medicines, Herb-drug interactions

Profiling of Bioactive Compounds, Molecular Docking, and *In Vitro* Antimicrobial Activity of n-Hexane bark extract from *Sclerocroton integerrimus*

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Sclerocroton integerrimus bark is ethnomedicinally used to treat oral infections. This research seeks to examine the bioactive compounds with antimicrobial properties of the n- Hexane bark extract from *Sclerocroton integerrimus*. Gas-Chromatography Mass Spectrometry (GC-MS) and Fourier Transform Infrared Spectroscopy (FT-IR) were utilised for the identification, separation and analysis of the non-polar constituents of n-Hexane bark extract. Molecular docking studies were conducted to discover potential drug leads and predict the binding affinity of compounds Linoleic acid, Palmitoleic acid, and Elaidic acid to 6MVM and 3VPA. Kirby-Bauer disc diffusion and microdilution was employed to assess antimicrobial susceptibility. *In vitro* cytotoxicity was assessed on Protox 3.0. The phytochemical analysis disclosed the detection of the following secondary metabolites: Linoleic acid, Palmitoleic acid, and Elaidic acid. The test bacteria showed to be susceptible against glycopeptide, fluoroquinolone, carbapenem and aminoglycoside classes of antibiotics. The extract showed to have antimicrobial activity against test bacteria *Staphylococcus aureus* and *Pseudomonas aeruginosa*. The functional groups revealed by the FT- R analysis validated the existence of phytochemicals identified by GC-MS.

Keywords: Antimicrobial Resistance, Molecular Docking, *Sclerecroton integerrimus*

Generative AI–Enhanced Optimization of Citric Acid Production from Potato Peel Waste Using Response Surface Methodology and Separate Hydrolysis and Fermentation

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The increasing global demand for environmentally sustainable citric acid (CA) production has intensified the search for renewable, low-cost feedstocks such as agricultural residues. This study investigates the use of potato peel waste (PPW), a starch-rich by-product of the potato processing industry as a substrate for CA production by *Aspergillus brasiliensis* under separate hydrolysis and fermentation (SHF) conditions. For process modeling and optimization, a Generative Artificial Intelligence (AI) framework integrating large language models (LLMs) and retrieval-augmented generation (RAG) was employed to identify the initial optimal ranges of key parameters—pH, temperature, and fermentation time, through combined literature synthesis. These AI-predicted ranges were subsequently fine-tuned and statistically validated using Response Surface Methodology (RSM) with a Box–Behnken experimental design. The combined AI–RSM approach modeled the effects of pH (4.5–7.0), temperature (24–34 °C), and fermentation time (48–168 h) on CA concentration. The statistical analysis (ANOVA) confirmed the model's significance (R^2 = 0.91), indicating a strong fit between predicted and experimental data. Validation under the optimized conditions, pH 6.99, 24 °C, and 48 h yielded a CA concentration of 4.194 g/L, closely matching the predicted value (4.336 g/L). This hybrid framework demonstrates how Generative AI-guided process identification, when coupled with RSM-based experimental refinement, can accelerate optimization in fungal bioprocessing. The results affirm PPW as a viable, sustainable substrate for citric acid production and highlight the potential of AI-driven modelling in advancing intelligent, resource-efficient biomanufacturing.

Keywords: Citric Acid, Potato Peel Waste, *Aspergillus brasiliensis*, Separate Hydrolysis and Fermentation (SHF), Generative AI, Large Language Models (LLM), Response Surface Methodology (RSM)

Generative AI–Assisted Response Surface Methodology for Cost-Effective Production of Plant Probiotics Using Agro-Waste Substrates.

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The increasing adoption of plant probiotics in modern agriculture has become essential for enhancing soil health, suppressing soil-borne pathogens, and improving crop productivity. These are the key pillars of sustainable food security. However, large-scale production of these beneficial microbes remains challenged by high fermentation costs and inconsistent process performance. This study introduces a Generative Artificial Intelligence (AI)-assisted optimization framework that integrates large language models (LLMs) and retrieval- augmented generation (RAG) with Response Surface Methodology (RSM) to enhance the production efficiency of *Bacillus amyloliquefaciens* strains U and M, employed as biocontrol agents against *Rhizoctonia solani*. Potato peel waste (PPW) served as a sustainable, low-cost fermentation substrate, pretreated via acid and enzymatic hydrolysis to improve fermentable sugar yield. The Generative AI framework was first used to identify preliminary optimal ranges for critical process parameters—temperature (25–35 °C), pH (6.0–7.2), and fermentation time (18–48 h)—through literature-informed and data-driven inference. These ranges were subsequently refined and validated experimentally using RSM with a second- order polynomial regression model. Thirty batch fermentations were conducted under varying conditions, and analysis of variance (ANOVA) confirmed model significance (p < 0.05) with high predictive reliability (R^2 = 0.97 for strain U; R^2 = 0.90 for strain M). Optimal conditions were determined as pH 7.2, 25 °C, and 33 h for strain U (zone of inhibition: 3.75 cm) and pH 6.0, 25 °C, and 33 h for strain M (zone of inhibition: 3.25 cm). This study demonstrates that coupling Generative AI–guided parameter identification with RSM-based optimization can substantially improve process efficiency and consistency. The hybrid AI–RSM framework offers a scalable, cost-effective strategy for sustainable plant probiotic production using agro-industrial waste, advancing the development of intelligent bioprocessing systems for agricultural biocontrol applications.

Keywords: Plant Probiotics, *Bacillus amyloliquefaciens*, Potato Peel Waste, Generative AI, Large Language Models (LLM), Response Surface Methodology (RSM), Agricultural Biocontrol, Sustainable Fermentation

Optogenetically Controlled Expression of Small Laccase (SLAC) and Its Application in Dye Decolourisation: A Dual Promoter Approach

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Enzyme yield is a major challenge in the potential industrial application of the small laccase (SLAC). This study aimed to develop an optogenetic-based dual promoter system for enhanced recombinant production of SLAC. To construct the dual promoter system for optoregulated expression of SLAC, previously constructed dark-induced recombinant plasmid vector, pDusk-SLAC, and the light-induced recombinant plasmid vector, pET28a- pD-SLAC, were combined. Briefly, the SLAC gene and its light-regulated promoter pFixK2-cl-pR (pD-SLAC) from pET28a-pD-SLAC were amplified by PCR and ligated into the PshAI restriction site of pDusk-SLAC, resulting in a dual optogenetic promoter plasmid vector pDusk-SLAC-pD-SLAC. The constructed dual promoter plasmid vector was transformed into E. coli T7 Express LysY host cells for overexpression of SLAC. The expression levels and yield of SLAC were investigated and compared with controls consisting of single promoter systems of pDusk-SLAC (dark) and pET28a-pD-SLAC (light). The dual promoter system, pDusk-SLAC-pD-SLAC, cultivated under blue light, had higher specific enzyme activity (0.235 U/mg) than the control for light (0.025 U/mg) or the control for dark (0.015 U/mg). The produced enzyme was applied in dye decolourisation. The SLAC protein produced by the dual promoter system revealed significant decolourisation ability of 98.80 ± 0.751%, 86.5 ± 1.901%, and 21.5 ± 4.130% for Congo red, indigo, and methyl orange, respectively. The dual promoter system has potential for enhanced production yields of SLAC and other proteins of industrial importance.

Keywords: Optoregulation, Small laccase (SLAC), Dye decolourisation

Optimization of Growth Parameters to Increase Chitin-Glucan Complex Production from *Pleurotus sajor-caju*

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Most fungal cells contain chitin-glucan complex (CGC) in their cell wall, which is responsible for flexibility and tensile strength and protects against osmotic pressure. This complex has properties such as biocompatibility, antioxidant, and other biological activities. *Pleurotus sojor-caju* is a highly adaptable and fast-growing mushroom widely cultivated for human consumption because of its nutritional and medicinal benefits. However, its CGC extraction has not been widely studies compared to that of other

species, such as *P. ostreatus*. This study aimed to optimize the growth parameters of *P. sajor-caju* to improve biomass production in submerged fermentation. The effect of different carbon sources (molasses, and sucrose), nitrogen sources (yeast extract, malt extract, and peptone), pH levels (4.5, 6.5, and 7.5) and incubation temperatures (22, 28, and 32 ^{O}C) on biomass and CGC production was also studied. The conditions that resulted in the highest CGC yields were 20 g/L molasses, 5 g/L peptone, pH 7.5 and 32 ^{O}C. theses optimized parameters were then used in flask fermentation, and the results were compared with those obtained from the standard medium. The results of this study indicated that the amount of biomass produced is not directly proportional to the CGC yield. The results suggest that there is an opportunity to further investigate ways to improve CGC production from *P. sajor-caju*, as an increase in biomass does not directly correlate with the amount of CGC produced.

Keywords: Chitin-glucan complex, Submerged fermentation, *Pleutotus sajor-caju*, CGC

EXPLORATION OF ENDOPHYTIC BACTERIA ASSOCIATED WITH *TETRADENIA RIPARIA* AS A SOURCE OF BIOACTIVE COMPOUNDS

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Antimicrobial resistance (AMR) poses serious global health challenges, prompting investigations into endophytic microorganisms from medicinal plants that produce bioactive metabolites with therapeutic potential. This study explored endophytic bacteria associated with *Tetradenia riparia*, a medicinal plant widely recognized for its bioactive compounds. Fresh plant samples were collected from KwaDlangezwa, KwaZulu-Natal, surface sterilized, and endophytes isolated on nutrient agar. Antimicrobial activity was screened using the cross-streak method against Staphylococcus aureus and Pseudomonas aeruginosa. Phytochemical and antioxidant analyses and MIC tests were conducted using qualitative assays, DPPH free radical scavenging, and serial dilution methods. 16S rRNA gene sequencing identified isolates as *Bacillus weihenstephanensis*, *Bacillus proteolyticus*, and *Bacillus cereus*. Isolates exhibited limited antibacterial activity; however, phytochemical analysis of *T. riparia* extracts revealed alkaloids, flavonoids, phenolics, glycosides, saponins, and terpenoids. Methanol extract demonstrated an MIC of 0.63 mg/mL against *S. aureus*, and chloroform extract exhibited the same MIC against *P. aeruginosa*. Methanol extract revealed strong antioxidant activity (IC_{50} = 0.00894 mg/mL). The findings highlight poor antibacterial

activity of the isolates but notable antioxidant potential of the plant, supporting its traditional medicinal use.

Keywords: *Tetradenia riparia*, Endophytic bacteria, Antimicrobial activity, *Bacillus* spp, Antioxidant