

Examining the Effect of Fermentation Medium on the Antibiotic Activity of Fungal Extracts

by

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A Thesis Submitted in Partial Fulfillment of the Requirements for the Degree of

Bachelor of Science with Honours in Biology-Psychology

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THE UNIVERSITY OF NEW BRUNSWICK

April 2025

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Abstract

Fermentation medium can influence the production of bioactive compounds by endophytes, affecting their antimicrobial properties. This study employed a panel of 10 microbial pathogens to examine the antimicrobial activity of extracts derived from 35 endophytes grown in potato dextrose broth (PDB) and malt extract broth (MEB). Factorial ANOVAs showed that extract bioactivity was significantly influenced by growth medium ($p < 0.001$ for 31 isolates) and pathogen ($p < 0.001$ for all isolates) and revealed a significant interaction between these factors ($p < 0.001$ for all isolates). Extracts of isolates grown in PDB generally exhibited greater activity in our bioassays, suggesting that it promotes the production of antimicrobial compounds. However, no consistent trend linked medium-dependant activity to pathogen type, indicating that metabolite production is also influenced by the metabolic ability of individual endophytes. These findings highlight the importance of selecting an appropriate fermentation medium to maximize the bioactivity of endophyte extracts.

Acknowledgements

I want to express my sincere gratitude to my supervisor, Dr. Christopher Gray, for his guidance and support throughout this project.

I also extend my appreciation to Dr. Rémy Rochette for his valuable direction in determining the appropriate statistical analyses to implement in this research.

Additionally, I thank Dr. Kurt Samways for his insightful feedback and encouragement.

A special thanks to the Natural Products Research Group graduate students Janet Debly and Ally Bos for their assistance and support throughout this process. I greatly appreciate their willingness to share their knowledge and expertise.

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List of Symbols, Nomenclature, or Abbreviations

ATCC	American Type Culture Collection
BGC	Biogenic gene cluster
DMSO	Dimethyl sulfoxide
CFU	Colony-forming unit
C. a	<i>Candida albicans</i>
df	Degrees of freedom
E. c	<i>Escherichia coli</i>
E. f	<i>Enterococcus faecium</i>
MEB	Malt extract broth
mg	Milligram
ml	Millilitre
MRSA	Methicillin resistant <i>Staphylococcus aureus</i>
M. s	<i>Mycobacterium smegmatis</i>
M. t	<i>Mycobacterium tuberculosis</i>
nm	Nanometer
OD	Optical density
PDB	Potato dextrose broth
P. a	<i>Pseudomonas aeruginosa</i>
S. a	<i>Staphylococcus aureus</i>
S. c	<i>Saccharomyces cerevisiae</i>
VRE	Vancomycin resistant <i>Enterococcus faecium</i>
Vs.	Versus
°C	Degrees Celsius
μL	Microlitre
μm	Micrometer

Introduction

Endophytes are fungi that reside within plant tissues and benefit their host by producing biomolecules known as natural products or secondary metabolites (Gouda et al., 2016; Reshi et al., 2023). These compounds play vital roles in plant defence, communication, competition, and responses to environmental stress (Gouda et al., 2016; Reshi et al., 2023). Natural products encompass various classes of chemical compounds, such as alkaloids, terpenoids, and polyketides, among others, which exhibit wide-ranging biological activities (Huang et al., 2022; Khosla, 1997). Beyond their ecological roles involving defence and signalling, natural products have played a pivotal role in drug discovery, with notable examples including taxol from the endophytic fungus *Taxomyces andreanae*, and citreoviridin derived from the endophytic fungus *Penicillium citreonigrum* (Chaachouay et al., 2024; Zou et al., 2022). These examples highlight the potential of natural products derived from endophytes in addressing medicinal and ecological challenges.

Fungi synthesize a wide array of natural products through various metabolic pathways, such as polyketide, non-ribosomal protein synthesis, and alkaloid biosynthesis pathways (Bhattarai et al., 2021). The production of these secondary metabolites is regulated by biosynthetic gene clusters (BGCs), which are groups of genes that encode the enzymes, regulatory proteins, and transporters necessary for their synthesis (Amoretti et al., 2002; Atanasov et al., 2021). Some fungi harbour dozens of BGCs, with each cluster typically specialized to produce a specific type of metabolite (Rokas et al., 2020). However, the production of metabolites is not uniform; environmental conditions, including nutrient availability and culture media, can modulate which BGCs are

activated, thereby influencing the range and diversity of metabolites synthesized (Amoretti et al., 2002; Atanasov et al., 2021).

The diversity of secondary metabolites produced by BGCs arises from several factors, including tailoring enzymes, promiscuity of core enzymes, modular assembly lines, and gene regulation, which is influenced by environmental signals or culture conditions (Fischbach et al., 2008; Geller-McGrath et al., 2023). Some clusters share or have overlapping functions, producing different metabolites when enzymes or intermediates from one pathway in a cluster interact with another (Castellana et al., 2014). In other cases, hybrid clusters that encode distinct biosynthetic pathways within a single cluster can lead to the synthesis of hybrid products (Fischbach et al., 2008). This biosynthetic flexibility highlights the dynamic nature of secondary metabolite production and suggests that varying environmental factors could influence which BGCs are activated and consequently the range of metabolites produced by fungi.

Despite the potential for diverse metabolite production, a significant portion of the biosynthetic capacity contained in the fungal genome can remain hidden or dormant under standard growth conditions due to the presence of silent or cryptic BGCs (Hoskisson et al., 2020; Lim et al., 2012). Silent BGCs encode secondary metabolites that are not actively produced unless triggered by specific stimuli (Amoretti et al., 2002; Atanasov et al., 2021). This silence is often regulated by evolutionary and metabolic mechanisms that conserve energy and resources, ensuring production occurs only under necessary circumstances (Amoretti et al., 2002; Nützmann et al., 2018). The regulation of energy and enzymatic activity required to activate these clusters underscores the importance of strategic resource allocation in metabolism (Amoretti et al., 2002;

Nützmann et al., 2018). Cryptic BGCs, a subset of silent clusters, often remain obscure and poorly characterized, requiring novel techniques to activate or express them (Reen et al., 2015). These clusters may produce minimal amounts of metabolite under standard conditions or encode entirely unknown functions (Lim et al., 2012). Fully activating silent and cryptic clusters could reveal a wealth of untapped natural products with significant pharmaceutical and ecological potential.

Nutrient availability plays a pivotal role in the production of bioactive secondary metabolites synthesized by biosynthetic gene clusters (Reshi et al., 2023). The One Strain Many Compounds (OSMAC) approach offers a promising strategy to address the challenges posed by silent and cryptic BGCs (Schwarz et al., 2021; Zhang et al., 2024). By altering culture conditions, such as nutrient composition, pH, temperature, or aeration, this method activates different BGCs within a single strain and uncovers a broader spectrum of secondary metabolites (Reshi et al., 2023; Schwarz et al., 2021). For example, glucose-rich media can suppress secondary metabolite production through catabolite repression, where cells prioritize preferred energy sources like glucose and repress genes involved in metabolizing less-preferred substrates, often leading to silent BGCs (Schwarz et al., 2021). In contrast, nitrogen-limited media frequently enhance the expression of these clusters and boosts metabolite production (Ibrahim et al., 2011; Schwarz et al., 2021). Other environmental factors such as pH, temperature, and light exposure further influence the activation of BGCs and, consequently, secondary metabolite production (Amoretti et al., 2002). Additionally, microbial competition can trigger stress responses in endophytes, activating defensive or competitive BGCs with antibiotic properties (Chaudhary et al., 2022; Verma et al., 2022). These findings

underscore the potential of silent BGCs as a reservoir of bioactive compounds and emphasize the importance of optimizing growth media and cultivation conditions to maximize the discovery and production of natural products with pharmaceutical and agricultural applications.

In my study, endophytes were cultivated in liquid media to allow them to produce natural products; ethyl acetate was then used to extract the compounds that the fungi produced, and the extracts were tested for activity against various microbial pathogens. The choice of growth medium is significant, as it includes different sources of nutrients that influence the metabolic pathways activated by endophytes, ultimately affecting the production of bioactive compounds (Ali et al., 2011; Gakuubi et al., 2021). My research aims to answer the question: Does the choice of growth medium influence the antibiotic activity of fungal extracts against specific pathogens? Specifically, this study compares the bioactivity profiles of fungal extracts produced in two different growth media: potato dextrose broth (PDB) and malt extract broth (MEB). It is hypothesized that fungi will produce extracts that exhibit contrasting bioactivity profiles depending on the growth medium used. Fungi grown in both MEB and PDB are expected to produce extracts with varying levels of activity when tested against ten different pathogens. By analyzing and comparing the bioactivity profiles of these extracts, my study aims to determine whether cultivation conditions influence the antimicrobial potential of the natural products produced.

Methods

Biological Assay Procedures

Antifungal Bioassay

Antifungal activity against *C. albicans* (ATCC 14053) and *S. cerevisiae* (ATCC 9763) was evaluated using a microbroth dilution antibiotic susceptibility assay modified from McCulloch et al. (2019). Immediately prior to use, stock solutions of fungal extracts (5.0 mg/mL) were prepared in sterile-filtered dimethyl sulfoxide (DMSO, 40 μ L) and diluted with either Difco Sabouraud dextrose broth (*C. albicans*, 960 μ L) or yeast mold broth (*S. cerevisiae*, 960 μ L) (Becton Dickinson, Mississauga, Ontario). The resulting test solutions (100 μ L; 4% DMSO) were transferred to the non-peripheral wells of a clear, non-tissue cultured 96-well microtiter plate in triplicate (BD Falcon, Becton Dickinson, Mississauga, Ontario). Each plate contained three positive control wells (*C. albicans*: nystatin, 5.0 μ g/mL; *S. cerevisiae*: amphotericin B, 5.0 μ g/mL, 100 μ L per well). Wells were then inoculated with suspensions of either *C. albicans* or *S. cerevisiae* (100 μ L; 1×10^6 colony forming units [CFU]/mL), to obtain a cell density of 5×10^5 CFU/mL. Sterile water (200 μ L) was added to all perimeter wells to reduce evaporation from experimental wells. Each plate contained three negative control wells (4% DMSO in appropriate broth [100 μ L] inoculated with appropriate fungi [100 μ L; 1×10^6 CFU/mL]) and three untreated blank wells (2% DMSO in appropriate broth [200 μ L]). Initial and final optical densities (OD) were determined for each well by recording absorbance at 600 nm immediately before and after incubation for 24 hours at 37°C using a Molecular Devices Emax microplate reader (Molecular Devices; Sunnyvale, CA, USA). Initial OD readings were subtracted from the final readings for each well to obtain the change in OD (Δ OD).

Δ OD values were corrected for background absorbance of the media by subtracting the mean Δ OD readings of the blanks from the mean Δ OD readings of the control and test wells. The percentage inhibition of fungal growth is defined as: $[1 - (\text{mean test or positive control } \Delta\text{OD}/\text{mean negative control } \Delta\text{OD})] \times 100$

Antibacterial Bioassay

Antibacterial activity against *S. aureus* (ATCC 29213), *P. aeruginosa* (ATCC 10145), *E. coli* (ATCC 25922), and *E. faecium* (ATCC 35667) was evaluated in the same manner as described for antifungal assay. The growth medium used for *P. aeruginosa*, *E. coli*, and *S. aureus* was BBL Mueller Hinton II cation adjusted broth (Becton Dickinson, Mississauga, Ontario), whereas BBL Brain Heart Infusion broth (Becton Dickinson, Mississauga, Ontario) was used for *E. faecium*. Positive controls consisted of a triplicate concentration of antibiotic (1.25 $\mu\text{g}/\text{mL}$, erythromycin for *S. aureus*; 20 $\mu\text{g}/\text{mL}$ and 2.5 $\mu\text{g}/\text{mL}$, gentamicin for *P. aeruginosa* and *E. coli*, respectively; 1.25 $\mu\text{g}/\text{mL}$, tetracycline for *E. faecium*; 100 μL per well) inoculated with a suspension of the appropriate pathogen (100 μL).

Antimycobacterial Bioassay

Antimycobacterial activity against *M. tuberculosis* strain H37Ra (ATCC 25177) and *M. smegmatis* (ATCC 70084) was evaluated using a microplate resazurin assay according to O'Neill et al. (2020). Immediately prior to use, stock solutions of fungal extracts were prepared at the desired concentration in sterile-filtered DMSO (40 μL) and were diluted with modified Middlebrook 7H9 broth (960 μL , BBL MGIT, Becton Dickinson, Mississauga, Ontario). The resulting test solutions (100 μL) were transferred to the non-peripheral wells of a black non-tissue culture treated, low-binding, 960-well microtiter

plate (VWR, Mississauga, Ontario) in triplicate and inoculated with suspensions of the appropriate organism (100 μ L) of cell density 2.0×10^6 cells/mL. Sterile water (200 μ L) was added to all perimeter wells to reduce evaporation from experimental wells. The positive control consisted of rifampin (*M. tuberculosis*, 0.02 μ g/mL) or ciprofloxacin (*M. smegmatis*, 2.5 μ g/mL). In addition to the positive controls, negative controls (4% DMSO in modified Middlebrook 7H9 broth [100 μ L] inoculated with suspensions of the appropriate organism [100 μ L]), blanks (2% DMSO in modified Middlebrook 7H9 broth [200 μ L]), and test solutions [100 μ L] with modified Middlebrook 7H9 broth [100 μ L]) were included in triplicate in each plate. *M. smegmatis* plates were incubated for 1 day (37°C) and *Mycobacterium tuberculosis* plates were incubated (37°C; 5% CO₂) for three days, in a humid environment. Following incubation, a solution of resazurin (0.0625 mg/mL) in aqueous Tween 80 (50 μ L) was added to all non-peripheral wells. Plates were then incubated for a further 24 hours, sealed with an adhesive polyester film (50 μ m; VWR, Mississauga, Ontario), and mycobacterial growth was assessed fluorometrically at 37°C (Molecular Devices Gemini EM dual-scanning microplates spectrofluorometer with a 530 nm excitation filter and a 590 nm emission filter operating in top-scan mode). Fluorescence values were corrected for any background fluorescence of the media and test samples by subtracting mean fluorescence readings of the appropriate blanks from the mean fluorescence readings of the control and test wells. The percentage inhibition of mycobacterial growth was then defined as: $[1 - (\text{mean test or positive control fluorescence} / \text{mean negative control fluorescence})] \times 10$

Statistical Analysis

Factorial ANOVAs were used to assess the effects of growth medium (PDB vs. MEB) on the bioactivity of the extracts obtained from each isolate. Simple main effects tests were also conducted to evaluate if the impact of growth medium varied depending on the pathogen being tested. This analysis indicated whether the impact of the growth medium was consistent across all pathogens or if certain pathogens responded differently to extracts derived from different media. To evaluate whether the distribution of bioactivity results deviated from an expected pattern, a Chi-squared goodness-of-fit test was performed. This test was applied after both the factorial ANOVA and simple main effects tests to assess whether observed bioactivity outcomes differed significantly from what would be expected under the assumption of no preferential bioactivity distribution across conditions. This method provides a straightforward way to evaluate overall trends while maintaining statistical rigour. Statistical significance was assessed at a p-value threshold of 0.05, and all analyses were conducted using SPSS.

Results

The bioassay results for each isolate extract (comprising a total of 700 bioassays performed in triplicate) are presented numerically in Table 1 and graphically as bioactivity profiles in the appendix (Figure 4). Factorial ANOVAs were conducted for each isolate to determine whether the bioactivity of the extracts was significantly influenced by the growth medium and/or the pathogen being tested. Table 2 summarizes the results of the factorial ANOVAs (the full ANOVA tables are provided in the appendix as Tables 4 – 38), which revealed that bioactivity was significantly affected by the fermentation medium. Specifically, 31 out of the 35 isolates exhibited differences at the $p < 0.001$ level; one isolate at $p < 0.05$ and three isolates showed no significant differences. This indicates that the choice of growth medium (PDB vs. MEB) has a major impact on bioactivity for most isolates.

Additionally, extract bioactivity varied significantly depending on the pathogen, with all 35 isolates displaying significant differences at the $p < 0.001$ significance level. This suggests that the effectiveness of each isolate's extract differs across pathogens, some may be more susceptible (strong inhibition), while others may be more resistant (weak or no inhibition). The interaction between pathogen and growth medium was also significant for all 35 isolates at the $p < 0.001$ significance level, indicating that the effect of growth medium on bioactivity depends on the specific pathogen being tested. In other words, an isolate's extract may better inhibit a particular pathogen in one medium but exhibit increased inhibition in another medium against a different pathogen. This highlights how bioactivity profiles can change based on fermentation medium, influencing different pathogens in distinct ways.

Table 1: Mean inhibition values (\pm standard error of the mean) for isolate extracts tested in triplicate against each pathogen. For each isolate – pathogen combination, extracts with significantly increased inhibition based on the results of simple main effects tests are shown in bold. Where there was no significant difference between extract bioactivities the values are underlined.

Isolate	Extract	C. a	S. c	S. a	P. a	E. c	E. f	MRSA	VRE	M. t	M. s	Winner
<i>KF2-175AQ</i>	MEB	<u>-6.8(±1.7)</u>	<u>2(±0.9)</u>	<u>-27.2(±3.6)</u>	<u>10.2(±0.5)</u>	<u>-8.3(±3.9)</u>	<u>-46(±2.5)</u>	<u>-6.6(±2.3)</u>	<u>-12.4(±0.8)</u>	<u>56.4(±1.6)</u>	<u>48.8(±1.7)</u>	None
	PDB	<u>5.3(±0.6)</u>	<u>10.6(±2.5)</u>	<u>-9.4(±3.9)</u>	<u>11.7(±0.9)</u>	<u>-11.4(±3.1)</u>	<u>-10.7(±5)</u>	<u>18.9(±2.2)</u>	<u>-0.5(±1.9)</u>	<u>4.4(±1.7)</u>	<u>-9.2(±0.5)</u>	
<i>KF2-175AI</i>	MEB	-8.5(±2.5)	<u>-1(±2)</u>	-0.1(±0.1)	-15.5(±2.7)	<u>-1.3(±0.6)</u>	-56.4(±3.2)	18.6(±1.4)	-10.5(±2)	<u>53.3(±1.4)</u>	<u>-1.5(±1.2)</u>	PDB
	PDB	2.9(±0.4)	<u>5.3(±1.4)</u>	21.1(±2.3)	9(±2.1)	<u>-4.4(±1)</u>	-16.7(±1.4)	45.5(±1)	10.4(±1.4)	<u>52.3(±0.8)</u>	<u>0.6(±2)</u>	
<i>KF2-175AV</i>	MEB	-4.4(±0.4)	<u>12.2(±0.8)</u>	5.9(±0.6)	<u>11.1(±3.9)</u>	-33.3(±2.1)	-35.3(±3.9)	-3.1(±3.1)	<u>-9.1(±3.7)</u>	<u>47.4(±0.7)</u>	2.5(±0.4)	PDB
	PDB	2.9(±2)	<u>13(±3)</u>	59.5(±1.3)	<u>1.9(±0.2)</u>	-47.7(±0.8)	-10.3(±1.2)	58.8(±1.3)	<u>-6.5(±2.9)</u>	<u>39.7(±1.9)</u>	20(±0.3)	
<i>KF2-175AW</i>	MEB	-4.7(±1.3)	<u>6.8(±3.7)</u>	-22.9(±1.4)	<u>8.8(±2.3)</u>	<u>-9.7(±2.3)</u>	-31.8(±1.4)	-8(±4.8)	<u>-2.6(±3.2)</u>	43.9(±2.9)	-0.8(±0.3)	PDB
	PDB	6.8(±1.5)	<u>7.8(±2.5)</u>	-2.4(±1.3)	<u>-7.7(±6.9)</u>	<u>-25.3(±7.3)</u>	-1.2(±2.1)	24(±1.5)	<u>5.4(±1.2)</u>	72(±1.2)	-6.5(±1.3)	
<i>KF2-175AX</i>	MEB	<u>1.8(±3.1)</u>	-12.3(±1.7)	-30(±1.5)	<u>14.9(±1.2)</u>	-32.5(±2.1)	10.7(±2.6)	<u>7.3(±3.6)</u>	-12.1(±1.6)	2.8(±1.7)	<u>-16.7(±1.6)</u>	PDB
	PDB	<u>10.3(±0.5)</u>	11.1(±3.7)	3.3(±2.2)	<u>12(±1.7)</u>	-10.7(±4.2)	-8.9(±3.2)	<u>7.4(±1.5)</u>	1.4(±0.5)	37.7(±6.7)	<u>-14.5(±0.3)</u>	
<i>KF2-175AR</i>	MEB	<u>-0.5(±0)</u>	<u>-0.2(±0.8)</u>	<u>-5.7(±3)</u>	<u>30.8(±1.3)</u>	<u>-32.8(±3.4)</u>	<u>4.9(±4.1)</u>	<u>11.5(±3.4)</u>	<u>0.5(±0.5)</u>	<u>67.8(±0.5)</u>	<u>3.6(±0.6)</u>	None
	PDB	<u>6.2(±1)</u>	<u>0.9(±4.8)</u>	<u>21.5(±4.1)</u>	<u>-16.9(±0.3)</u>	<u>-0.7(±0.1)</u>	<u>-5.4(±0.7)</u>	<u>53(±4.2)</u>	<u>8.6(±3)</u>	<u>34.8(±7)</u>	<u>-3.3(±1)</u>	
<i>KF2-175AT</i>	MEB	<u>-1.9(±2.2)</u>	<u>14.1(±1.7)</u>	<u>59(±4.1)</u>	<u>16.2(±4.5)</u>	<u>-4.8(±2.4)</u>	<u>-40.2(±2.5)</u>	<u>51.9(±1)</u>	<u>-0.1(±2)</u>	<u>99.5(±0.2)</u>	<u>70.2(±0.7)</u>	None
	PDB	<u>7.1(±1.4)</u>	<u>12.8(±1.6)</u>	<u>75.5(±1.9)</u>	<u>5.5(±1.8)</u>	<u>-22.1(±3.8)</u>	<u>-12.3(±3.4)</u>	<u>75.2(±3.3)</u>	<u>0.9(±2.7)</u>	<u>100.8(±0)</u>	<u>24.5(±2.6)</u>	
<i>KF2-175AG</i>	MEB	8.7(±1.2)	11.1(±1.3)	65.5(±3.5)	18.6(±0.3)	7.8(±3.3)	-15.5(±1)	66.5(±4.2)	-10.3(±0.5)	12.2(±1.1)	1.1(±1.4)	MEB
	PDB	-4.4(±0.3)	-13(±3.3)	45.7(±1.7)	-1(±1.1)	-66.3(±2)	-23.7(±1.8)	53(±2.5)	-1.3(±2.5)	64.7(±0.8)	27.5(±1.2)	
<i>KF2-175N</i>	MEB	-4.8(±2.7)	-29(±0.9)	100.9(±0.2)	<u>0.2(±2.7)</u>	<u>-6.4(±2.8)</u>	100.2(±0.2)	96.7(±0.7)	99.1(±0.1)	69.3(±1.6)	89(±0.5)	TIE
	PDB	6.5(±2.5)	17.7(±1.9)	100(±0.1)	<u>6.1(±1.8)</u>	<u>-7.9(±1.6)</u>	16.1(±1.3)	100.7(±0.2)	59.4(±4.4)	97.9(±0.2)	-2.1(±3.4)	

Table 1: Mean inhibition values (\pm standard error of the mean) for isolate extracts tested in triplicate against each pathogen.

Isolate	Extract	C. a	S. c	S. a	P. a	E. c	E. f	MRSA	VRE	M. t	M. s	Winner
<i>KF2-175A</i>	MEB	5.8 (\pm 0.8)	<u>16 (\pm0.4)</u>	<u>98.4 (\pm0.2)</u>	<u>9.9 (\pm4)</u>	<u>-10.2(\pm0.3)</u>	5.1 (\pm 1.2)	<u>92.6 (\pm3.8)</u>	16.8 (\pm 1.8)	98.2 (\pm 0.1)	-14.4(\pm 0.6)	PDB
	PDB	81.7 (\pm3.3)	<u>23.4 (\pm3.8)</u>	<u>97.7 (\pm0.4)</u>	<u>8.9 (\pm2)</u>	<u>-4.8 (\pm3)</u>	97.5 (\pm0.2)	<u>97.5 (\pm0.5)</u>	97.7 (\pm0.2)	99.7 (\pm0)	12.8 (\pm5.5)	
<i>KF2-175AH</i>	MEB	2.3 (\pm 0.5)	7.5 (\pm 1.2)	<u>99.4 (\pm0.6)</u>	<u>6.5 (\pm2.5)</u>	<u>-7.6 (\pm2.1)</u>	97.4 (\pm0.1)	<u>97.3 (\pm0.1)</u>	96.1 (\pm0.3)	99.4 (\pm 0)	-16.4(\pm 0.7)	PDB
	PDB	7.9 (\pm1.3)	21.4 (\pm2.1)	<u>98.3 (\pm0)</u>	<u>6.7 (\pm0.5)</u>	<u>-12 (\pm4.7)</u>	6.7 (\pm 3)	<u>97.5 (\pm0.7)</u>	36.2 (\pm 7)	99.8 (\pm0)	13.4 (\pm4.6)	
<i>KF2-175W</i>	MEB	-1.6 (\pm 0.6)	-8.1 (\pm 2.1)	54.2 (\pm 3.4)	8.8 (\pm 1.4)	-14.3(\pm 1.1)	97.6 (\pm0.3)	99.4 (\pm1)	-4.5 (\pm 4.4)	99.9 (\pm0.2)	-17.1(\pm 0.1)	PDB
	PDB	1.3 (\pm0.6)	9.7 (\pm4.2)	97.4 (\pm5)	19.8 (\pm1.7)	5.5 (\pm1.4)	10.3 (\pm 2.7)	88 (\pm 1.4)	33.2 (\pm3)	98.9 (\pm 0.1)	7.4 (\pm1.1)	
<i>KF2-175AO</i>	MEB	2.2 (\pm 2.1)	<u>-2.6 (\pm2.2)</u>	66.9 (\pm 1.5)	<u>8.2 (\pm0.9)</u>	-20 (\pm 3.9)	<u>97.5 (\pm0.2)</u>	97.1 (\pm 0.1)	13.5 (\pm 4)	99.1 (\pm0.1)	-16.2(\pm 2.1)	PDB
	PDB	33.7 (\pm2.3)	<u>11.7 (\pm4.8)</u>	99.7 (\pm1.7)	<u>11 (\pm2.4)</u>	7.1 (\pm0.7)	<u>97.7 (\pm0.3)</u>	102 (\pm0.1)	98.9 (\pm0.4)	99.5 (\pm 0.1)	13.4 (\pm5.3)	
<i>KF2-175BF</i>	MEB	<u>2.3 (\pm1.5)</u>	-4.5 (\pm 1.7)	-34.9(\pm 3.7)	5.5 (\pm 0.5)	-10.8(\pm 0.1)	<u>0.1 (\pm1.4)</u>	8.6 (\pm 3.9)	-13 (\pm 4.3)	41.1 (\pm 1.4)	<u>10.7 (\pm0.9)</u>	PDB
	PDB	<u>3.7 (\pm1)</u>	22 (\pm4.8)	19.8 (\pm3.7)	17.3 (\pm0.9)	1.3 (\pm0.7)	<u>4.3 (\pm1.4)</u>	45.6 (\pm0.6)	2.7 (\pm2.1)	97.7 (\pm0.1)	<u>3.4 (\pm3.3)</u>	
<i>KF2-175AZ</i>	MEB	-3.5 (\pm 0.7)	-18.1(\pm 2.3)	-29.1 (\pm 4)	<u>3.9 (\pm2.2)</u>	-8.6 (\pm 0.9)	<u>-8.1 (\pm3)</u>	-10 (\pm 3.4)	<u>-4.3 (\pm1.4)</u>	60.7 (\pm 1.8)	-18.2(\pm 0.2)	PDB
	PDB	1.6 (\pm1.2)	13.2 (\pm1.6)	-6.8 (\pm1.9)	<u>7 (\pm1.1)</u>	1.4 (\pm0.5)	<u>-11.7(\pm0.7)</u>	4.2 (\pm2.5)	<u>-6.5 (\pm1.1)</u>	97.1 (\pm0.3)	7.5 (\pm0.8)	
<i>KF2-175G</i>	MEB	3.1 (\pm 0.6)	<u>-3 (\pm2.3)</u>	98.2 (\pm0.1)	6.7 (\pm3.1)	5.9 (\pm0.9)	37.7 (\pm 0.6)	<u>96.9 (\pm0.7)</u>	87.8 (\pm 1)	97.9 (\pm 0.1)	-15.5(\pm 0.1)	PDB
	PDB	86.4 (\pm1)	<u>2.3 (\pm1)</u>	92.6 (\pm 1)	-13 (\pm 4.6)	2.4 (\pm 0.7)	93.9 (\pm0.7)	<u>98.8 (\pm1.4)</u>	96.9 (\pm0.8)	98.9 (\pm0.1)	-5.9 (\pm1.3)	
<i>KF2-175S</i>	MEB	1.7 (\pm 0.7)	<u>5.2 (\pm3.5)</u>	<u>101.9 (\pm1.8)</u>	4.1 (\pm 2.8)	13.1 (\pm1)	97.5 (\pm0.2)	98.6 (\pm 0.2)	97 (\pm 1.1)	99.6 (\pm0.2)	-19 (\pm 0.4)	TIE
	PDB	9 (\pm0.5)	<u>6.6 (\pm3.1)</u>	<u>97.6 (\pm0.2)</u>	13.6 (\pm1.6)	-6.1 (\pm 0.9)	22.1 (\pm 3.1)	<u>98.7 (\pm0.5)</u>	<u>95.7 (\pm0.2)</u>	97.2 (\pm 0.2)	7.4 (\pm0.9)	
<i>KF2-175M</i>	MEB	<u>-1.1 (\pm1.7)</u>	-15.4(\pm 0.5)	98.8 (\pm0.3)	<u>-10.6(\pm3.9)</u>	-1.2 (\pm 1.3)	<u>12.6 (\pm4.9)</u>	97.7 (\pm0.3)	54.6 (\pm2.8)	81.9 (\pm 1)	91.6 (\pm1)	MEB
	PDB	<u>4.1 (\pm1)</u>	-1.7 (\pm0.2)	11.4 (\pm 3)	<u>1.1 (\pm6.5)</u>	9.2 (\pm0.6)	<u>-0.5 (\pm0.9)</u>	47 (\pm 3.7)	7.6 (\pm 2.1)	85.4 (\pm0.4)	4.9 (\pm 1.6)	

Table 1: Mean inhibition values (\pm standard error of the mean) for isolate extracts tested in triplicate against each pathogen.

Isolate	Extract	C. a	S. c	S. a	P. a	E. c	E. f	MRSA	VRE	M. t	M. s	Winner
<i>KF2-175BS</i>	MEB	-8.1 (\pm 0.6)	6.2 (\pm 2.8)	-33.5(\pm 2.9)	7.8 (\pm0.8)	-30.6(\pm 0.4)	-31.3(\pm 4.6)	-25.2 (\pm 4.2)	-15.4(\pm 4.1)	17.2 (\pm 0.9)	8.4 (\pm0.6)	PDB
	PDB	100.7 (\pm0.2)	93.1 (\pm0.1)	42.8 (\pm1.3)	-21.6(\pm 3.5)	3.3 (\pm0.3)	9.9 (\pm2.5)	16.3 (\pm1.1)	24 (\pm0.6)	84.9 (\pm3.3)	2.2 (\pm 1.6)	
<i>KF2-175C</i>	MEB	86.2 (\pm1.4)	99 (\pm0.1)	-24.5(\pm 0.3)	-10.8(\pm 1.4)	<u>-8.4 (\pm2.9)</u>	-21 (\pm 1.9)	1.8 (\pm 2.6)	<u>-5.7 (\pm1.9)</u>	61.5 (\pm1.4)	101.5 (\pm0.9)	TIE
	PDB	-0.6 (\pm 0.3)	25.3 (\pm 2.4)	12 (\pm3)	5.3 (\pm1)	<u>-1.5 (\pm0.5)</u>	-1.2 (\pm0.7)	25.5 (\pm1.4)	<u>-0.2 (\pm2.1)</u>	-4.2 (\pm 0.5)	-1.6 (\pm 2.2)	
<i>KF2-175BC</i>	MEB	-4 (\pm 0.9)	0.6 (\pm 0.4)	18 (\pm 2)	11.9 (\pm 1.4)	-3.9 (\pm 2.4)	-1.3 (\pm1.3)	12.4 (\pm 4.6)	-5.1 (\pm 0.5)	25.6 (\pm 1.4)	79.1 (\pm2)	PDB
	PDB	94.4 (\pm0.4)	93.3 (\pm0.7)	57.5 (\pm6.9)	26.1 (\pm0.6)	32.6 (\pm0.5)	-14.1(\pm 3.2)	84.3 (\pm1.6)	-1.3 (\pm0.7)	65.1 (\pm1)	30 (\pm 1.1)	
<i>KF2-175BA</i>	MEB	-0.4 (\pm 0.5)	<u>19.5 (\pm2.2)</u>	<u>48.7 (\pm1.5)</u>	<u>16.1 (\pm1.4)</u>	-24.7(\pm1.9)	-41.7(\pm 1.8)	<u>56.1 (\pm2.7)</u>	-19 (\pm 2.4)	58.9 (\pm 1.3)	8.5 (\pm 2)	PDB
	PDB	2.7 (\pm0.2)	<u>15.7 (\pm3.7)</u>	<u>43.3 (\pm1.7)</u>	<u>11.4 (\pm1)</u>	-39.7(\pm 1.9)	-10.4(\pm1.7)	<u>55 (\pm0.5)</u>	-3.3 (\pm1.8)	78.8 (\pm1.8)	26.4 (\pm0.7)	
<i>KF2-175AK</i>	MEB	<u>0.4 (\pm1.6)</u>	-16.4(\pm 2.2)	-20.8(\pm 4.8)	3.9 (\pm 1)	-4.1 (\pm 1.1)	17.1 (\pm0.9)	10.1 (\pm 3.7)	<u>1.6 (\pm1.7)</u>	10.9 (\pm 4)	-12.4(\pm 1.6)	PDB
	PDB	<u>8.5 (\pm2.5)</u>	17.3 (\pm3.4)	-1.9 (\pm3.4)	7.2 (\pm0.1)	10.6 (\pm1.4)	-5.3 (\pm 2.1)	21.3 (\pm0.9)	<u>-0.7 (\pm1.8)</u>	98.1 (\pm0.1)	10.7 (\pm1.1)	
<i>KF2-175Y</i>	MEB	<u>-1.1 (\pm1.2)</u>	-7.9 (\pm 1.6)	-18.5 (\pm 2)	<u>10.4 (\pm1.5)</u>	-8.6 (\pm 2.3)	-7.5 (\pm0.6)	10.2 (\pm 2.8)	<u>-2.8 (\pm0.3)</u>	63.1 (\pm 2)	-8.9 (\pm 0.6)	PDB
	PDB	<u>1 (\pm2.4)</u>	20 (\pm1.4)	3.7 (\pm4.4)	<u>9.6 (\pm0.9)</u>	15.7 (\pm1.6)	-12.8. (\pm 1)	23.6 (\pm3.9)	<u>-3.9 (\pm1)</u>	98.7 (\pm0.3)	7.8 (\pm1.1)	
<i>KF2-175I</i>	MEB	<u>-1.3 (\pm1.4)</u>	-10.7(\pm 1.6)	-28.5(\pm 0.7)	<u>-1.3 (\pm2.5)</u>	-2.2 (\pm 2.5)	-12.8(\pm 3.6)	-17.7 (\pm 1.3)	<u>2.1 (\pm2.4)</u>	-15.1(\pm 0.4)	-20.9 (\pm 2.1)	PDB
	PDB	<u>-0.5 (\pm0.5)</u>	12 (\pm1.2)	13.3 (\pm2.6)	<u>7 (\pm1.9)</u>	10.4 (\pm1.2)	2.6 (\pm0.5)	42.9 (\pm2.5)	<u>4.1 (\pm1.5)</u>	96.9 (\pm0)	6 (\pm2.1)	
<i>KF2-175AY</i>	MEB	<u>2 (\pm0.9)</u>	-5 (\pm 3.2)	-35.7(\pm 3.2)	<u>11.1 (\pm2)</u>	-24.1(\pm 3.4)	13.9 (\pm1.2)	<u>13 (\pm2.2)</u>	<u>3 (\pm1.2)</u>	73.3 (\pm 1.1)	-17.5 (\pm 0.6)	PDB
	PDB	<u>2.6 (\pm1.5)</u>	13.7 (\pm2.4)	7.8 (\pm2.4)	<u>8.7 = (\pm0.5)</u>	8.4 (\pm1.3)	-2.9 (\pm 1.9)	<u>17.1 (\pm1.2)</u>	<u>1.5 (\pm2.8)</u>	97.9 (\pm0.2)	7.9 (\pm3.1)	
<i>KF2-175L</i>	MEB	-2.6 (\pm 1.2)	0.2 (\pm 3.8)	-41.1(\pm 2.5)	14.2 (\pm 1.9)	-12.3 (\pm 2)	-36.2(\pm 1.3)	-34 (\pm 2.3)	-5.7 (\pm 2.8)	-22.5 (\pm 1.3)	56.2 (\pm2.7)	PDB
	PDB	88.3 (\pm3)	86.1 (\pm2.7)	55.8 (\pm1)	26.1 (\pm0.3)	18.2 (\pm2)	-9.4 (\pm1)	76.3 (\pm2.7)	3.4 (\pm0.9)	71.8 (\pm0.8)	2.6 (\pm 2.4)	

Table 1: Mean inhibition values for isolate extracts tested in triplicate against each pathogen.

Isolate	Extract	C. a	S. c	S. a	P. a	E. c	E. f	MRSA	VRE	M. t	M. s	Winner
<i>KF2-175BP</i>	MEB	96.9 (±2.9)	95.9 (±0.8)	93.9 (±2.2)	65.9 (±0.6)	47.8 (±0.9)	-53.6(±1.4)	92.1 (±2.6)	-18.3 (±2)	101.7 (±0)	10.4 (±0.9)	MEB
	PDB	-2.1 (±0.7)	13.6 (±1.8)	-14.6 (±3)	11.8 (±0.7)	-1.8 (±1.7)	-6.6 (±2.1)	2 (±3.4)	1.7 (±1)	13.5 (±0.7)	29.7 (±2.1)	
<i>KF2-175BL</i>	MEB	-2.8 (±1.8)	<u>8 (±0.4)</u>	<u>3.7 (±2)</u>	6.5 (±2.9)	0.3 (±2.3)	-45.4(±1.3)	10.2 (±1.9)	-7.3 (±1)	-16.9(±0.3)	94.7 (±3.2)	PDB
	PDB	6.9 (±0.8)	<u>9.4 (±2.8)</u>	<u>7.7 (±0.5)</u>	16.3 (±1.1)	11.5 (±1.4)	2.3 (±1.1)	46.3 (±2.8)	6.3 (±1.4)	23.2 (±1.1)	7.5 (±4.4)	
<i>KF2-175BQ</i>	MEB	-2 (±1.4)	10.1 (±2.5)	<u>24.2 (±3.1)</u>	<u>4.1 (±2.1)</u>	1.9 (±1.1)	-19.9(±3.6)	5 (±1.1)	-19.3 (±1)	54.9 (±0.6)	<u>9.7 (±0.6)</u>	PDB
	PDB	16.8 (±1.2)	19.3 (±0.8)	<u>28.5 (±0.9)</u>	<u>7.1 (±0.4)</u>	-19.1(±1.1)	-7.1 (±1.3)	71 (±1.4)	2.6 (±0.4)	88.6 (±0.1)	<u>12.2 (±3.2)</u>	
<i>KF2-175BU</i>	MEB	<u>0.9 (±2.1)</u>	<u>1 (±3.4)</u>	14.8 (±0.8)	<u>4.2 (±2)</u>	-15.7(±1.4)	-18.6(±2.4)	12.7 (±4.9)	-23.4(±4.8)	94 (±0.4)	83.2 (±0.4)	MEB
	PDB	<u>4 (±1.3)</u>	<u>-1.1 (±2.1)</u>	-22.2(±0.9)	<u>0.9 (±2)</u>	-3.6 (±1)	4.6 (±1)	-7.9 (±0.7)	2.5 (±0.7)	3.4 (±0.9)	8.8 (±0.9)	
<i>KF2-175BJ</i>	MEB	-2.2 (±0.4)	<u>4.9 (±1.7)</u>	-40.9(±1.8)	19.5 (±1.3)	-22.6(±2.3)	-35.3(±2.6)	-26.7 (±3.3)	-11.3(±3.3)	74.4 (±0.6)	<u>-2 (±1)</u>	PDB
	PDB	4.4 (±0.7)	<u>-0.9 (±7.7)</u>	-4 (±0.6)	4.8 (±1.8)	-9.7 (±0.6)	0.9 (±1.1)	9.4 (±0.7)	4.5 (±1.2)	25.1 (±1.9)	<u>0.0 (±2.1)</u>	
<i>KF2-175BY</i>	MEB	<u>3.1 (±2.7)</u>	-14.3(±0.8)	<u>2.5 (±1.6)</u>	<u>3.5 (±1.9)</u>	<u>-8.1 (±1.1)</u>	-38.1(±3.9)	-26.1 (±4.9)	<u>-8.4 (±3.5)</u>	65.7 (±2.7)	81.7 (±1.3)	PDB
	PDB	<u>9.1 (±1.3)</u>	-0.8 (±1.8)	<u>-8.3 (±3.8)</u>	<u>2.9 (±1)</u>	<u>-3.5 (±1.9)</u>	-10.8 (±3)	16.8 (±1.7)	<u>-1.6 (±3.6)</u>	92.3 (±0)	-0.2 (±0.7)	
<i>KF2-175BX</i>	MEB	-8.2 (±2)	<u>8.9 (±0.9)</u>	-28.1(±4.2)	4.8 (±2)	-4.5 (±2.5)	-42.9(±3.5)	-12.3 (±3.2)	<u>-9.6 (±0.7)</u>	48.9 (±0.9)	83.4 (±1)	TIE
	PDB	3.8 (±0.6)	<u>7.4 (±3.7)</u>	-0.2 (±0.6)	-16.7(±4.6)	12.3 (±0.4)	7.4 (±1.1)	-31.9 (±3.8)	<u>-9.5 (±0.9)</u>	9.9 (±1.6)	6.9 (±0.6)	
<i>KF2-175AU</i>	MEB	<u>-0.8 (±1.3)</u>	<u>5.9 (±1.7)</u>	-20.4 (±1)	<u>32.6 (±1.8)</u>	<u>-12.5 (±4.7)</u>	-40.7(±3.8)	<u>-10.5 (±4.7)</u>	-13 (±0.5)	<u>16.8 (±2.1)</u>	36.5 (±0.7)	PDB
	PDB	<u>5.4 (±2.5)</u>	<u>1.4 (±2.2)</u>	4.4 (±0.8)	<u>36.7 (±1)</u>	<u>-10.1 (±3.7)</u>	7.5 (±0.9)	<u>-6.5 (±0.6)</u>	-3.8 (±2.1)	<u>21.4 (±4.5)</u>	2.6 (±0.6)	

Table 2: A summary of the results of the factorial ANOVAs performed on each of the 35 fungal isolates showing the number and level of significant effects revealed by the analyses.

	<i>P</i> <0.05	<i>P</i> <0.001	<i>Non-significant</i>
<i>Media</i>	32	31	3
<i>Pathogen</i>	35	35	0
<i>Media x Pathogen</i>	35	35	0

To compare the effect of fermentation media on bioactivity, we assessed inhibition values for each extract against all pathogens. Overall, PDB-derived extracts exhibited higher inhibition against more pathogens than MEB-derived extracts (Table 1). This was determined by performing simple main effects tests to statistically compare inhibition values between media as shown in Table 3 and counting instances where an extract from one medium showed significantly greater inhibition ($p < 0.05$) than its counterpart. The number of times a PDB-derived extract exhibited greater inhibition against a pathogen was compared to the amount of times it's MEB-derived counterpart showed greater inhibition or no significant difference. The fermentation medium whose extracts inhibited more pathogens than the other was deemed the “winner,” as shown in Table 1. Specifically, PDB-derived extracts displayed higher bioactivity in 24 isolates, due to having greater values of inhibition against more pathogens than their MEB-derived counterparts as shown in Table 1, with the extract derived from the fermentation medium that exhibited increased activity being bolded. In contrast, MEB-derived extracts

exhibited higher inhibition than PDB in four isolates. Four isolates showed an equal number of inhibited pathogens in both media, while three isolates displayed no significant difference in bioactivity between media; these three were excluded from further analyses. A chi-squared goodness-of-fit test was performed on the results in Table 1, revealing that the increased activity observed for PDB extracts was highly significant (χ^2 goodness of fit, $\chi^2=14.29$, $df=1$, $p=0.00016$).

Simple main effects tests were also performed on the bioassay data obtained for the 32 isolates shown in Table 2 that exhibited significant differences in bioactivity with media type to give the results shown in Table 1. These analyses determined which medium produced superior extracts for inhibiting specific pathogens as shown in Table 3 and given this data we were able to determine the results shown in Table 2 ($p < 0.05$ indicating significance). For *C. albicans*, PDB-derived extracts demonstrated higher activity in 19 instances due to seeing significantly higher inhibition levels in the PDB extracts at $p < 0.05$ compared to three for MEB-derived extracts ($\chi^2 = 11.64$, $df = 1$, $p < 0.0006$). Against *S. cerevisiae*, PDB-derived extracts outperformed MEB-derived extracts in 16 cases, versus three, though this difference was non-significant ($\chi^2 = 8.89$, $df = 1$, $p < 0.0029$). A significant difference was observed for *S. aureus*, with PDB-derived extracts outperforming MEB-derived extracts 19 times versus six ($\chi^2 = 6.76$, $df = 1$, $p < 0.0093$). For *P. aeruginosa*, PDB-derived extracts exhibited higher activity in nine cases, while MEB-derived extracts did so in six cases, though this difference was not significant ($\chi^2 = 0.6$, $df = 1$, $p < 0.4386$). Against *E. coli*, the PDB-derived extracts had higher bioactivity in 17 instances compared to seven for MEB-derived extracts ($\chi^2 = 4.2$, $df = 1$, $p < 0.0412$). Against *E. faecium*, PDB-derived extracts showed higher activity in 19 cases,

while MEB-derived extracts were more active in nine cases, but this difference was not significant ($\chi^2 = 3.57$, $df = 1$, $p < 0.059$). For MRSA, the PDB-derived extracts exhibited higher activity in 17 cases compared to six for MEB-derived extracts ($\chi^2 = 5.26$, $df = 1$, $p < 0.0218$). Against VRE, PDB-derived extracts outperformed MEB-derived extracts in 18 cases, whereas MEB-derived extracts exhibited more significant activity in three cases ($\chi^2 = 10.7$, $df = 1$, $p < 0.0011$). A significant difference was observed in activity against *M. tuberculosis*, with PDB-derived extracts outperforming MEB-derived 21 times compared to eight times ($\chi^2 = 5.83$, $df = 1$, $p < 0.0158$). While PDB-derived extracts exhibited higher activity in 14 cases than 13 for MEB-derived extracts against *M. smegmatis*, this difference did not reach statistical significance ($\chi^2 = 0.04$, $df = 1$, $p < 0.8474$).

Data for each pathogen was analyzed using a chi-squared goodness-of-fit test and significant differences between media were observed for one pathogen at $p < 0.01$ (*C. albicans*) and six pathogens at $p < 0.05$ (*S. cerevisiae*, *S. aureus*, *E. coli*, *MRSA*, *VRE* and *M. tuberculosis*) with PDB-derived extracts showing increased inhibition in all cases. Three pathogens displayed no significant difference in inhibition levels based on media type (*P. aeruginosa*, *E. faecium*, and *M. smegmatis*). Table 3 summarizes these results.

Table 3: The number of isolate extracts that exhibited superior growth inhibition against a pathogen and the medium from which they were derived.

	<i>MEB</i>	<i>PDB</i>	<i>Non-Significant</i>
<i>C. albicans</i>	3	19	9
<i>S. cerevisiae</i>	3	15	13
<i>S. aureus</i>	6	19	6
<i>P. aeruginosa</i>	6	9	16
<i>E. coli</i>	7	17	7
<i>E. faecium</i>	9	18	4
<i>MRSA</i>	6	16	19
<i>VRE</i>	3	18	10
<i>M. tuberculosis</i>	8	20	3
<i>M. smegmatis</i>	12	14	5

The overall trend observed in the data was that PDB-derived extracts exhibited greater inhibition against more pathogens than MEB-derived extracts. Within this trend, three distinct patterns emerged. The most common pattern was that PDB-derived extracts demonstrated significantly higher inhibition compared to MEB-derived extracts, occurring with 24 out of 32 isolates. PDB-derived extracts consistently exhibited increased inhibition against more pathogens than MEB-derived extracts. Figure 1 exemplifies this pattern, where a PDB-derived extract inhibited nine pathogens more effectively than its MEB counterpart, which only inhibited one.

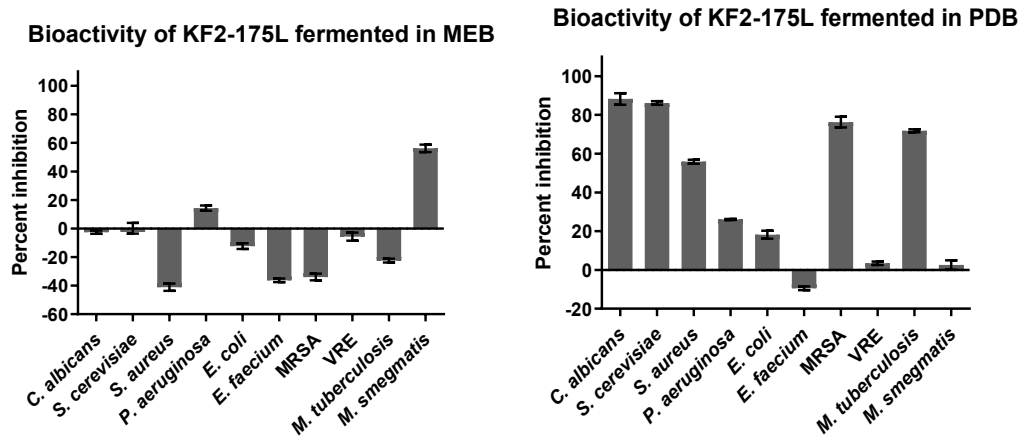


Figure 1: Bioactivity profiles for KF2-175L extracts derived from MEB (left) and PDB (right). Bars show the mean percentage growth inhibition of triplicate values and error bars show the standard error of the mean.

A second pattern, seen in four out of 32 isolates, showed the opposite effect, where MEB-derived extracts exhibited greater inhibition than PDB-derived extracts.

Figure 2 illustrates this, with an MEB-derived extract significantly inhibiting four pathogens, while the PDB-derived extract had superior activity against three pathogens and three pathogens showed no significant difference between media.

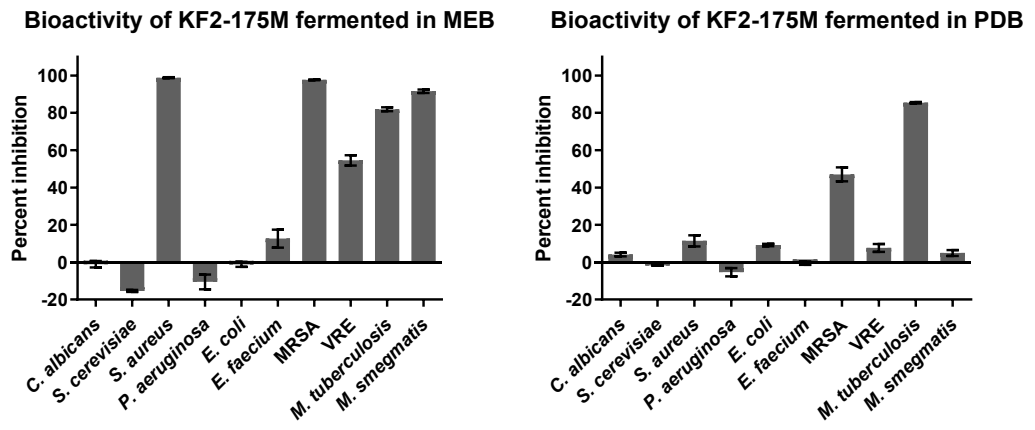


Figure 2: Bioactivity profiles for KF2-175M extracts derived from MEB (left) and PDB (right). Bars show the mean percentage growth inhibition of triplicate values and error bars show the standard error of the mean.

The third pattern observed was equal inhibition between media types, which occurred in seven out of 32 isolates. Figure 3 represents this scenario, where inhibition was evenly distributed (4:4), with two pathogens showing no significant differences in inhibition.

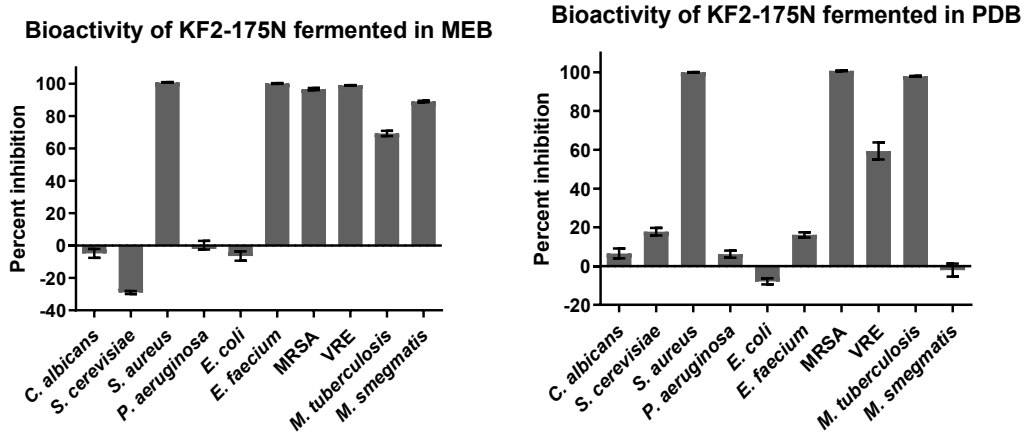


Figure 3: Bioactivity profiles for KF2-175N extracts derived from MEB (left) and PDB (right). Bars show the mean percentage growth inhibition of triplicate values and error bars show the standard error of the mean.

Discussion

In this study, the pathogens tested can be grouped into four broad categories: Gram-positive bacteria (*S. aureus*, *E. faecium*, MRSA, and VRE), Gram-negative bacteria (*E. coli* and *P. aeruginosa*), yeast (*C. albicans* and *S. cerevisiae*), and mycobacteria (*M. tuberculosis* and *M. smegmatis*) (Antonelli et al., 2021; Furuno et al., 2005; Sparks, 2023; Wilson, 2016). The results found in this study indicate that antimicrobial activity primarily depends on the endophytic isolate and its interaction with the growth medium rather than the targeted pathogen, since antimicrobial activity against *E. faecium*, *P. aeruginosa*, and *M. smegmatis* was not significantly different between media, it suggests that pathogen type does not determine which fermentation medium produces the most bioactive extract. These three pathogens represent different categories of pathogens (Gram-positive, Gram-negative, and mycobacteria, respectively) yet no consistent trend emerged where one medium was more effective against a specific pathogen group. Instead, antimicrobial activity appears to be driven by the metabolic preferences of individual endophytes rather than by the characteristics of the targeted pathogen. This reinforces the idea that selecting an appropriate fermentation medium is not a one-size-fits-all approach; some endophytes produce bioactive compounds more effectively in PDB, while others do so in MEB.

As a carbon-rich medium, PDB supports the biosynthesis of carbon-dependant secondary metabolites, leading to higher antimicrobial activity in many cases (Barajas et al., 2017; Zardecki, 2021). The sugars and organic compounds in PDB may enhance the production of metabolites such as polyketides and other carbohydrate-based antimicrobial compounds (Barajas et al., 2017). This likely explains why PDB-derived extracts

exhibited more significant inhibition in most isolates. However, MEB may favour the production of different metabolites, particularly nitrogen-containing compounds, which could be why a smaller subset of isolates demonstrated higher activity in MEB (Wattanachaisaereekul et al., 2014). The variation in antimicrobial activity across different isolates suggests that enhanced bioactivity in PDB is not solely due to pathogen susceptibility but rather to the metabolic responses of endophytes to the medium. This finding underscores the importance of optimizing culture conditions to maximize bioactive compound production, which is critical for the discovery of novel antimicrobial agents

Patterns in Antimicrobial Activity

The most common trend observed was greater antimicrobial activity in PDB compared to MEB, indicating that the bioactive compounds responsible for antimicrobial activity were more effectively produced in PDB. The likely explanation for this lies in the nutrient composition of PDB, which is rich in carbohydrates and organic compounds that promote microbial growth and secondary metabolite production (Zardecki, 2021). Specific endophytes may preferentially metabolize these nutrients, leading to a higher concentration of bioactive compounds such as polyketides and other carbon-based antimicrobials (Barajas et al., 2017).

Another observed trend was greater antimicrobial activity in MEB compared to PDB. A minority of isolates exhibited enhanced activity when fermented in MEB, suggesting that certain secondary metabolites were either more efficiently synthesized or more stable in this medium. MEB's higher nitrogen content may favour the production of peptide-based or nitrogen-containing antimicrobial metabolites less efficiently

synthesized in PDB (Barajas et al., 2017; Wattanachaisaereekul et al, 2014). This could explain why a subset of isolates showed enhanced activity in MEB, though this trend was far less prevalent.

The final trend observed was equal activity in both MEB and PDB. Some isolates exhibited no significant differences in inhibition between the two media, suggesting that their antimicrobial compound production is not strongly influenced by external nutrient availability. This implies that these isolates produce bioactive metabolites constitutively as part of their normal metabolism rather than in response to specific growth conditions (Kumari et al., 2023). These endophytes may have stable biosynthetic pathways that allow for consistent production of antimicrobial compounds regardless of the fermentation medium used (Kumari et al., 2023).

The findings of this study contribute to the broader understanding of how fermentation conditions influence the production of antimicrobial metabolites in endophytes. By demonstrating that different endophytes respond uniquely to PDB and MEB, this research highlights the necessity of optimizing culture conditions for maximizing bioactive compound production. This is particularly important in the search for novel antimicrobial agents, as media composition can significantly impact their efficacy. Identifying optimal conditions for secondary metabolite production in endophytes may improve the efficiency of screening natural products for pharmaceutical applications.

Conclusion

This study investigated the effects of two commonly used growth media, PDB and MEB, on the antimicrobial activity of endophyte-derived extracts. The results indicate that antimicrobial efficacy is primarily influenced by the metabolic interactions between endophytes and their growth environment, rather than by pathogen susceptibility alone. While PDB generally supported higher antimicrobial activity, some isolates exhibited greater inhibition in MEB, and others showed no significant difference between the two. These findings underscore the importance of media selection in antimicrobial research and suggest that different endophytes have distinct bio synthetic preferences.

Contributions to Thesis

Kirstyn Forgrave isolated all the endophytes used in this research, fermented them in PDB, prepared the PDB extracts and obtained the PDB bioassay data.

Janet Debly fermented the endophyte isolates in MEB and prepared the MEB extracts.

I conducted the bioassays on the MEB-derived extracts, compiled the bioactivity profiles of the extracts derived from both media types and performed all of the analyses described in this thesis.

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Appendix

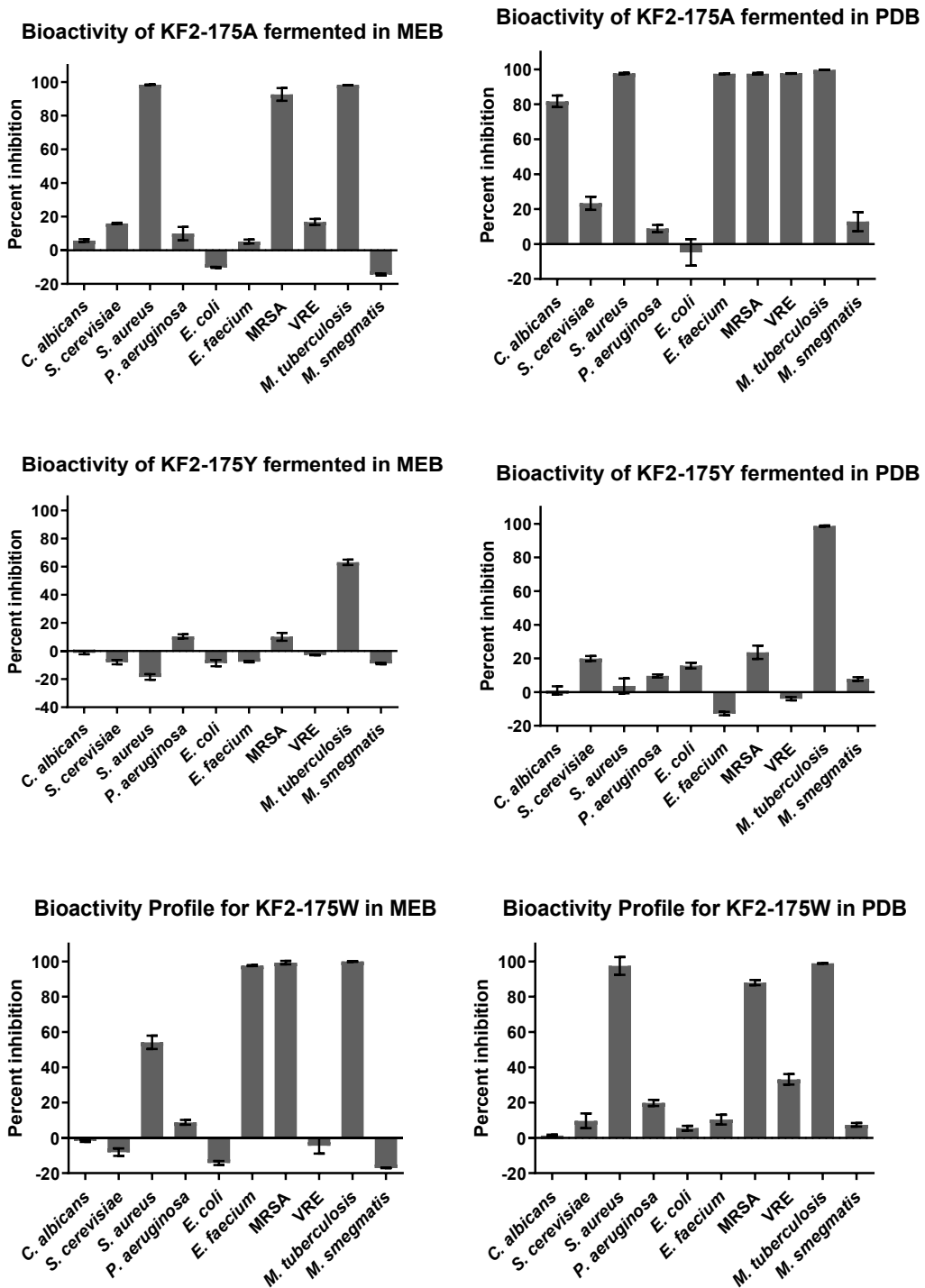


Figure 4: Bioactivity profiles of the 35 isolates fermented in MEB (left) and PDB (right).

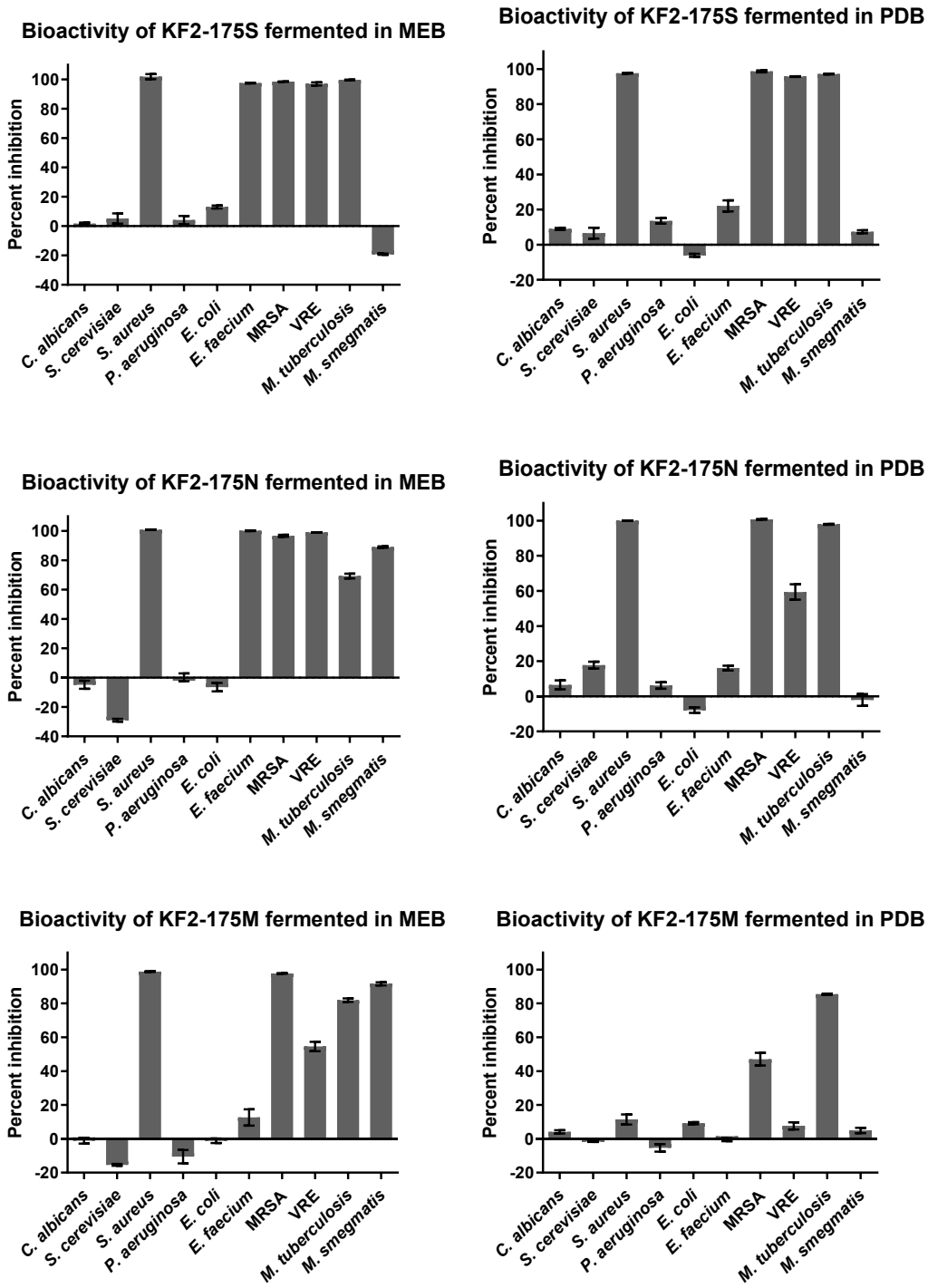


Figure 4: Bioactivity profiles of the 35 isolates fermented in MEB (left) and PDB (right).

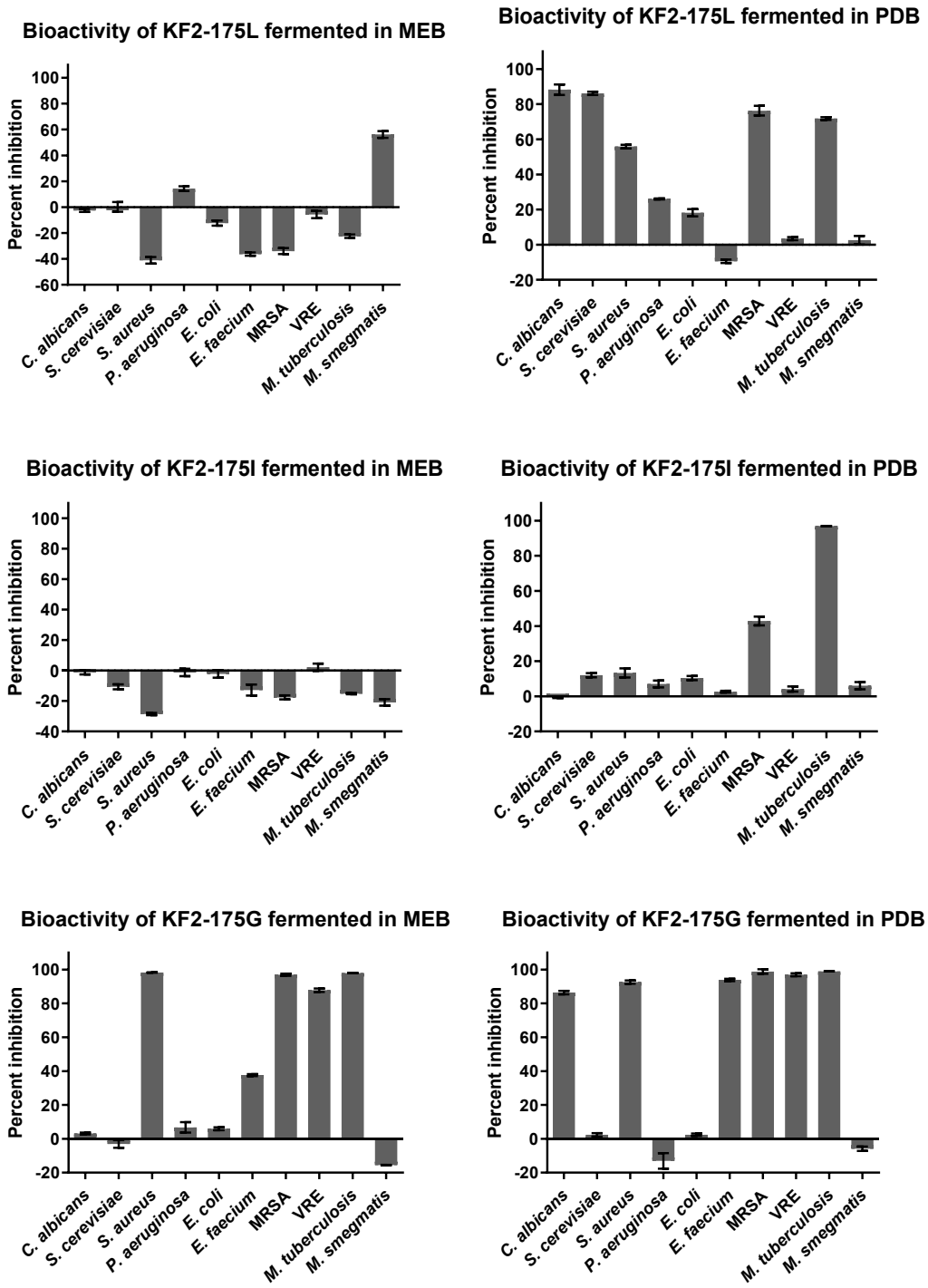


Figure 4: Bioactivity profiles of the 35 isolates fermented in MEB (left) and PDB (right).

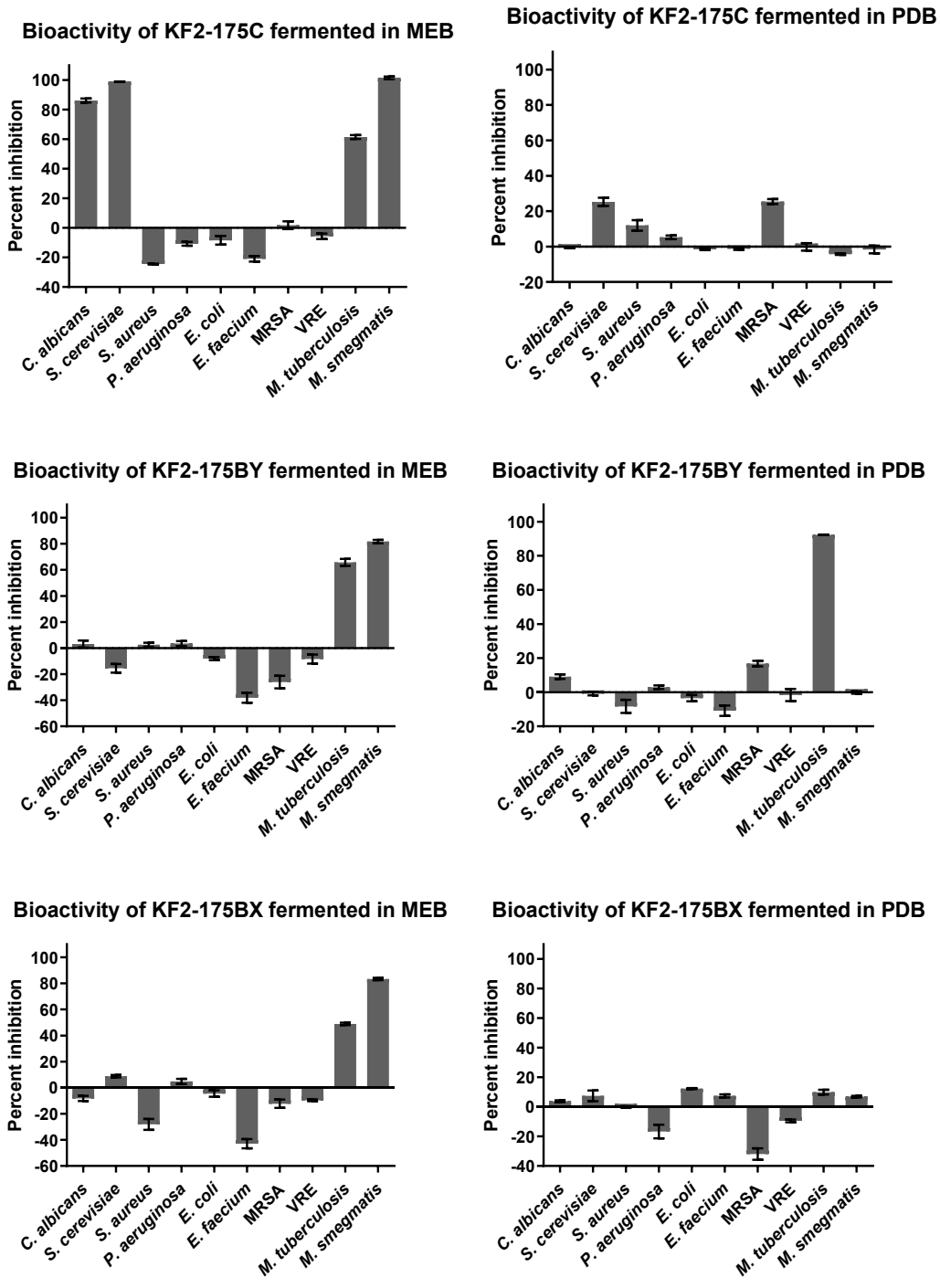
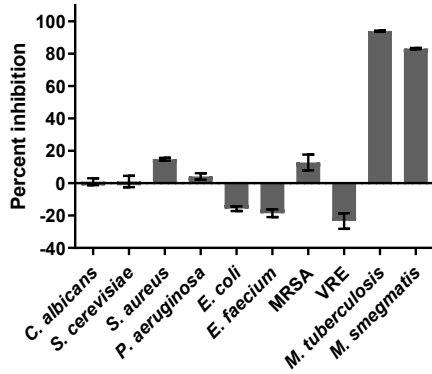
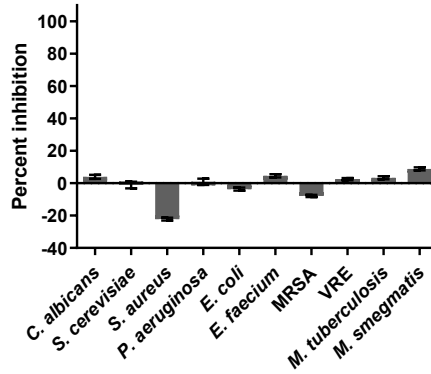


Figure 4: Bioactivity profiles of the 35 isolates fermented in MEB (left) and PDB (right).

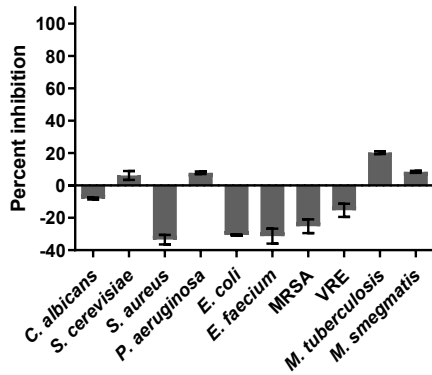
Bioactivity of KF2-175BU fermented in MEB



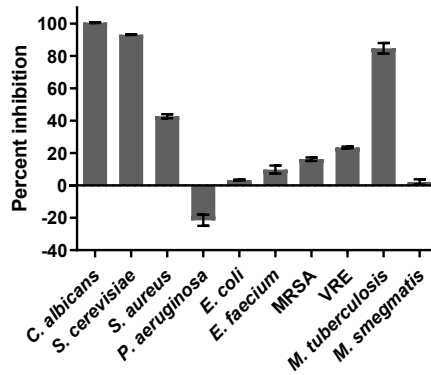
Bioactivity of KF2-175BU fermented in PDB



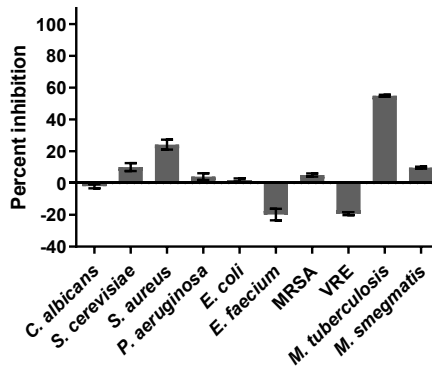
Bioactivity of KF2-175BS fermented in MEB



Bioactivity of KF2-175BS fermented in PDB



Bioactivity of KF2-175BQ fermented in MEB



Bioactivity of KF2-175BQ fermented in PDB

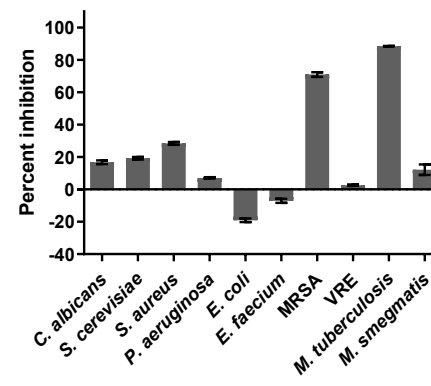
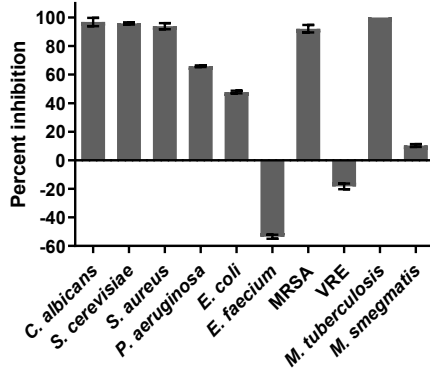
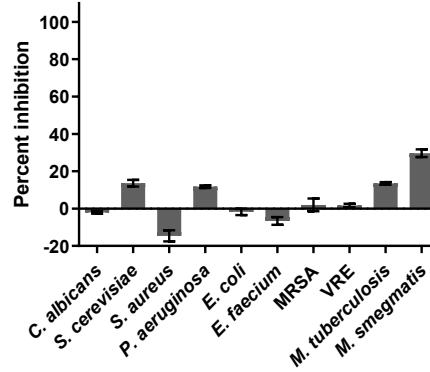


Figure 4: Bioactivity profiles of the 35 isolates fermented in MEB (left) and PDB (right).

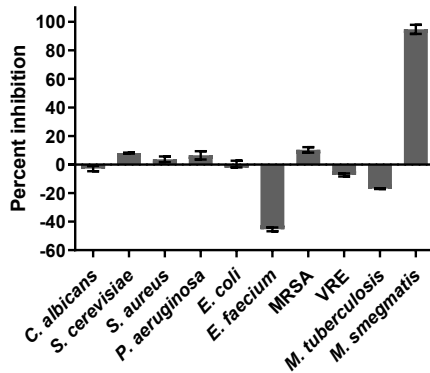
Bioactivity of KF2-175BP fermented in MEB



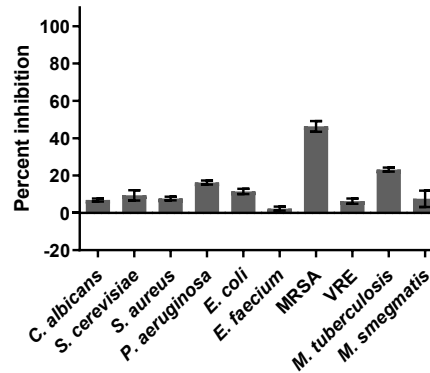
Bioactivity of KF2-175BP fermented in PDB



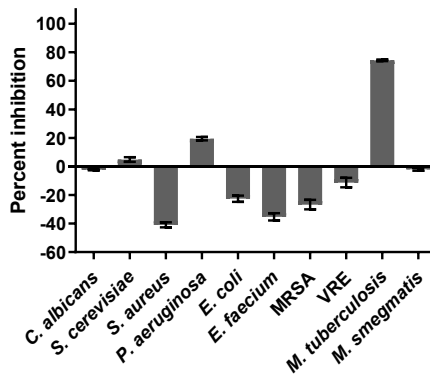
Bioactivity of KF2-175BL fermented in MEB



Bioactivity of KF2-175BL fermented in PDB



Bioactivity of KF2-175BJ fermented in MEB



Bioactivity of KF2-175BJ fermented in PDB

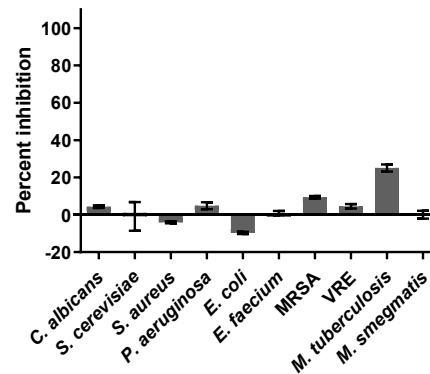
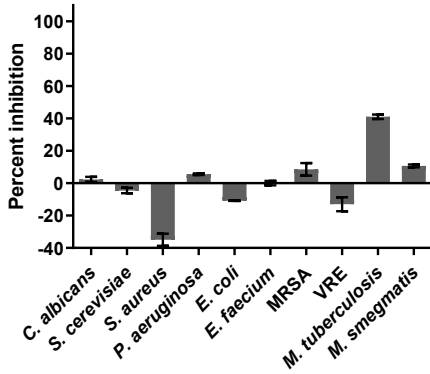
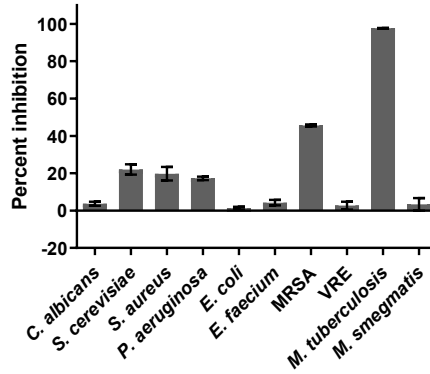


Figure 4: Bioactivity profiles of the 35 isolates fermented in MEB (left) and PDB (right).

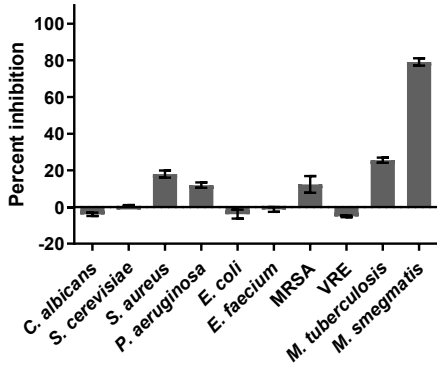
Bioactivity of KF2-175BF fermented in MEB



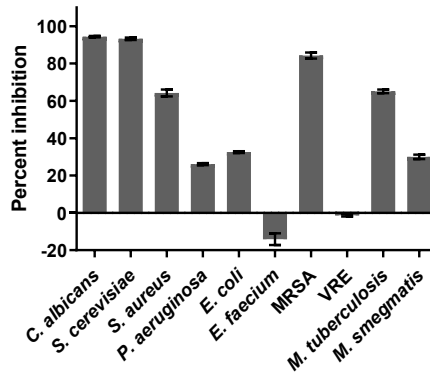
Bioactivity of KF2-175BF fermented in PDB



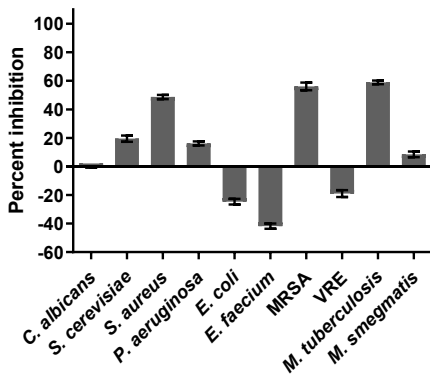
Bioactivity of KF2-175BC fermented in MEB



Bioactivity of KF2-175BC fermented in PDB



Bioactivity of KF2-175BA fermented in MEB



Bioactivity of KF2-175BA fermented in PDB

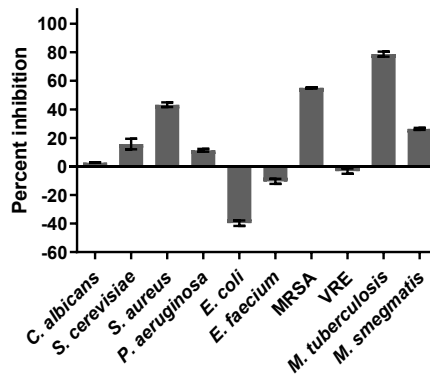
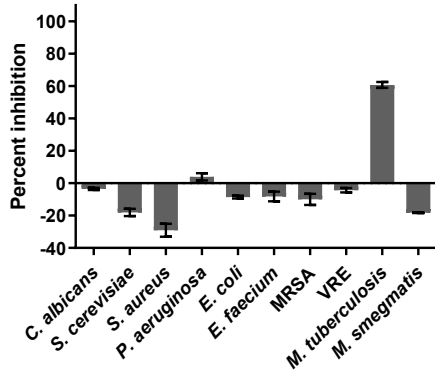
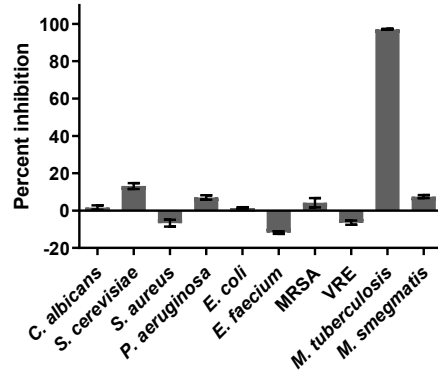


Figure 4: Bioactivity profiles of the 35 isolates fermented in MEB (left) and PDB (right).

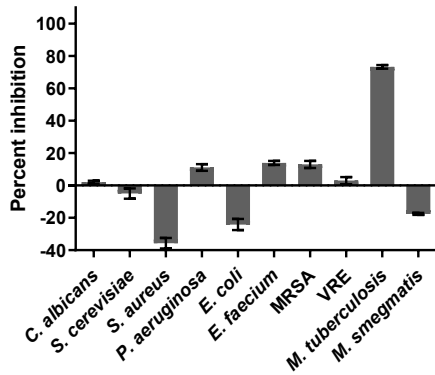
Bioactivity of KF2-175AZ fermented in MEB



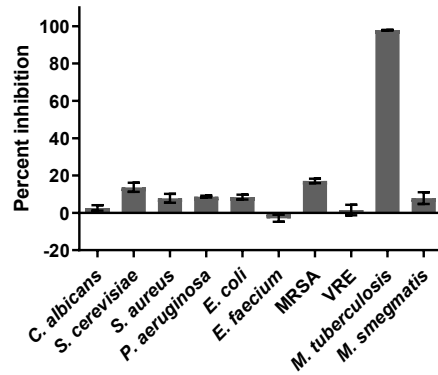
Bioactivity of KF2-175AZ fermented in PDB



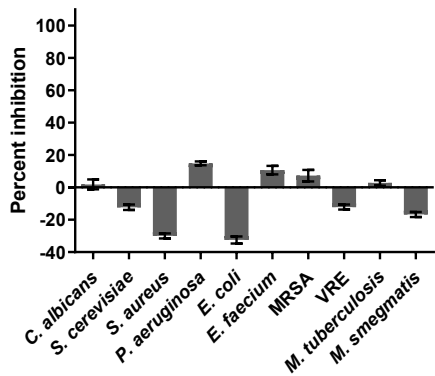
Bioactivity of KF2-175AY fermented in MEB



Bioactivity of KF2-175AY fermented in PDB



Bioactivity of KF2-175AX fermented in MEB



Bioactivity of KF2-175AX fermented in PDB

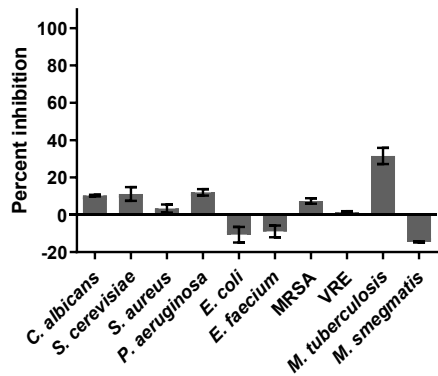
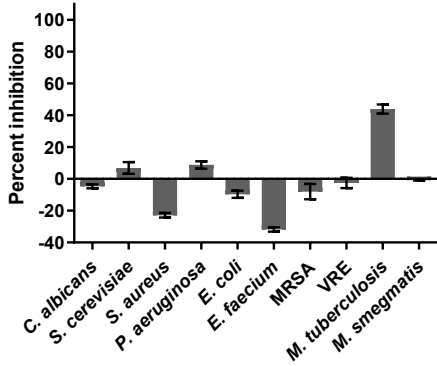
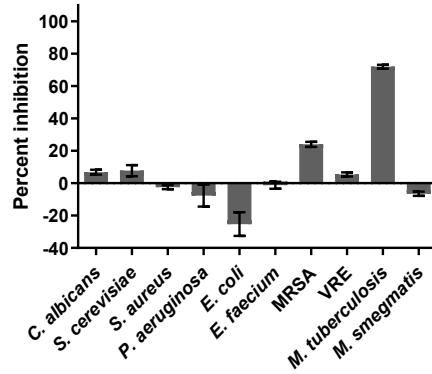


Figure 4: Bioactivity profiles of the 35 isolates fermented in MEB (left) and PDB (right).

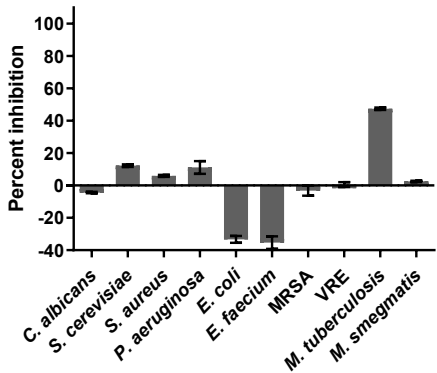
Bioactivity of KF2-175AW fermented in MEB



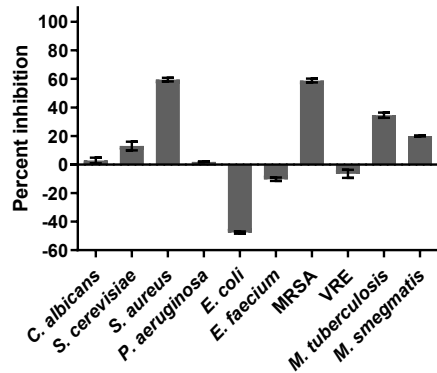
Bioactivity of KF2-175AW fermented in PDB



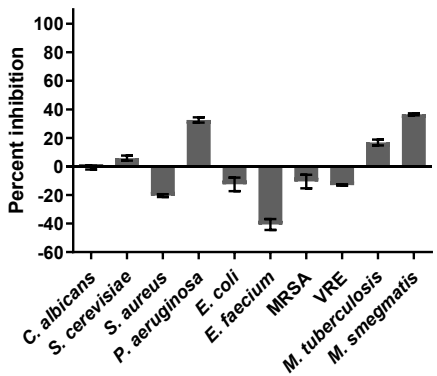
Bioactivity of KF2-175AV fermented in MEB



Bioactivity of KF2-175AV fermented in PDB



Bioactivity of KF2-175AU fermented in MEB



Bioactivity of KF2-175AU fermented in PDB

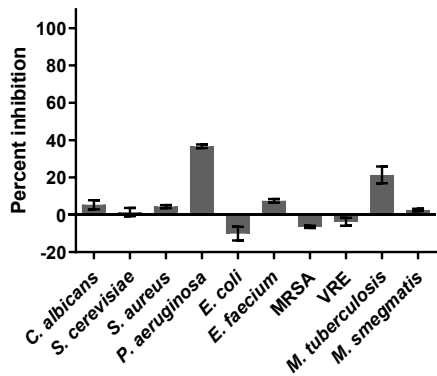
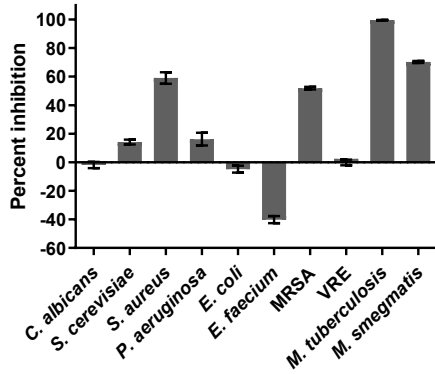
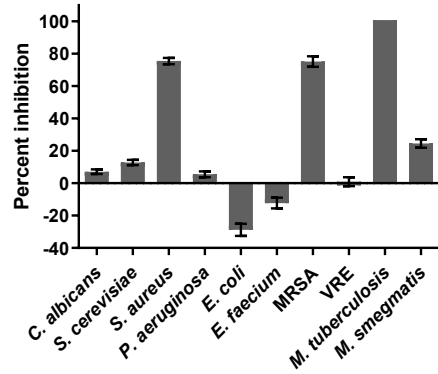


Figure 4: Bioactivity profiles of the 35 isolates fermented in MEB (left) and PDB (right).

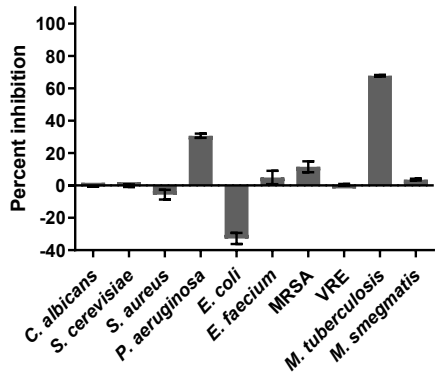
Bioactivity of KF2-175AT fermented in MEB



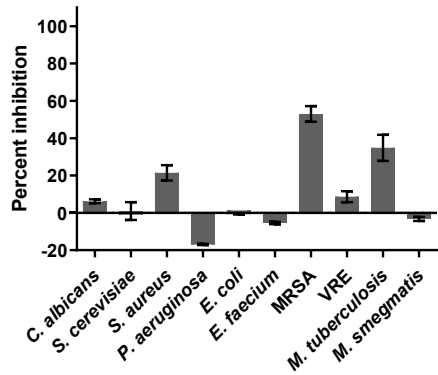
Bioactivity of KF2-175AT fermented in PDB



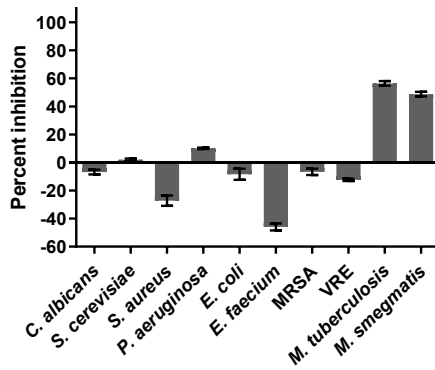
Bioactivity of KF2-175AR fermented in MEB



Bioactivity of KF2-175AR fermented in PDB



Bioactivity of KF2-175AQ fermented in MEB



Bioactivity of KF2-175AQ fermented in PDB

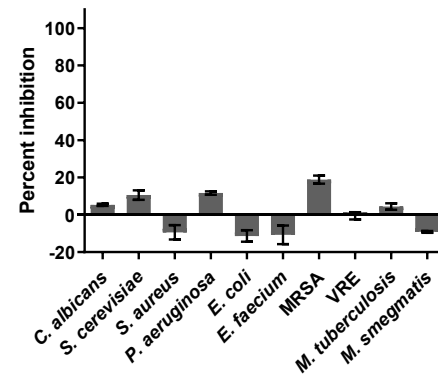
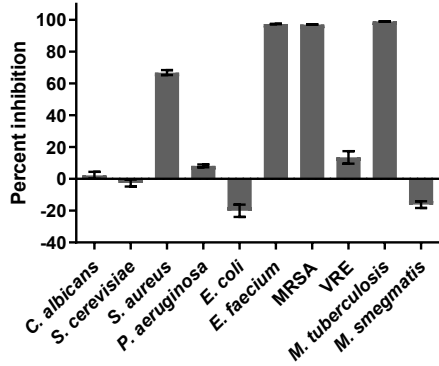
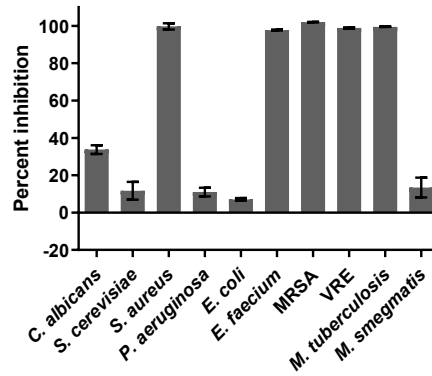


Figure 4: Bioactivity profiles of the 35 isolates fermented in MEB (left) and PDB (right).

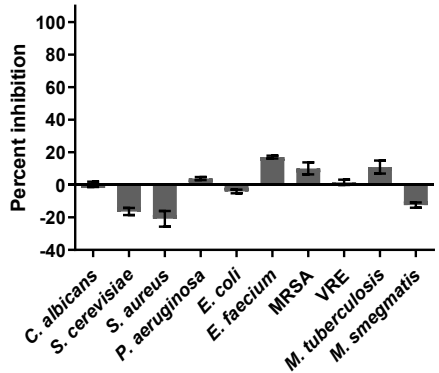
Bioactivity of KF2-175AO fermented in MEB



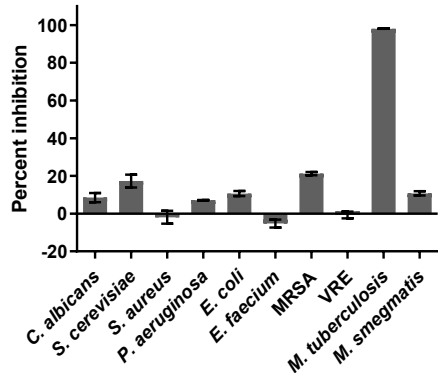
Bioactivity of KF2-175AO fermented in PDB



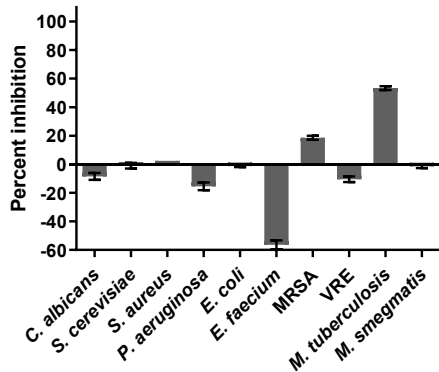
Bioactivity of KF2-175AK fermented in MEB



Bioactivity of KF2-175AK fermented in PDB



Bioactivity of KF2-175AI fermented in MEB



Bioactivity of KF2-175AI fermented in PDB

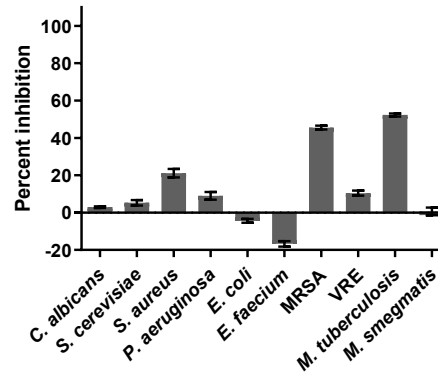
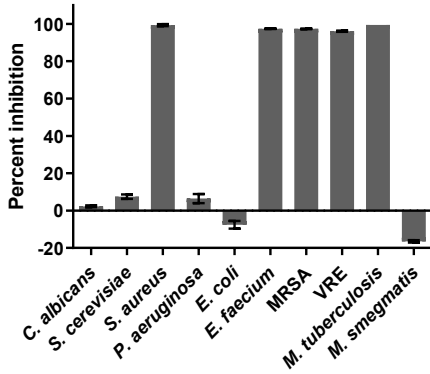
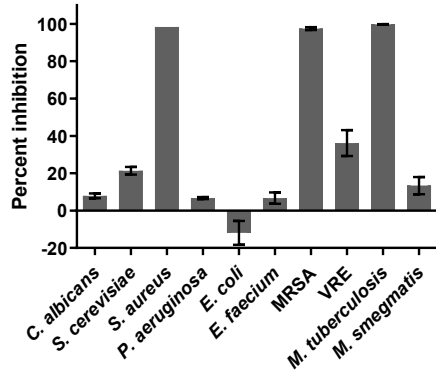


Figure 4: Bioactivity profiles of the 35 isolates fermented in MEB (left) and PDB (right).

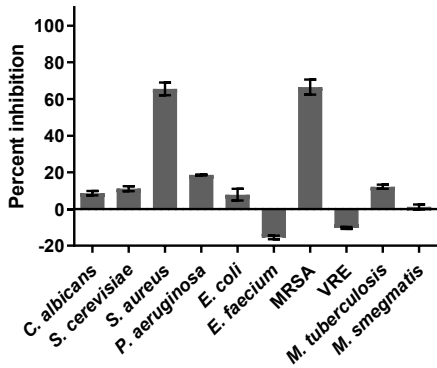
Bioactivity of KF2-175AH fermented in MEB



Bioactivity of KF2-175AH fermented in PDB



Bioactivity of KF2-175AG fermented in MEB



Bioactivity of KF2-175AG fermented in PDB

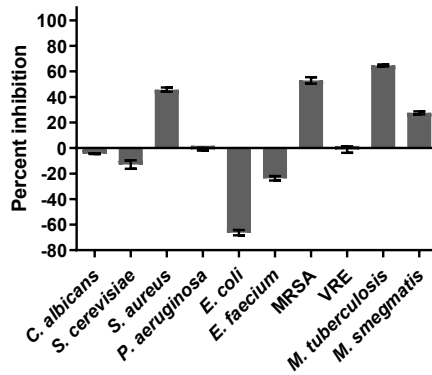


Figure 4: Bioactivity profiles of the 35 isolates fermented in MEB (left) and PDB (right).

Table 4: Factorial ANOVA results for isolate KF2-175AQ (VAR00001 = medium type, and VAR00003 = pathogen).

Source	Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared
Corrected Model	29655.125 ^a	19	1560.796	87.667	<.001	.977
Intercept	58.295	1	58.295	3.274	.078	.076
VAR00001	.021	1	.021	.001	.973	.000
VAR00003	16691.730	9	1854.637	104.172	<.001	.959
VAR00001 * VAR00003	12963.373	9	1440.375	80.903	<.001	.948
Error	712.147	40	17.804			
Total	30425.567	60				
Corrected Total	30367.272	59				

Table 5: Factorial ANOVA results for isolate KF2-175AI (VAR00001 = medium type, and VAR00003 = pathogen).

Source	Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared
Corrected Model	36106.720 ^a	19	1900.354	214.897	<.001	.991
Intercept	1552.603	1	1552.603	175.572	<.001	.818
VAR00001	3238.992	1	3238.992	366.274	<.001	.904
VAR00003	30083.272	9	3342.586	377.988	<.001	.989
VAR00001 * VAR00003	2603.733	9	289.304	32.715	<.001	.883
Error	344.880	39	8.843			
Total	37977.599	59				
Corrected Total	36451.600	58				

Table 6: Factorial ANOVA results for isolate KF2-175AV (VAR00001 = medium type, and VAR00003 = pathogen).

Source	Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared
Corrected Model	46849.586 ^a	19	2465.768	160.355	<.001	.987
Intercept	2730.384	1	2730.384	177.564	<.001	.816
VAR00001	2440.644	1	2440.644	158.721	<.001	.799
VAR00003	34697.225	9	3855.247	250.717	<.001	.983
VAR00001 * VAR00003	9711.716	9	1079.080	70.175	<.001	.940
Error	615.076	40	15.377			
Total	50195.046	60				
Corrected Total	47464.662	59				

Table 7: Factorial ANOVA results for isolate KF2-175AW (VAR00001 = medium type, and VAR00003 = pathogen).

Source	Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared
Corrected Model	29962.710 ^a	19	1576.985	75.483	<.001	.974
Intercept	384.359	1	384.359	18.398	<.001	.326
VAR00001	1260.629	1	1260.629	60.341	<.001	.614
VAR00003	24499.295	9	2722.144	130.297	<.001	.969
VAR00001 * VAR00003	4142.704	9	460.300	22.033	<.001	.839
Error	793.889	38	20.892			
Total	31369.618	58				
Corrected Total	30756.599	57				

Table 8: Factorial ANOVA results for isolate KF2-175AX (VAR00001 = medium type, and VAR00003 = pathogen).

Source	Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared
Corrected Model	15547.580 ^a	19	818.294	38.028	<.001	.948
Intercept	43.739	1	43.739	2.033	.162	.048
VAR00001	1992.084	1	1992.084	92.576	<.001	.698
VAR00003	9542.677	9	1060.297	49.274	<.001	.917
VAR00001 * VAR00003	4012.819	9	445.869	20.720	<.001	.823
Error	860.732	40	21.518			
Total	16452.050	60				
Corrected Total	16408.312	59				

Table 9: Factorial ANOVA results for isolate KF2-175AR (VAR00001 = medium type, and VAR00003 = pathogen).

Source	Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared
Corrected Model	29651.352 ^a	19	1560.597	58.036	<.001	.967
Intercept	4556.594	1	4556.594	169.451	<.001	.817
VAR00001	50.407	1	50.407	1.875	.179	.047
VAR00003	18955.481	9	2106.165	78.324	<.001	.949
VAR00001 * VAR00003	9597.402	9	1066.378	39.657	<.001	.904
Error	1021.832	38	26.890			
Total	35953.094	58				
Corrected Total	30673.184	57				

Table 10: Factorial ANOVA results for isolate KF2-175AT (VAR00001 = medium type, and VAR00003 = pathogen).

Source	Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared
Corrected Model	95920.442 ^a	19	5048.444	210.532	<.001	.990
Intercept	42434.987	1	42434.987	1769.641	<.001	.978
VAR00001	2.337	1	2.337	.097	.757	.002
VAR00003	89642.875	9	9960.319	415.369	<.001	.989
VAR00001 * VAR00003	6275.230	9	697.248	29.077	<.001	.867
Error	959.177	40	23.979			
Total	139314.606	60				
Corrected Total	96879.619	59				

Table 11: Factorial ANOVA results for KF2-175AG (VAR00001 = medium type, and VAR00003 = pathogen).

Source	Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared
Corrected Model	65252.375 ^a	19	3434.336	271.372	<.001	.992
Intercept	9143.753	1	9143.753	722.515	<.001	.948
VAR00001	1072.931	1	1072.931	84.780	<.001	.679
VAR00003	49043.466	9	5449.274	430.587	<.001	.990
VAR00001 * VAR00003	15135.977	9	1681.775	132.889	<.001	.968
Error	506.218	40	12.655			
Total	74902.346	60				
Corrected Total	65758.593	59				

Table 12: Factorial ANOVA results for KF2-175N (VAR00001 = medium type, and VAR00003 = pathogen).

Source	Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared
Corrected Model	136877.828 ^a	19	7204.096	632.452	<.001	.997
Intercept	124153.843	1	124153.843	10899.534	<.001	.996
VAR00001	2181.951	1	2181.951	191.555	<.001	.827
VAR00003	106680.848	9	11853.428	1040.619	<.001	.996
VAR00001 * VAR00003	28015.029	9	3112.781	273.273	<.001	.984
Error	455.630	40	11.391			
Total	261487.301	60				
Corrected Total	137333.458	59				

Table 13: Factorial ANOVA results for KF2-175A (VAR00001 = medium type, and VAR00003 = pathogen).

Source	Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared
Corrected Model	121344.514 ^a	19	6386.553	276.428	<.001	.993
Intercept	123599.631	1	123599.631	5349.748	<.001	.993
VAR00001	12340.964	1	12340.964	534.152	<.001	.934
VAR00003	90098.140	9	10010.904	433.301	<.001	.990
VAR00001 * VAR00003	18766.257	9	2085.140	90.251	<.001	.955
Error	877.945	38	23.104			
Total	247245.418	58				
Corrected Total	122222.460	57				

Table 14: Factorial ANOVA results for KF2-175AH (VAR00001 = medium type, and VAR00003 = pathogen).

Source	Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared
Corrected Model	128909.348 ^a	19	6784.703	324.030	<.001	.994
Intercept	110347.148	1	110347.148	5270.066	<.001	.992
VAR00001	1682.654	1	1682.654	80.362	<.001	.668
VAR00003	109497.377	9	12166.375	581.053	<.001	.992
VAR00001 * VAR00003	17729.317	9	1969.924	94.082	<.001	.955
Error	837.539	40	20.938			
Total	240094.035	60				
Corrected Total	129746.887	59				

Table 15: Factorial ANOVA results for KF2-175W (VAR00001 = medium type, and VAR00003 = pathogen).

Source	Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared
Corrected Model	114780.104 ^a	19	6041.058	366.873	<.001	.994
Intercept	70540.533	1	70540.533	4283.926	<.001	.991
VAR00001	487.114	1	487.114	29.582	<.001	.425
VAR00003	96072.167	9	10674.685	648.274	<.001	.993
VAR00001 * VAR00003	18220.823	9	2024.536	122.950	<.001	.965
Error	658.653	40	16.466			
Total	185979.290	60				
Corrected Total	115438.757	59				

Table 16: Factorial ANOVA results for KF2-175AO (VAR00001 = medium type, and VAR00003 = pathogen).

Source	Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared
Corrected Model	128984.613 ^a	19	6788.664	399.489	<.001	.995
Intercept	127035.835	1	127035.835	7475.607	<.001	.995
VAR00001	7879.158	1	7879.158	463.660	<.001	.921
VAR00003	112175.788	9	12463.976	733.461	<.001	.994
VAR00001 * VAR00003	8929.667	9	992.185	58.387	<.001	.929
Error	679.735	40	16.993			
Total	256700.183	60				
Corrected Total	129664.348	59				

Table 17: Factorial ANOVA results for KF2-175BF (VAR00001 = medium type, and VAR00003 = pathogen).

Source	Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared
Corrected Model	41216.781 ^a	19	2169.304	124.191	<.001	.983
Intercept	7092.654	1	7092.654	406.049	<.001	.910
VAR00001	6453.342	1	6453.342	369.449	<.001	.902
VAR00003	28718.563	9	3190.951	182.679	<.001	.976
VAR00001 * VAR00003	6044.876	9	671.653	38.452	<.001	.896
Error	698.700	40	17.467			
Total	49008.135	60				
Corrected Total	41915.481	59				

Table 18: Factorial ANOVA results for KF2-175AZ (VAR00001 = medium type, and VAR00003 = pathogen).

Source	Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared
Corrected Model	45529.483 ^a	19	2396.289	224.962	<.001	.991
Intercept	771.156	1	771.156	72.395	<.001	.644
VAR00001	3030.322	1	3030.322	284.484	<.001	.877
VAR00003	39816.110	9	4424.012	415.323	<.001	.989
VAR00001 * VAR00003	2683.051	9	298.117	27.987	<.001	.863
Error	426.080	40	10.652			
Total	46726.718	60				
Corrected Total	45955.562	59				

Table 19: Factorial ANOVA results for KF2-175G (VAR00001 = medium type, and VAR00003 = pathogen).

Source	Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared
Corrected Model	133311.349 ^a	19	7016.387	963.875	<.001	.998
Intercept	137433.219	1	137433.219	18879.873	<.001	.998
VAR00001	2773.350	1	2773.350	380.989	<.001	.907
VAR00003	118925.121	9	13213.902	1815.258	<.001	.998
VAR00001 * VAR00003	12724.078	9	1413.786	194.219	<.001	.978
Error	283.895	39	7.279			
Total	267747.402	59				
Corrected Total	133595.244	58				

Table 20: Factorial ANOVA results for KF2-175S (VAR00001 = medium type, and VAR00003 = pathogen).

Source	Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared
Corrected Model	131953.311 ^a	19	6944.911	910.915	<.001	.998
Intercept	132840.442	1	132840.442	17423.737	<.001	.998
VAR00001	504.081	1	504.081	66.117	<.001	.623
VAR00003	121563.742	9	13507.082	1771.628	<.001	.997
VAR00001 * VAR00003	9885.489	9	1098.388	144.068	<.001	.970
Error	304.964	40	7.624			
Total	265098.717	60				
Corrected Total	132258.276	59				

Table 21: Factorial ANOVA results for KF2-175M (VAR00001 = medium type, and VAR00003 = pathogen).

Source	Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared
Corrected Model	93137.587 ^a	19	4901.978	255.101	<.001	.992
Intercept	50061.851	1	50061.851	2605.235	<.001	.985
VAR00001	8665.334	1	8665.334	450.947	<.001	.919
VAR00003	62298.849	9	6922.094	360.228	<.001	.988
VAR00001 * VAR00003	22173.404	9	2463.712	128.212	<.001	.966
Error	768.635	40	19.216			
Total	143968.073	60				
Corrected Total	93906.222	59				

Table 22: Factorial ANOVA results for KF2-175BS (VAR00001 = medium type, and VAR00003 = pathogen).

Source	Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared
Corrected Model	89235.370 ^a	18	4957.521	218.146	<.001	.991
Intercept	8082.663	1	8082.663	355.662	<.001	.908
VAR00001	23470.954	1	23470.954	1032.794	<.001	.966
VAR00003	34458.201	9	3828.689	168.474	<.001	.977
VAR00001 * VAR00003	20533.209	8	2566.651	112.940	<.001	.962
Error	818.125	36	22.726			
Total	98460.882	55				
Corrected Total	90053.495	54				

Table 23: Factorial ANOVA results for KF2-175C (VAR00001 = medium type, and VAR00003 = pathogen).

Source	Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared
Corrected Model	85028.963 ^a	19	4475.209	527.793	<.001	.996
Intercept	17169.049	1	17169.049	2024.869	<.001	.981
VAR00001	7330.656	1	7330.656	864.557	<.001	.956
VAR00003	39224.574	9	4358.286	514.004	<.001	.991
VAR00001 * VAR00003	38473.733	9	4274.859	504.165	<.001	.991
Error	339.164	40	8.479			
Total	102537.176	60				
Corrected Total	85368.126	59				

Table 24: Factorial ANOVA results for KF2-175BC (VAR00001 = medium type, and VAR00003 = pathogen).

Source	Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared
Corrected Model	73816.524 ^a	19	3885.080	246.192	<.001	.992
Intercept	54199.226	1	54199.226	3434.527	<.001	.988
VAR00001	16772.461	1	16772.461	1062.847	<.001	.964
VAR00003	27820.428	9	3091.159	195.882	<.001	.978
VAR00001 * VAR00003	29223.635	9	3247.071	205.762	<.001	.979
Error	631.228	40	15.781			
Total	128646.979	60				
Corrected Total	74447.752	59				

Table 25: Factorial ANOVA results for KF2-175BA (VAR00001 = medium type, and VAR00003 = pathogen).

Source	Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared
Corrected Model	64417.076 ^a	19	3390.372	341.932	<.001	.994
Intercept	13322.568	1	13322.568	1343.632	<.001	.972
VAR00001	486.913	1	486.913	49.107	<.001	.557
VAR00003	60689.096	9	6743.233	680.081	<.001	.994
VAR00001 * VAR00003	2828.705	9	314.301	31.698	<.001	.880
Error	386.698	39	9.915			
Total	77395.486	59				
Corrected Total	64803.774	58				

Table 26: Factorial ANOVA results for KF2-175AK (VAR00001 = medium type, and VAR00003 = pathogen).

Source	Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared
Corrected Model	32826.442 ^a	19	1727.707	105.053	<.001	.980
Intercept	3643.575	1	3643.575	221.547	<.001	.847
VAR00001	4627.522	1	4627.522	281.375	<.001	.876
VAR00003	17009.268	9	1889.919	114.916	<.001	.963
VAR00001 * VAR00003	11189.652	9	1243.295	75.598	<.001	.944
Error	657.844	40	16.446			
Total	37127.861	60				
Corrected Total	33484.286	59				

Table 27: Factorial ANOVA results for KF2-175Y (VAR00001 = medium type, and VAR00003 = pathogen).

Source	Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared
Corrected Model	42788.525 ^a	19	2252.028	193.671	<.001	.989
Intercept	5522.722	1	5522.722	474.946	<.001	.922
VAR00001	2724.090	1	2724.090	234.268	<.001	.854
VAR00003	37370.931	9	4152.326	357.094	<.001	.988
VAR00001 * VAR00003	2693.504	9	299.278	25.738	<.001	.853
Error	465.124	40	11.628			
Total	48776.371	60				
Corrected Total	43253.649	59				

Table 28: Factorial ANOVA results for KF2-175I (VAR00001 = medium type, and VAR00003 = pathogen).

Source	Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared
Corrected Model	40426.942 ^a	19	2127.734	205.286	<.001	.990
Intercept	1121.877	1	1121.877	108.240	<.001	.730
VAR00001	13792.895	1	13792.895	1330.753	<.001	.971
VAR00003	10909.352	9	1212.150	116.949	<.001	.963
VAR00001 * VAR00003	15724.695	9	1747.188	168.571	<.001	.974
Error	414.589	40	10.365			
Total	41963.408	60				
Corrected Total	40841.531	59				

Table 29: Factorial ANOVA results for KF2-175AY (VAR00001 = medium type, and VAR00003 = pathogen).

Source	Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared
Corrected Model	49449.486 ^a	19	2602.605	195.926	<.001	.989
Intercept	5810.108	1	5810.108	437.389	<.001	.916
VAR00001	2483.372	1	2483.372	186.950	<.001	.824
VAR00003	42151.601	9	4683.511	352.578	<.001	.988
VAR00001 * VAR00003	4814.513	9	534.946	40.271	<.001	.901
Error	531.344	40	13.284			
Total	55790.938	60				
Corrected Total	49980.830	59				

Table 30: Factorial ANOVA results for KF2-175L (VAR00001 = medium type, and VAR00003 = pathogen).

Source	Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared
Corrected Model	99025.232 ^a	19	5211.854	413.013	<.001	.995
Intercept	16879.536	1	16879.536	1337.617	<.001	.971
VAR00001	37930.224	1	37930.224	3005.777	<.001	.987
VAR00003	22781.890	9	2531.321	200.594	<.001	.978
VAR00001 * VAR00003	38313.118	9	4257.013	337.347	<.001	.987
Error	504.764	40	12.619			
Total	116409.532	60				
Corrected Total	99529.996	59				

Table 31: Factorial ANOVA results for KF2-175BP (VAR00001 = medium type, and VAR00003 = pathogen).

Source	Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared
Corrected Model	106339.448 ^a	18	5907.747	564.358	<.001	.996
Intercept	39072.734	1	39072.734	3732.561	<.001	.990
VAR00001	26315.765	1	26315.765	2513.906	<.001	.985
VAR00003	38929.869	9	4325.541	413.213	<.001	.990
VAR00001 * VAR00003	40922.884	8	5115.361	488.663	<.001	.990
Error	397.787	38	10.468			
Total	142823.536	57				
Corrected Total	106737.235	56				

Table 32: Factorial ANOVA results for KF2-175BL (VAR00001 = medium type, and VAR00003 = pathogen).

Source	Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared
Corrected Model	39606.795 ^a	19	2084.568	166.185	<.001	.988
Intercept	5197.316	1	5197.316	414.338	<.001	.914
VAR00001	1085.857	1	1085.857	86.566	<.001	.689
VAR00003	19650.672	9	2183.408	174.064	<.001	.976
VAR00001 * VAR00003	18822.650	9	2091.406	166.730	<.001	.975
Error	489.203	39	12.544			
Total	45366.974	59				
Corrected Total	40095.998	58				

Table 33: Factorial ANOVA results for KF2-175BQ (VAR00001 = medium type, and VAR00003 = pathogen).

Source	Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared
Corrected Model	46072.909 ^a	19	2424.890	274.272	<.001	.993
Intercept	12185.887	1	12185.887	1378.308	<.001	.972
VAR00001	3331.950	1	3331.950	376.866	<.001	.906
VAR00003	35550.027	9	3950.003	446.773	<.001	.990
VAR00001 * VAR00003	7098.304	9	788.700	89.207	<.001	.954
Error	344.807	39	8.841			
Total	58298.788	59				
Corrected Total	46417.715	58				

Table 34: Factorial ANOVA results for KF2-175BU (VAR00001 = medium type, and VAR00003 = pathogen).

Source	Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared
Corrected Model	50937.087 ^a	19	2680.899	196.670	<.001	.989
Intercept	3046.024	1	3046.024	223.455	<.001	.848
VAR00001	4026.690	1	4026.690	295.396	<.001	.881
VAR00003	25571.841	9	2841.316	208.438	<.001	.979
VAR00001 * VAR00003	21338.556	9	2370.951	173.932	<.001	.975
Error	545.259	40	13.631			
Total	54528.370	60				
Corrected Total	51482.346	59				

Table 35: Factorial ANOVA results for KF2-175BJ (VAR00001 = medium type, and VAR00003 = pathogen).

Source	Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared
Corrected Model	33314.955 ^a	19	1753.419	100.151	<.001	.979
Intercept	9.006	1	9.006	.514	.477	.013
VAR00001	886.395	1	886.395	50.629	<.001	.559
VAR00003	22635.986	9	2515.110	143.657	<.001	.970
VAR00001 * VAR00003	9792.573	9	1088.064	62.147	<.001	.933
Error	700.311	40	17.508			
Total	34024.271	60				
Corrected Total	34015.265	59				

Table 36: Factorial ANOVA results for KF2-175BY (VAR00001 = medium type, and VAR00003 = pathogen).

Source	Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared
Corrected Model	63960.592 ^a	19	3366.347	181.109	<.001	.989
Intercept	3621.768	1	3621.768	194.851	<.001	.833
VAR00001	171.647	1	171.647	9.235	.004	.191
VAR00003	48270.331	9	5363.370	288.549	<.001	.985
VAR00001 * VAR00003	14926.189	9	1658.465	89.225	<.001	.954
Error	724.908	39	18.587			
Total	68197.515	59				
Corrected Total	64685.500	58				

Table 37: Factorial ANOVA results for KF2-175BX (VAR00001 = medium type, and VAR00003 = pathogen).

Source	Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared
Corrected Model	42494.465 ^a	19	2236.551	133.281	<.001	.984
Intercept	132.913	1	132.913	7.921	.008	.165
VAR00001	388.130	1	388.130	23.130	<.001	.366
VAR00003	24568.037	9	2729.782	162.674	<.001	.973
VAR00001 * VAR00003	17538.297	9	1948.700	116.127	<.001	.963
Error	671.228	40	16.781			
Total	43298.606	60				
Corrected Total	43165.693	59				

Table 38: Factorial ANOVA results for KF2-175AU (VAR00001 = medium type, and VAR00003 = pathogen).

Source	Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared
Corrected Model	21470.783 ^a	19	1130.041	59.659	<.001	.966
Intercept	418.742	1	418.742	22.107	<.001	.356
VAR00001	637.263	1	637.263	33.644	<.001	.457
VAR00003	15035.098	9	1670.566	88.196	<.001	.952
VAR00001 * VAR00003	5798.422	9	644.269	34.013	<.001	.884
Error	757.663	40	18.942			
Total	22647.188	60				
Corrected Total	22228.446	59				

