

**THE DEVELOPMENT AND APPLICATION OF A SIMPLIFIED BIOACTIVITY  
PROFILING STRATEGY TO NATURAL PRODUCT DISCOVERY**

by

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## **ABSTRACT**

Natural products continue to be a promising source of new drug leads, however, better prioritization techniques are constantly needed. Bioactivity profiling is a technique that facilitates the discovery of bioactive compounds with unique modes of action or targets from natural product extracts. This study aimed to investigate a simplified method of bioactivity profiling to determine the potential limitations with respect to screening concentrations, and matrix effects due to the extract surrounding. The results showed that the screening concentration affected bioactivity profiles and that bioactivity profiles of antimicrobial compounds in an extract matrix were different to those of antimicrobials alone. This study improved the Natural Product Research Group's bioactivity profiling technique and could be a promising tool for prioritizing collections of natural product extracts.

## **DEDICATION**

To Mila and Kiro, for always giving me a reason to smile.

## **ACKNOWLEDGEMENTS**

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## List of Abbreviations

ATCC	American Type Culture Collection
BioMAP	Antibiotic mode of action profile
DMSO	Dimethyl sulfoxide
MIC	Minimum inhibitory concentration
MGIT	Mycobacteria growth indicator tube
NPRG	Natural Products Research Group
OD	Optical density
PC	Principal component
PCA	Principal component analysis
$\Delta$ OD	Change in optical density

## **Chapter 1: General introduction**

Natural products continue to be an excellent source of new drug leads (Aminov, 2017; Davies and Davies, 2010; Kirst, 2013; Lewis, 2017; Walsh and Wencewicz, 2014). Natural products are non-essential organic compounds that play an important role in the defense mechanism of the living organisms that produces them, and these naturally occurring organic molecules have been the major source of new antimicrobials since the discovery of penicillin in 1932 (Aminov, 2017; Davies and Davies, 2010; Kirst, 2013; Lewis, 2017, Walsh and Wencewicz, 2014). Approximately two-thirds of current antimicrobial agents are from natural products and most were discovered during the ‘golden age of antibiotic discovery’, between 1940 and 1960 (Aminov, 2017; Durand et al., 2019; Wright et al., 2014). Since the late 20th century, many pharmaceutical companies have abandoned their natural product program and instead invested in the creation of large collections of small synthetic molecules, resulting in an overall decline in the discovery rate of new drug leads (David et al., 2015; Harvey et al., 2015; Katz and Baltz, 2016; Li and Vederas, 2009; Shen, 2015). However, decades of research have produced many collections of extracts, and any one of them could have chemical compounds with potential as new therapeutic drugs (Atanasov et al., 2015; Kirst, 2013; Molinari, 2009). Researchers are now faced with the challenge of investigating different approaches to effectively and efficiently pursue these collections in order to identify new antimicrobial lead compounds (Gaudêncio and Pereira, 2015; Harvey et al., 2015; Hubert et al., 2017; Wong et al., 2012).

One approach to finding new drug leads is to prioritise collections of extracts by rapidly recognizing and disregarding extracts with known compounds and biological activity (Harvey et al., 2015; Higginbotham et al., 2014; Jacob et al., 2019; Molinari, 2009; Wong et al., 2012; ). Members of the Linington research group at the University of

California addressed the need for finding new antimicrobial lead compounds from natural products (Wong et al., 2012). Wong and colleagues (2012) developed a method of prioritisation using the biological activity of natural product extracts to find not only new antibiotic compounds, but antibiotic compounds with modes of action that were different from current therapeutics (Wong et al., 2012). The bioactivity of each extract was tested against a panel of bacterial pathogens to create a unique biological fingerprint or barcode that is known as the bioactivity profile for that extract (Wong et al., 2012). By comparing the bioactivity profiles of extracts and commercial antimicrobial compounds, Wong and colleagues (2012) were able to test the hypothesis that antibiotic mode of action profile (BioMAP) screening could be used to identify extracts that had novel compounds with antibiotic activity, thus helping prioritise collections of natural product extracts (Wong et al., 2012).

In BioMAP screening, the bioactivity data of extracts and commercial antimicrobials was normalized to generate unique bioactivity profiles (Wong et al., 2012). Natural product extracts are complicated mixtures of compounds (Harvey et al., 2015; Hubert et al., 2017). In an attempt to control for any concentration differences among different compounds in a given extract, the bioactivity data was normalized or standardized to the highest percent inhibition obtained (Wong et al., 2012). Extracts and antimicrobial compounds were serially diluted and screened against a panel of fifteen pathogens to obtain percent inhibition values (Wong et al., 2012). The percent inhibition values were then used to determine minimum inhibitory concentration (MIC) values for all extracts and antimicrobial compounds against all target organisms (Wong et al., 2012). The MIC values were normalized to generate a unique, concentration-independent, bioactivity profile or

“fingerprint” profile for each extract (Wong et al., 2012). The bioactivity profiles could then be used to prioritise the collection of natural product extracts (Wong et al., 2012).

Overall, the BioMAP technique proved to be an effective method for rapidly prioritizing a collection of natural product extracts. BioMAP profiles of extracts were compared to BioMAP profiles generated for 72 known antibiotic compounds in a hierarchical cluster analysis (Wong et al., 2012). Using the BioMAP technique, the Linington group could then focus on extracts that did not group or cluster with known antibiotic compounds and further investigate these extracts for their unique bioactivity (Wong et al., 2012). The investigation of one of these extracts led to the isolation of a structurally unique naphthoquinone antimicrobial, arromycin (Wong et al., 2012). The Linington research group utilized robotics which helped them screen their collection of natural product extracts at different concentrations in serial dilutions to determine the MIC values for each extract quickly (Wong et al., 2012). Unfortunately, while effective, the approach used by this research group is expensive and not accessible to most natural product research groups.

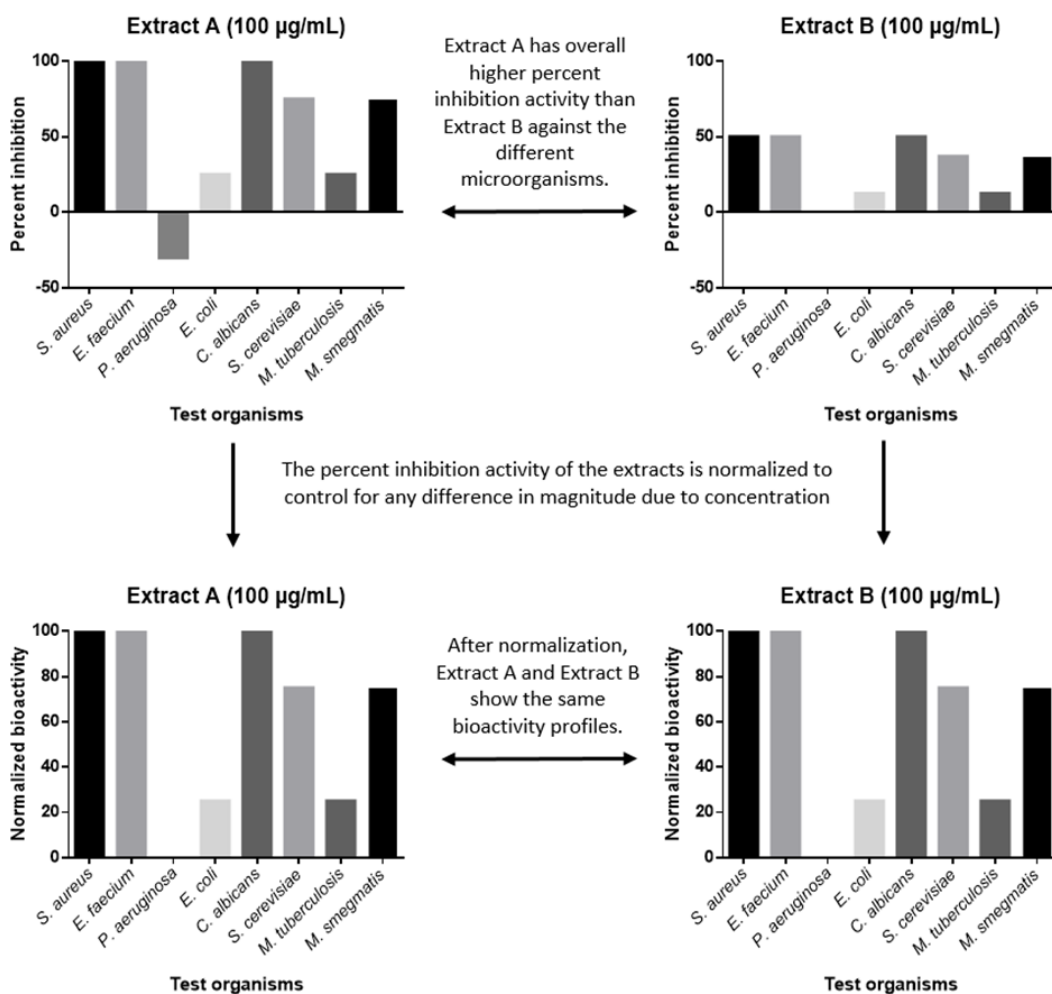
The Linington group technique inspired Andrew Flewelling (2018) to develop a simplified approach to bioactivity profiling. Flewelling made some modifications to the BioMAP technique so that it would be less labour intensive and costly in order to make it more accessible to a wider range of research laboratories (Flewelling, 2018). One of the modifications that Flewelling investigated was using the bioactivity generated from screening all extracts and commercial antimicrobials at a single concentration (Flewelling, 2018). Instead of finding the MIC values of extracts and commercial antimicrobials, all extracts were screened at 100 µg/mL and all antimicrobial compounds were screened at

1% of the extract concentration (1 µg/mL) to approximate the concentration of a bioactive compound within an extract (Flewelling, 2018). This would allow for the comparison of bioactivity profiles of extracts and antimicrobials (Flewelling, 2018). Since the concentration of bioactive compounds within complex natural product extracts is unknown, bioactivity data is normalized to avoid any bias due to the concentration of compounds within an extract, and to allow for comparison bioactivity data for antimicrobial compounds is also normalized. Figure 1.1 depicts the process of normalizing bioactivity data of extracts.

The MIC allows us to determine the lowest concentration of an extract or an antimicrobial that will inhibit activity. A disadvantage of not determining the MIC is that extracts or antimicrobials screened above their MIC could have artificially inflated bioactivity profiles and this could be a problem when determining outliers using multivariate statistical analysis. Some extracts that should have been identified as outliers could be missed in the analysis, while others will be identified as outliers when in fact they should not have been. However, the extent of the problem from screening all extracts and antimicrobials at a single concentration is unknown and warrants further investigation.

Another modification of Flewelling's technique was to use fewer antimicrobial standards to compare against the collection of natural product extracts (Flewelling, 2018). Seventeen commercial antimicrobials from six different antimicrobial classes representing a wide range of modes-of-action as standards were used for comparison against the extracts, compared to the 72 antibiotics used by Linington (Flewelling, 2018). Despite these modifications, a 3R,5R-dihydroxyhexanoic acid oligomer that had only been previously isolated once from the *Manilkara* sp. endophyte *Daldinia concentrica* was

isolated using the Flewelling method (Flewelling, 2018; Maloney, 2007). Therefore, this simplified approach to bioactivity profiling developed by Flewelling did show limited success (Flewelling, 2018).



**Figure 1.1.** Process depicting the normalization of extracts at the Natural Product Research Group. While Extract A and Extract B have different percent inhibition values, once they are normalized they have the same bioactivity profiles. The difference in magnitude due to the concentration of the compounds within the extract is eliminated by taking the maximum inhibition value and dividing all the percent inhibition by that value. Furthermore, any negative percent inhibition values are converted to zero as the Natural Product Research Group (NPRG) is interested in finding compounds within extracts that inhibit the growth of the pathogens that are being tested, not ones promoting growth.

While the bioactivity profiling technique developed by Flewelling (2018) did show some promise, it requires further investigation of some unknown effects based on concentration and the matrix effect of complex extracts and commercial antimicrobial compounds. Additional tests would determine if using this modified technique is an effective way to prioritize collections of natural product extracts based on their bioactivity, and thus help find compounds with antimicrobial activity within extracts. The objectives of this thesis were to investigate the effect of changing the screening concentration of extracts and antimicrobial compounds and to conduct a test to verify whether antimicrobial compounds can be detected in an extract mixture. Furthermore, this research will provide a tool to help future work focus on promising extracts showing unique antimicrobial activity and potential as new drug leads.

## **Chapter 2: Investigating concentration effects in bioactivity profiles**

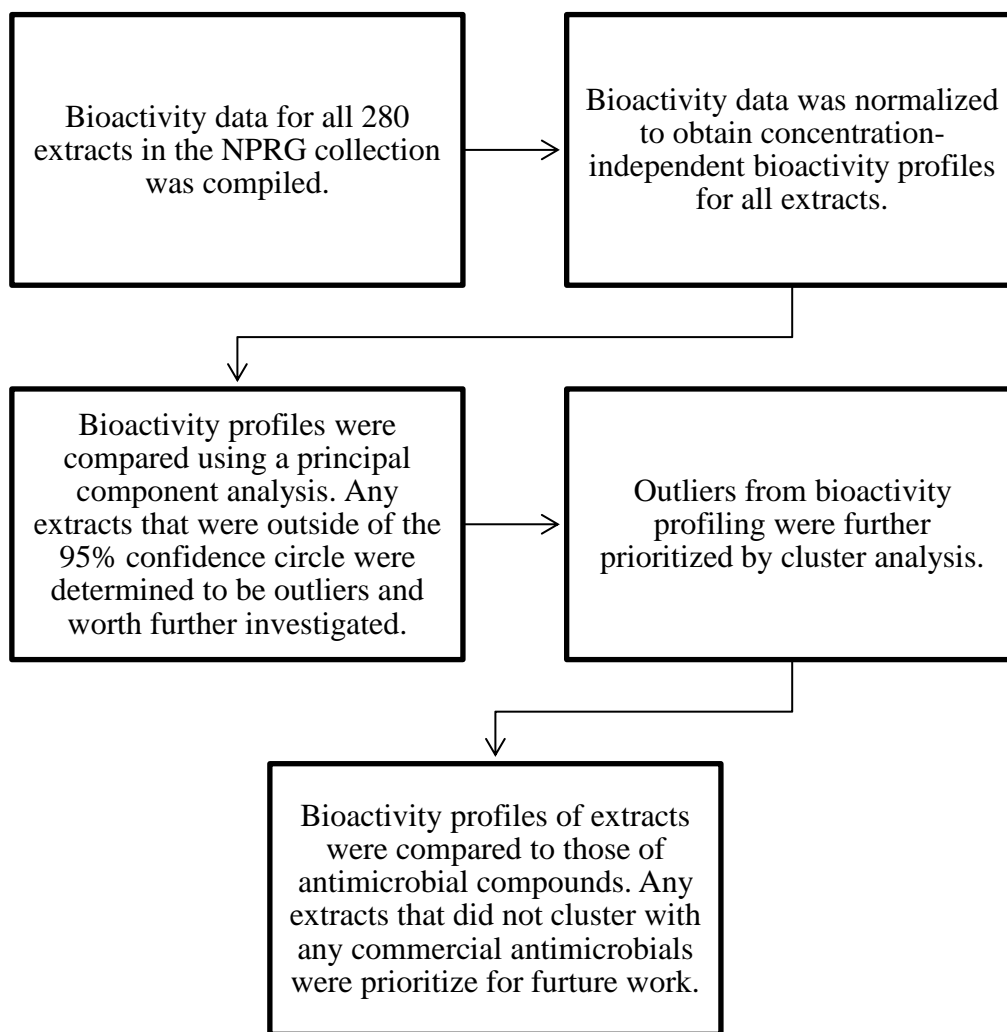
## **2.1 Introduction**

The bioactivity profiling technique developed by Flewelling (2018) was used to prioritise a collection of 280 natural product extracts from the NPRG collection (Appendix 1). Further investigation of this technique has led to the consideration of effects not yet investigated. For example, some extracts showed 100% inhibition when screened at 100 µg/mL, and their bioactivity profiles could be artificially inflated as a result of screening the extracts above their MIC. To test whether this effect was a potential limitation, the effect of diluting an extract or an antimicrobial on the resulting bioactivity profile was investigated. Therefore, the two objectives of this chapter were to apply Flewelling's bioactivity profiling technique to a collection of natural product extracts and to investigate concentration effects in bioactivity profiles of commercial antimicrobials and extracts by screening them at different concentrations, comparing their bioactivity profiles using a cluster analysis.

## 2.2 Experimental

### 2.2.1 The application of a bioactivity profiling strategy to a collection of natural product extracts

The bioactivity profiling technique developed by Flewelling (2018) was applied to prioritize the NPRG collection of 280 extracts (Appendix 1). The detailed experimental procedure for this bioactivity profiling approach is described below. However, a summary of the steps is shown in Figure 2.1.



**Figure 2.1.** An overview of the bioactivity profiling approach developed by Flewelling (2018); used to prioritise the NPRG collection of 280 extracts.

### **2.2.1.1 Creation of bioactivity profiles for a collection of natural product extracts**

The Natural Products Research Group (NPRG) has a collection of 280 fungal extracts (Appendix 1). The percent inhibition value for each extract when screened at 100 µg/mL against eight test microorganisms (*Staphylococcus aureus* (ATCC 29213), *Enterococcus faecium* (ATCC 35667), *Pseudomonas aeruginosa* (ATCC 10145), *Escherichia coli* (ATCC 25922), *Saccharomyces cerevisiae* (ATCC 9763), *Candida albicans* (ATCC 14053), *Mycobacterium tuberculosis* strain H37Ra (ATCC 25177) and *Mycobacterium smegmatis* (ATCC 700084)) was compiled and used to create bioactivity profiles as follows:

1. All negative percentage inhibition values were converted to zero.
2. The maximum percentage inhibition value for an extract against the eight test pathogens was identified.
3. All percentage inhibition values for each extract were divided by the maximum value of that extract to create a concentration-independent ratio of activities between 0 and 1.

### **2.2.1.2 Identification of unique bioactivity profiles from a collection of natural product extracts**

Principal component analysis (PCA) was performed on the bioactivity profiles of all extracts to identify unique bioactivity profiles within the data set (Unscrambler 10.3, CAMO Software). The data was mean-centered and eight principal components (one for each test microorganism) were created for the analysis. When data is mean centered, the mean of each variable is subtracted from the variable's data value, so that the empirical mean (average) is zero (Dufour, 2008). Mean-centering allows the minimization of

collinearity or correlations between the independent variables. Every combination of principal component (PC) was plotted (PC1 vs. PC2 through PC7 vs. PC8) and unique bioactivity profiles or outliers were identified as those located outside of the 95% confidence interval of each PCA plot.

### **2.2.1.3 Comparison of unique extract bioactivity profiles to those of commercial antimicrobials**

Hierarchical cluster analysis using Cluster 3.0 (de Hoon et al. 2004) with a Euclidean distance measure and average linkage clustering method was performed to compare the bioactivity profiles of 17 commercial antimicrobials with the bioactivity profiles of extracts identified as outliers in the NPRG collection using PCA. These 17 commercial antimicrobials were actinomycin D, amoxicillin, amphotericin B, chloramphenicol, ciprofloxacin, erythromycin, ethambutol, gentamicin, isoniazid, kanamycin, miconazole, nystatin, rifampin, streptomycin, sulfamethazine, tetracycline, and vancomycin. The hierarchical cluster analysis dendrogram was visualised using Java Treeview (Saldanha 2004), whereby clusters were identified where bioactivity profiles of extracts and/or antimicrobial standards possessed correlations  $\geq 0.80$ .

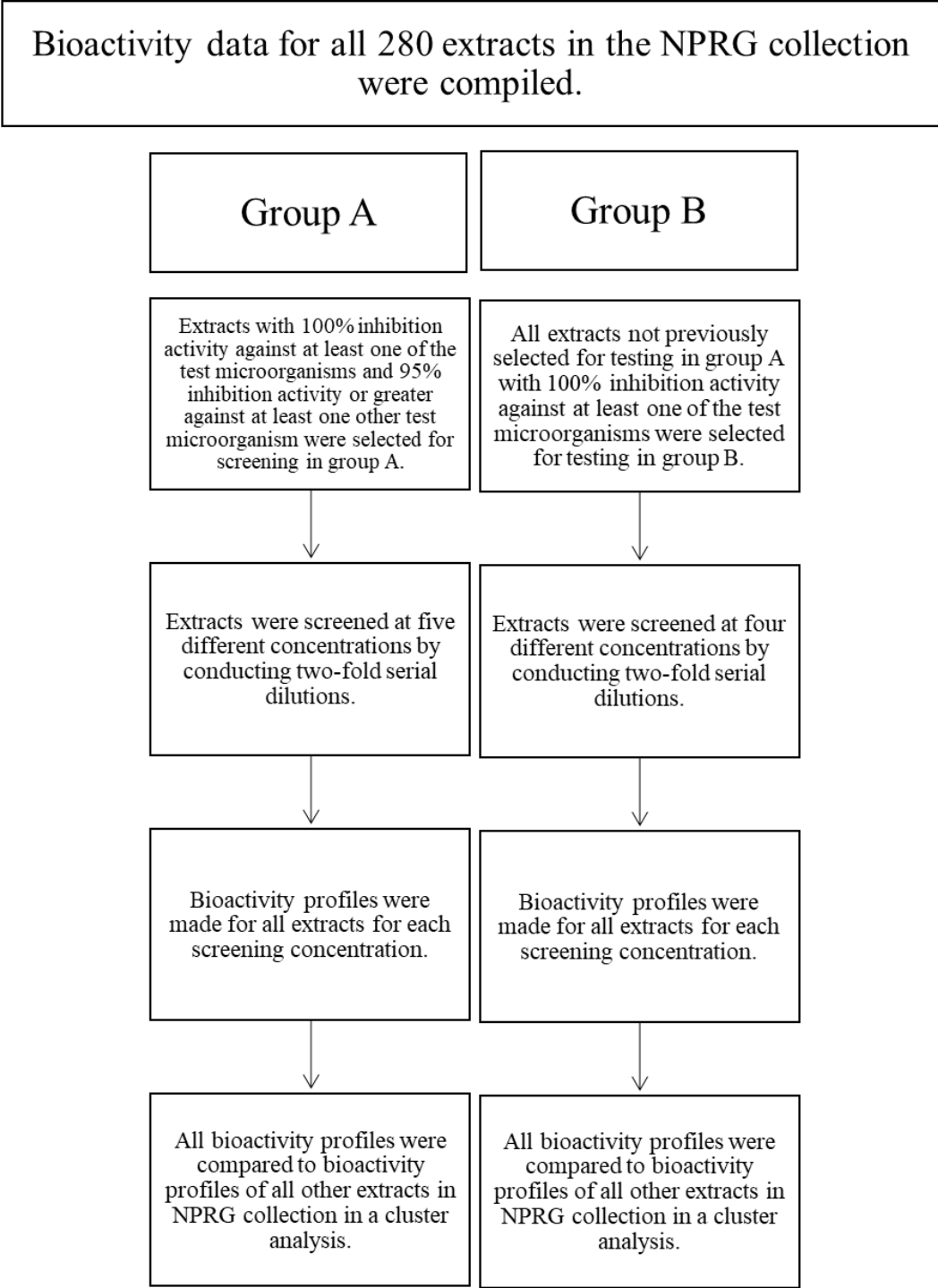
## 2.2.2 Testing concentration effects in bioactivity profiles for natural product extracts and commercial antimicrobial compounds

The screening concentration of extracts and antimicrobials was investigated to determine whether it is a potential limitation of Flewelling's bioactivity profiling technique. To test the effect of concentration in bioactivity profiles, three groups of samples were tested at different concentrations in dilution testing experiments (Table 2.1).

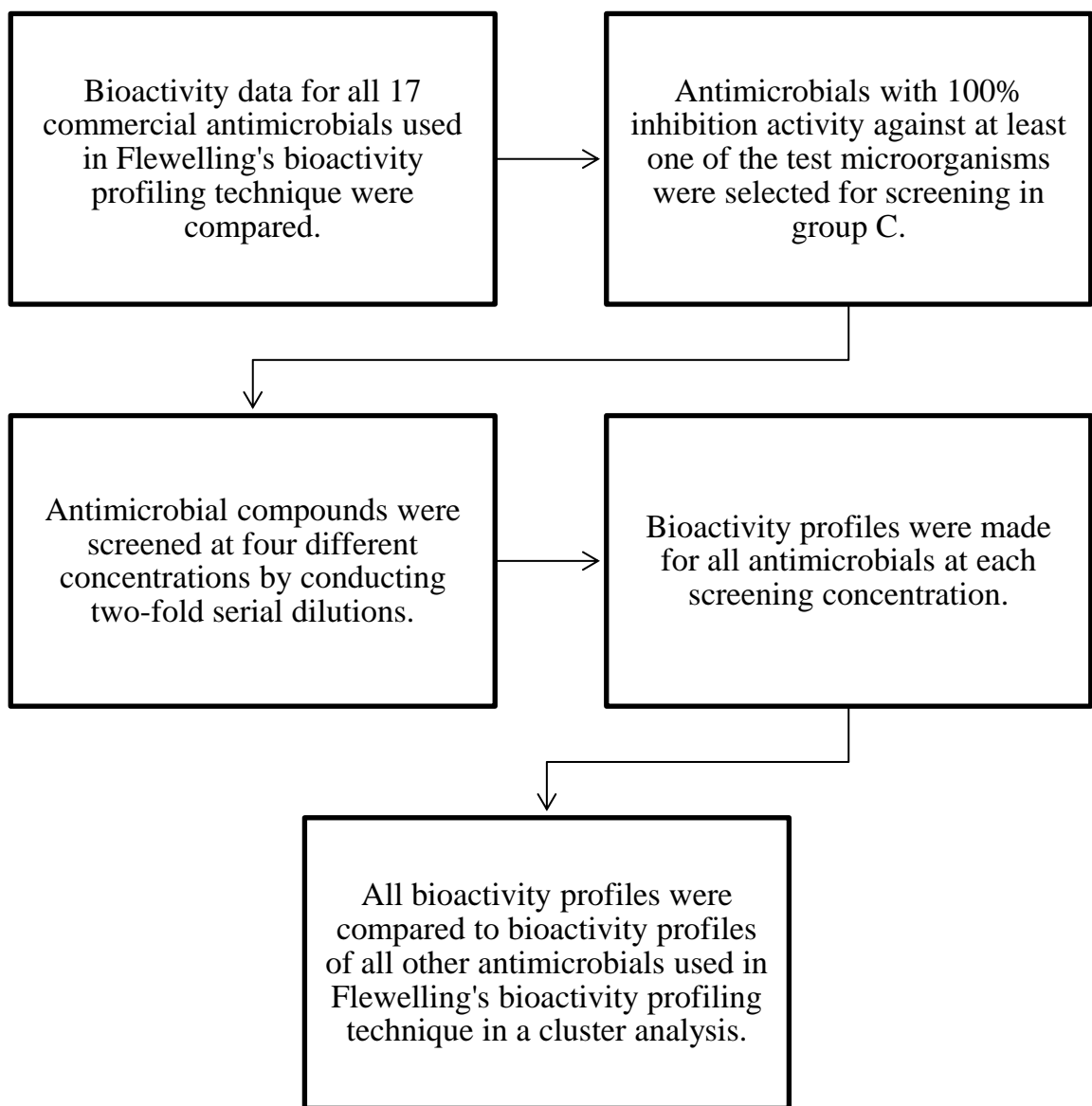
**Table 2.1.** Samples from dilution testing experiments. All samples (natural product extracts from NPRG collection and commercial antimicrobial compounds) were separated into three groups (A, B, and C) and were serially diluted to obtain the different test concentrations. Samples from group A and B are natural product extracts from NPRG collection, while samples from group C are commercial antimicrobial compounds used in Flewelling's bioactivity profiling technique.

Group	Samples	Test concentrations
A	MYEX2-090	100 µg/mL
	MYEX3-110	50 µg/mL
	MYEX4-177	25 µg/mL
		12.5 µg/mL
		6.125 µg/mL
B	MYEX2-069	100 µg/mL
	MYEX2-073	50 µg/mL
	MYEX7-320	25 µg/mL
	MYEX7-334	12.5 µg/mL
	MYEX8-384	
C	Actinomycin D	1.000 µg/mL
	Amoxicillin	0.500 µg/mL
	Ciprofloxacin	0.250 µg/mL
	Gentamicin	0.125 µg/mL
	Tetracycline	
	Vancomycin	

Samples from group A and B were selected from the NPRG collection of 280 extracts, and samples from group C were selected from the panel of 17 commercial antimicrobial compounds used in Flewelling's bioactivity profiling technique. The detailed experimental procedure explaining all the steps in testing concentration effects in bioactivity profiles is described below. However, a summary of the steps is shown in Figure 2.2 and Figure 2.3.



**Figure 2.2.** An overview of the experimental steps for testing concentration effects in bioactivity profiles of natural product extracts in the NPRG collection.



**Figure 2.3.** An overview of the experimental steps for testing concentration effects in bioactivity profiles of commercial antimicrobial compounds used in Flewelling's bioactivity profiling technique.

### **2.2.2.1 Sample selection for dilution testing experiments**

To investigate concentration effects in bioactivity profiles, samples with 100% inhibition were selected from the NPRG collection of 280 extracts, and from the panel of 17 commercial antimicrobial compounds used in Flewelling's bioactivity profiling technique (Table 2.1).

Samples in group A consisted of natural product extracts from the NPRG collection. Extracts screened at 100 $\mu$ g/mL with 100% inhibition activity against at least one of the test microorganisms and 95% inhibition activity or greater against at least one other test microorganism were selected for testing. By selecting extracts with very high (greater than 95%) inhibition activity across at least two of the test microorganisms, the effect of concentration in bioactivity profiles for the same extract can be observed in different microorganisms.

Samples in group B were also natural product extracts from the NPRG collection. Extracts screened at 100 $\mu$ g/mL with 100% inhibition activity against at least one of the test microorganisms were selected for screening. However, extracts previously tested in group A were not tested again in group B.

For group C, commercial antimicrobial compounds used in Flewelling's bioactivity profiling technique screened at 1 $\mu$ g/mL with 100% inhibition activity against at least one of the test microorganisms were selected for testing.

### **2.2.2.2 Sample preparation for dilution testing experiments**

Group A samples were prepared as follows:

From the extracts in the NPRG collection, MYEX2-090, MYEX3-110, and MYEX4-177 were investigated further. Samples for bioassays were prepared from a stock solution of 5 mg/mL in dimethyl sulfoxide (DMSO) and were tested at five different concentrations (100 µg/mL, 50 µg/mL, 25 µg/mL, 12.5 µg/mL, and 6.25 µg/mL) in a microbroth dilution antimicrobial assay.

Group B samples were prepared as follows:

Five extracts (MYEX2-069, MYEX2-073, MYEX7-320, MYEX7-334, and MYEX8-384) from the NPRG collection were selected. Samples for bioassays were prepared from a stock solution of 5 mg/mL in DMSO and were tested at four different concentrations (100 µg/mL, 50 µg/mL, 25 µg/mL, 12.5 µg/mL) in a microbroth dilution antimicrobial assay.

Group C samples were prepared as follows:

Six antimicrobials (actinomycin D, amoxicillin, ciprofloxacin, gentamicin, tetracycline, vancomycin) that were used in Flewelling's bioactivity profiling technique were tested for concentration effects. Samples for bioassays were prepared from a stock solution of 1 mg/mL in DMSO and were tested at four different concentrations (1 µg/mL, 0.500 µg/mL, 0.250 µg/mL, 0.125 µg/mL) in a microbroth dilution antimicrobial assay.

### 2.2.2.3 Source of antimicrobial standards

All antimicrobial standards, including the panel of 17 antimicrobial standards used in Flewelling's bioactivity profiling technique and antimicrobials used as positive controls in bioassays, with the exception of rifampin, were obtained from Sigma Aldrich (Oakville, Ontario). Rifampin was obtained from Fisher Scientific (Ottawa, Ontario).

Stock solutions for each antimicrobial for positive control use (100 µg/mL) were prepared with either Difco™ Sabouraud dextrose broth (*C. albicans*), yeast mold broth (*S. cerevisiae*), BBL™ Mueller Hinton II cation-adjusted broth (*P. aeruginosa*, *E. coli* and *S. aureus*), BBL™ Brain Heart Infusion broth (*E. faecium*), or BBL™ modified Middlebrook 7H9 broth (*M. tuberculosis* and *M. smegmatis*) and stored at 4 °C prior to use.

### 2.2.2.4 Antifungal activity assay

Antifungal activity against *C. albicans* and *S. cerevisiae* was evaluated using a microbroth dilution antimicrobial assay. All procedures of the antimicrobial susceptibility tests, except for plate absorbance measurements, were performed in a Class II biological safety cabinet (Labconco, Kansas City, Missouri, USA). Immediately prior to use, stock solutions of each extract (5 mg/mL) and commercial antimicrobial (1 mg/mL) were serially diluted with either Difco™ Sabouraud dextrose broth (*C. albicans*) or yeast mold broth (*S. cerevisiae*; Becton Dickinson, Mississauga, Ontario) to make all concentration samples in an 4% DMSO solution in the respective broth. The samples (100 µL) were then transferred to the non-peripheral wells of a clear, 96-well microtitre plate, in triplicate (BD Falcon™, Becton Dickinson, Mississauga, Ontario). Each plate contained three positive control wells

(*C. albicans*: nystatin, 2.7  $\mu$ M; *S. cerevisiae*: amphotericin B, 2.7  $\mu$ M, 100  $\mu$ L per well). Wells were then inoculated with suspensions of either *C. albicans* or *S. cerevisiae* (100  $\mu$ L). To reduce evaporation, sterile water (200  $\mu$ L) was added to all perimeter wells. Each plate contained three negative control wells [4% DMSO in broth (100  $\mu$ L) inoculated with suspensions of the appropriate organism (100  $\mu$ L)] and three untreated blank control wells [2% DMSO in broth (200  $\mu$ L)]. Initial absorbance measurements (600 nm) were recorded prior to a 24 h incubation period (37 °C) where a final absorbance measurement was then taken for percentage inhibition determination. Optical densities (OD) were measured using a Molecular Devices Emax microplate reader. Final absorbance readings were subtracted from the initial readings to obtain the change in optical density ( $\Delta$ OD). The change in optical density values were corrected for background absorbance of the media by subtracting the mean  $\Delta$ OD readings of the blanks from the mean  $\Delta$ OD readings of the control and crude extracts wells, while the percentage inhibition of fungal growth was defined as:

$$[1 - (\text{mean test or positive control } \Delta\text{OD}/\text{mean negative control } \Delta\text{OD})] \times 100$$

#### **2.2.2.5 Antibiotic activity assay**

Antibiotic activity against *P. aeruginosa*, *E. coli*, *S. aureus*, and *E. faecium* was evaluated in the same manner as described for the antifungal activity assay. The growth medium 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 (21  $\mu$ M & 2.6  $\mu$ M, gentamicin

for *P. aeruginosa* and *E. coli* respectively; 0.85  $\mu\text{M}$  erythromycin for *S. aureus*; 1.4  $\mu\text{M}$  tetracycline for *E. faecium*; 100  $\mu\text{L}$  per well) inoculated with suspensions of the appropriate pathogen (100  $\mu\text{L}$ ).

#### **2.2.2.6 Antimycobacterial activity assay**

Antimycobacterial activity against *M. tuberculosis* and *M. smegmatis* was evaluated using a microplate resazurin assay. Immediately prior to use, stock solutions of each crude extract (5 mg/mL) and commercial antimicrobial (1 mg/mL) were serially diluted with modified Middlebrook 7H9 broth (BBL™ MGIT™, Becton Dickinson, Mississauga, Ontario) to make all concentration samples 4% DMSO solutions in broth. The samples (100  $\mu\text{L}$ ) were then transferred to the non-peripheral wells of a black, non-tissue culture treated, low-binding, 96-well microtitre plate (VWR, Mississauga, Ontario) in triplicate and inoculated with suspensions of the appropriate organism (100  $\mu\text{L}$ ). To reduce evaporation from the plates, sterile water (200  $\mu\text{L}$ ) was added to all perimeter wells. The positive control consisted of rifampin (*M. tuberculosis*, 0.012  $\mu\text{M}$ ) or ciprofloxacin (*M. smegmatis*, 3.8  $\mu\text{M}$ ) in triplicate. In addition to the positive controls, negative controls [4% DMSO in modified Middlebrook 7H9 broth (100  $\mu\text{L}$ ) inoculated with suspensions of the appropriate organism (100  $\mu\text{L}$ )], blanks [2% DMSO in modified Middlebrook 7H9 broth (200  $\mu\text{L}$ )], and test solutions (100  $\mu\text{L}$ ) in modified Middlebrook 7H9 broth (100  $\mu\text{L}$ ) were included in triplicate in each plate. *M. tuberculosis* was incubated (37 °C; 5% CO<sub>2</sub>) for three days in a humid environment and *M. smegmatis* plates were incubated for one day in an incubator set at 37 °C. Following incubation, a solution of resazurin (0.0625 mg/mL) in 5% aqueous Tween 80 (50  $\mu\text{L}$ ) was added to all non-peripheral wells. Plates were then

incubated for a further 24 h and sealed with an adhesive polyester film (50 µm; VWR, Mississauga, Ontario), and mycobacterial growth was assessed fluorometrically at 37 °C (Molecular Devices Gemini EM dual-scanning microplate 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 crude extracts by subtracting the mean fluorescence readings of the appropriate blanks from the mean fluorescence readings of the control and crude extract wells. The percentage inhibition of mycobacterial growth was then defined as:

$$[1 - (\text{mean test or positive control fluorescence} / \text{mean negative control fluorescence})] \times 100.$$

#### **2.2.2.7 Creation of bioactivity profiles for samples in dilution testing experiments**

The percentage inhibition values obtained for each extract or antimicrobial against the eight test microorganisms were used to create bioactivity profiles as previously described.

#### **2.2.2.8 Identification of clusters using hierarchical cluster analysis**

Bioactivity profiles of serially diluted extracts and antimicrobials were compared in hierarchical cluster analyses as previously described.

Samples from groups A and B were compared against all 280 extracts in the NPRG collection in a hierarchical cluster analysis, while the samples from group C were compared to all 17 antimicrobial standards used in Flewelling's bioactivity profiling technique.

## **2.3 Results and discussion**

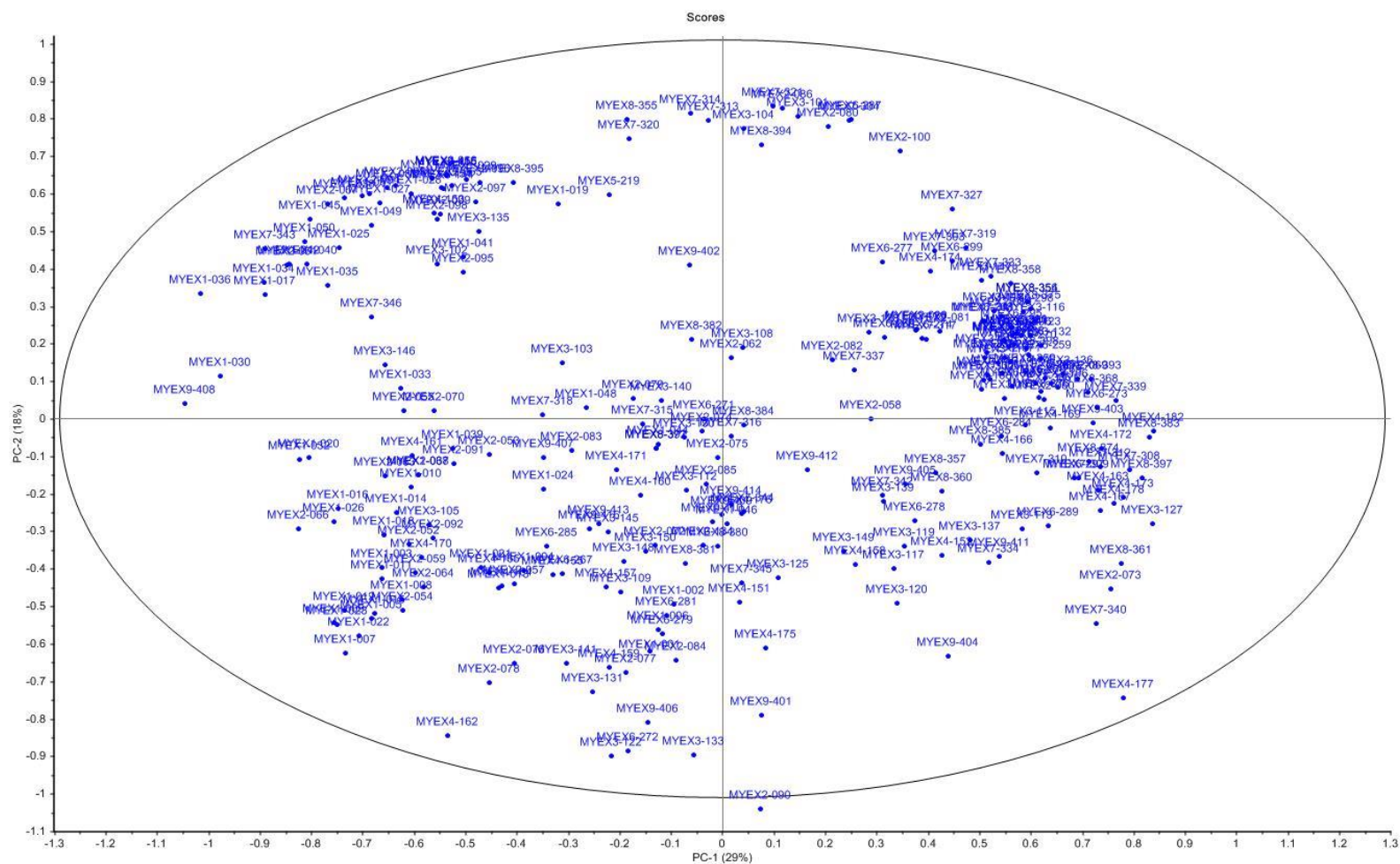
### **2.3.1 Creation of bioactivity profiles for all fungal extracts in the NPRG collection**

The percent inhibition for all 280 extracts in the NPRG collection (Appendix 2; Table A2.1) against the eight test microorganisms (*S. aureus*, *E. faecium*, *P. aeruginosa*, *E. coli*, *S. cerevisiae*, *C. albicans*, *M. tuberculosis*, and *M. smegmatis*) was compiled to create bioactivity profiles.

Bioactivity data was compiled in order to prioritize the NPRG collection of fungal extracts and in order to identify which extracts had 100% inhibition against any of the test microorganisms. When the bioassay data for all extracts in the collection was normalized, it was then used in prioritization techniques to allow for the identification of extracts that could contain new compounds with new bioactivities (Flewelling et al., 2016; Wong et al., 2012).

### **2.3.2 Principal component analysis of bioactivity profiles derived from fungal extracts in the NPRG collection**

To identify extracts within the NPRG collection of 280 fungal extracts that display unique profiles, a principal component analysis (PCA) was performed. PCA is a valuable tool for prioritizing multivariate data such as bioactivities from collections of natural product extracts, as it reduces the dimensionality and allows the simultaneous visualization of the full data set (Baker, 2016; Flewelling, 2018). While PCA is generally used to determine patterns in large data matrices, the NPRG has applied this statistical tool in a different way to help find unique extracts within a large collection (Flewelling, 2018; Wold et al., 1987). Eight principal components (one for each test microorganism) were created for the analysis, with every combination of principal component (PC) plotted (PC1 vs. PC2 through PC7 vs. PC8; example PCA plot in Figure 2.4). After every combination of principal component was investigated, 89 extracts were identified as outliers as they were located outside of the 95% confidence circle determined for the dataset within the PCA plots (Appendix 5: Table A5.1.). This analysis suggests that from the 280 endophytic fungal isolates in the NPRG collection these 89 extracts have the most unique bioactivity profiles and warrant further investigation.



**Figure 2.4.** An example of output obtained from the principal component analysis (PCA) of bioactivity profiles obtained from endophytic fungal extract from the NPRG collection. Bioactivity profiles considered outliers were identified as those found outside the 95% confidence circle for each PCA plot.

### **2.3.3 Cluster analysis of bioactivity profiles derived from fungal extracts and antimicrobial standards**

Hierarchical cluster analysis was used to measure the similarity of values between bioactivity profiles for extracts from the NPRG collection and commercial antimicrobials. The similarity is measured by the distance between clusters, which is computed based on the length of a straight line drawn from one cluster to another. A hierarchical cluster analysis (Appendix 6; Figure A6.1; Euclidean distance measure with average linkage) was used to assess the similarity between the bioactivity profiles of the 89 extracts identified as outliers in PCA and the bioactivity profiles of 17 commercial antimicrobial compounds. These antimicrobials were actinomycin D, amoxicillin, amphotericin B, chloramphenicol, ciprofloxacin, erythromycin, ethambutol, gentamicin, isoniazid, kanamycin, miconazole, nystatin, rifampin, streptomycin, sulfamethazine, tetracycline, and vancomycin. The average linkage took the average distance between each point in one cluster to every point in the other cluster. Since the Euclidean distance measure is best used for continuous data, it is used to evaluate the normalized percent inhibition data that is on a continuous scale from 0 to 1 (Almeida et al. 2007; Flewelling, 2018).

In order to determine which extracts in the NPRG collection possessed bioactivity profiles different than those of the commercial antimicrobials, clusters were identified in the dendrogram where bioactivity profiles of extracts and/or antimicrobials possessed correlations  $\geq 0.80$  (Flewelling, 2018). Previous research has described data with very strong correlations using correlation coefficient values between 0.7 and 0.9 (Chan, 2003; Flewelling, 2018; Kozak, 2009; Mukaka, 2012; Ratner, 2009). Therefore, Flewelling

(2018) chose  $\geq 0.80$  as a cut-off point for determining clusters as it was half way between the limits of this range (Chan, 2003; Flewelling, 2018; Kozak, 2009; Mukaka, 2012; Ratner, 2009).

Overall, 49 clusters were formed, and 12 of the clusters contained at least one of the antimicrobials in the NPRG training set. From the 37 clusters that did not contain any commercial antimicrobials, there were 70 extracts that did not cluster with the bioactivity profiles of the commercial antimicrobials, making these 70 extracts the top priority for future bioassay-guided fractionation.

### **2.3.4 Creation of bioactivity profiles for extracts in group A of dilution testing experiments**

Bioactivity profiles (Figure 2.5; Figure 2.6; Figure 2.7) were collated for samples in group A of dilution testing (Appendix 2; Table A2.2., and Table A2.3.). Overall, there were differences in the bioactivity profiles of each extract (MYEX2-090, MYEX3-110, and MYEX4-177) at the different concentrations (100 µg/mL, 50 µg/mL, 25 µg/mL, 12.5 µg/mL, and 6.25 µg/mL), and these will be further explained below. Bioactivity profiles for extracts at 50 µg/mL and 25 µg/mL showed the most similarities within each sample (Figure 2.5; Figure 2.6; Figure 2.7). This suggests that the bioactivity profiles of extracts that reach 100% inhibition at 100 µg/mL are different than those of the same extracts diluted to a lower concentration that do not show 100% inhibition. Therefore, future work should consider diluting extracts that reach 100% inhibition at 100 µg/mL to obtain an accurate bioactivity profile for that extract.

Extracts previously tested at 100 µg/mL had their maximum inhibition activity (100% inhibition) against some of the test microorganisms that would artificially inflate the bioactivity profile of the extract against some of the target microorganisms and therefore not represent the true shape of the bioactivity profile (Flewelling, 2018). This phenomenon was observed in MYEX2-090 with respect to *S. aureus*, *E. faecium*, and *S. cerevisiae* activity at 100 µg/mL and *E. faecium* activity at 50 µg/mL; they showed 100% inhibition activity prior to normalizing the bioactivity data (Table A2.2). This suggests that the bioactivity profile for MYEX2-090 observed at 25 µg/mL is a better representation of the bioactivity profile of that extract as the bioactivity profile is not artificially inflated since the difference in magnitude observed between the different pathogens is based on

percent inhibition readings below 100%. For MYEX3-110, 100% inhibition was observed in *S. aureus* activity at 100 µg/mL and *E. faecium* activity at 50 µg/mL, suggesting once again that the bioactivity profile at 25 µg/mL better depicts the overall bioactivity of that extract. For MYEX4-177, 100% inhibition was only observed in *S. aureus* activity at 100 µg/mL and this would indicate the bioactivity profile at 50 µg/mL is the best representation of the bioactivity profile for that extract.

Bioactivity profiles for all three extracts at 12.5 µg/mL and 6.25 µg/mL were different than all other normalized bioactivity at 100 µg/mL, 50 µg/mL, and 25 µg/mL (Figure 2.5; Figure 2.6; Figure 2.7). Sometimes, activity against a specific pathogen which was not present at a stronger concentration would reappear at a more dilute concentration, as seen in the biological activity against *E. faecium* of MYEX2-090 tested at 12.5 µg/mL, and 6.25 µg/mL (Figure 2.5). The percent inhibitions for MYEX2-090 against *E. faecium* at 12.5 µg/mL and 6.25 µg/mL were -1.9% (promotes growth) and 3.5%, respectively. Looking at the bioactivity profile for MYEX2-090 at 12.5 µg/mL and 6.25 µg/mL, it is not evident that levels of activity against *E. faecium* are within 5.4% of each other at these two concentrations. In fact, since normalized bioactivity profiles exclude negative inhibition values and the overall highest percent inhibition value is used to normalize all remaining bioactivity data, small changes from positive to negative inhibition will appear more drastic after the data is normalized than the actual values would show. For example, the percent inhibition value tends to drop as the concentration of the extract decreases, and low percent inhibition values (<25% inhibition) could be assigned a value of 1 in a normalized bioactivity profile if that activity is the highest one observed for that specific extract and concentration against all the pathogens. This explains some results where at the most dilute

concentration (6.25  $\mu\text{g/mL}$ ) the extract only has high bioactivity against a single microorganism from the test microorganisms, as was seen with MYEX4-177 (Figure 2.7).

Natural product extracts are complex mixtures and their biological activity is a result of the different compounds within them that interact together to promote and/or mask different biological activities (Caesar and Cech, 2019; Efferth and Koch, 2011; Vuuren and Viljoen, 2011; Wagner and Ulrich-Merzenich, 2009). There are many examples of not only single compounds but natural product extracts which contain many constituents producing a bimodal effect in inhibition curves (Barreto et al., 2002; Cragg et al., 1998; Dayan et al., 2000; Netikova et al., 2013; Stermitz et al., 1999). While some of the effects of double peaks in inhibition curves are a result of time-dependent inhibition or a change of drug potency over time, this phenomenon is also found in concentration-dependent inhibition curves (Aliabadi and Lees, 2000; Bell et al, 2015; Netikova et al. 2013; Yang et al, 2016).

Research has shown that there exist many different shapes and relationships other than the sigmoidal and logarithmic curves in concentration-dependent inhibition curves (Barreto et al., 2002; Netikova et al., 2013; Stebbing, 1932; Stermitz et al., 1999). Many examples have been reported where low concentrations of an extract or a single compound have shown non-linear response against various bacteria, plants, fungi, algae, and animals (Barreto et al., 2002; Stebbing, 1982). There are examples of concentration-dependent inhibition curves where there was growth promotion (positive inhibition) at low concentrations while inhibition of growth at high concentrations, and various double-peak effects where at even lower concentrations (past the point where positive inhibition occurs) the extract will inhibit growth again before promoting it at even lower extract concentrations (Barreto et al., 2002; Stebbing, 1982). This phenomenon is known as

hormesis and is best described as the stimulation of a biological response (e.g. growth) at low concentrations of an inhibitor (Luckey et al., 1975). There are many synonyms for hormesis, including paradoxical effect, double inhibition maximum, zone phenomena, inversion phenomena, bi-modal dosage response, polymodal curve, biphasic activity curve, folded curves, and second-growth stimulation (Wainwright, 1994). Hormesis effects can explain some of the results that are being found in the concentration effect test where either there are multiple peaks in the inhibition activity or growth promotion (negative inhibition) at the low concentrations. Therefore, oscillations in growth rates are a normal response to low levels of a toxin, and researchers should consider the effects of hormesis rather than blaming poor laboratory practices for their occurrences (Barreto et al., 2002; Cragg, 1998; Dayan et al., 2000; Farnsworth and Bingel, 1997).

There are two explanations for hormesis (Barreto et al., 2002). The first is a common theory for all organisms and toxicants, since the effect is seen in all species (Barreto et al., 2002; Stebbing, 1997). It states that hormesis is due to the response of an organism to it sensing its environment and over-responding to stimulus (Barreto et al., 2002; Stebbing, 1997). The second theory suggests that each organism is unique in its response to its environment, although the end response is similar in different circumstances (Hart and Frame, 1996). Each case of hormesis may be due to a unique defense mechanism (e.g. DNA-repair, stress-protein responses, and cell differentiation), and each organism may have its own unique way of reacting to the different chemical stressors (Hart and Frame, 1996).

In recent years, research that has investigated the mechanistic basis of hormesis has found that environmental phenotypic plasticity controls more than underlying receptor and

cell-signaling pathways (Calabrese and Mattson, 2017). However, ethanolic extracts from algae, methanolic wood extracts, and hot water extracts of *Thrija plicata* are only a few examples of natural product extracts shown to exhibit hormesis dissolved in different solvents (Barreto et al., 2002; Osborne and Thrower, 1964; Southam and Erlich, 1943). Furthermore, DMSO extract of propolis (a bee saliva and wax mixture) against *E. coli* revealed a double peak effect in the inhibition activity, with the first peak being the highest test concentration of 1969  $\mu\text{g}$ , and the other peak (the more intense) being observed at a lower concentration of 246  $\mu\text{g}$  (Netikova et al. 2013). Also, DMSO Czech propolis extract showed a decrease in inhibition activity against *Microsporium canis* and *Listeria monocytogenes* at the highest tested concentration of 1969  $\mu\text{g}$  (Netikova et al. 2013).

Conventional drug screening processes miss many bioactive compounds due to their low concentrations, and there is a general lack of understanding with respect to hormesis effects as most research on toxic substances focuses on the effects of high doses on test organisms (Calabrese and Mattson, 2010; Davies et al., 2006). However, the pharmaceutical industry is now more and more interested in the entire dose-response continuum and is investigating the effects of different therapeutics at low concentrations (Calabrese and Agathokleousa, 2019; Calabrese, 2018; Calabrese and Mattson, 2017).

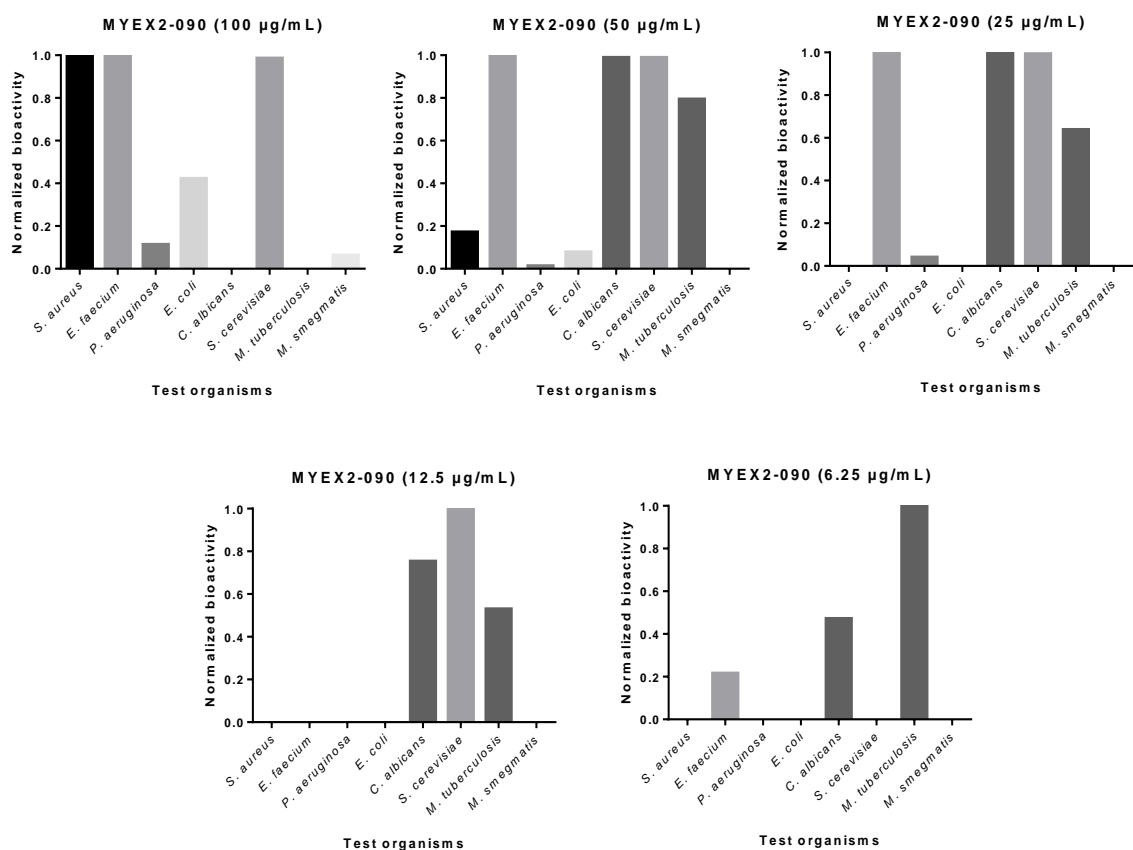


Figure 2.5. Bioactivity profiles from concentration effect testing for MYEX2-090.

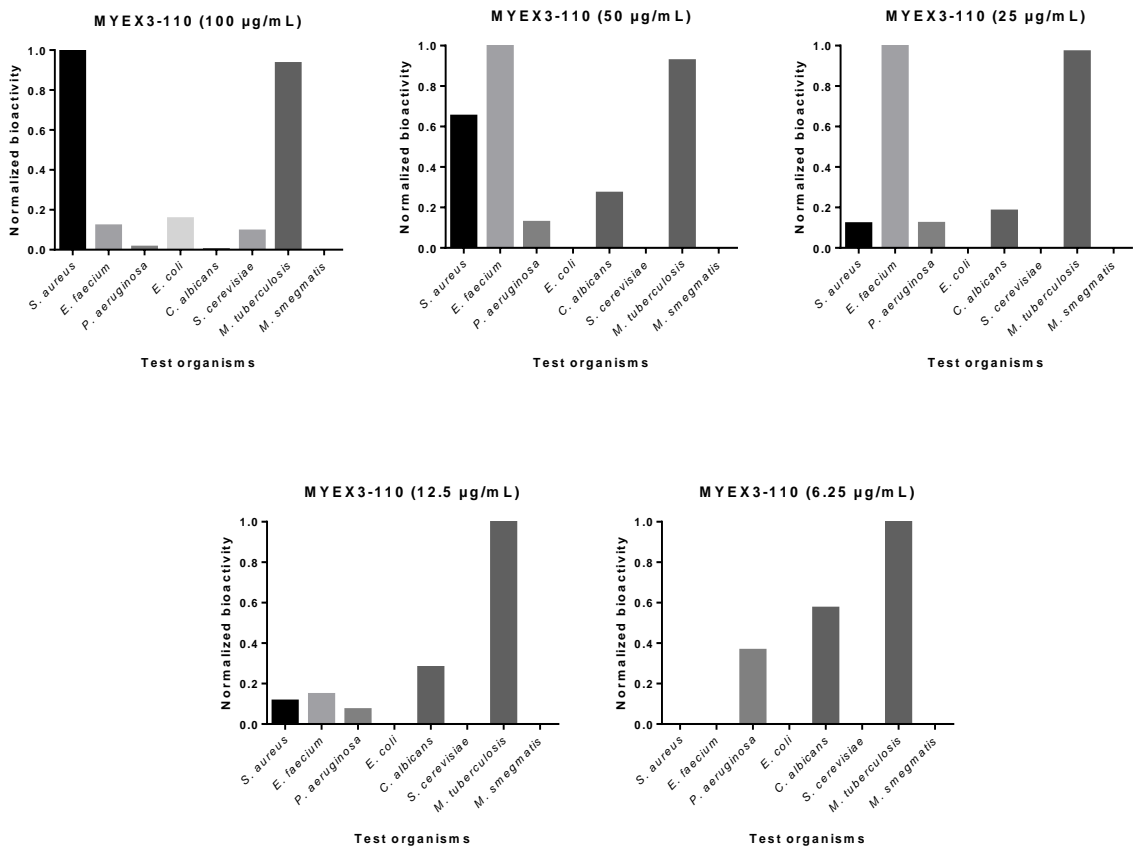
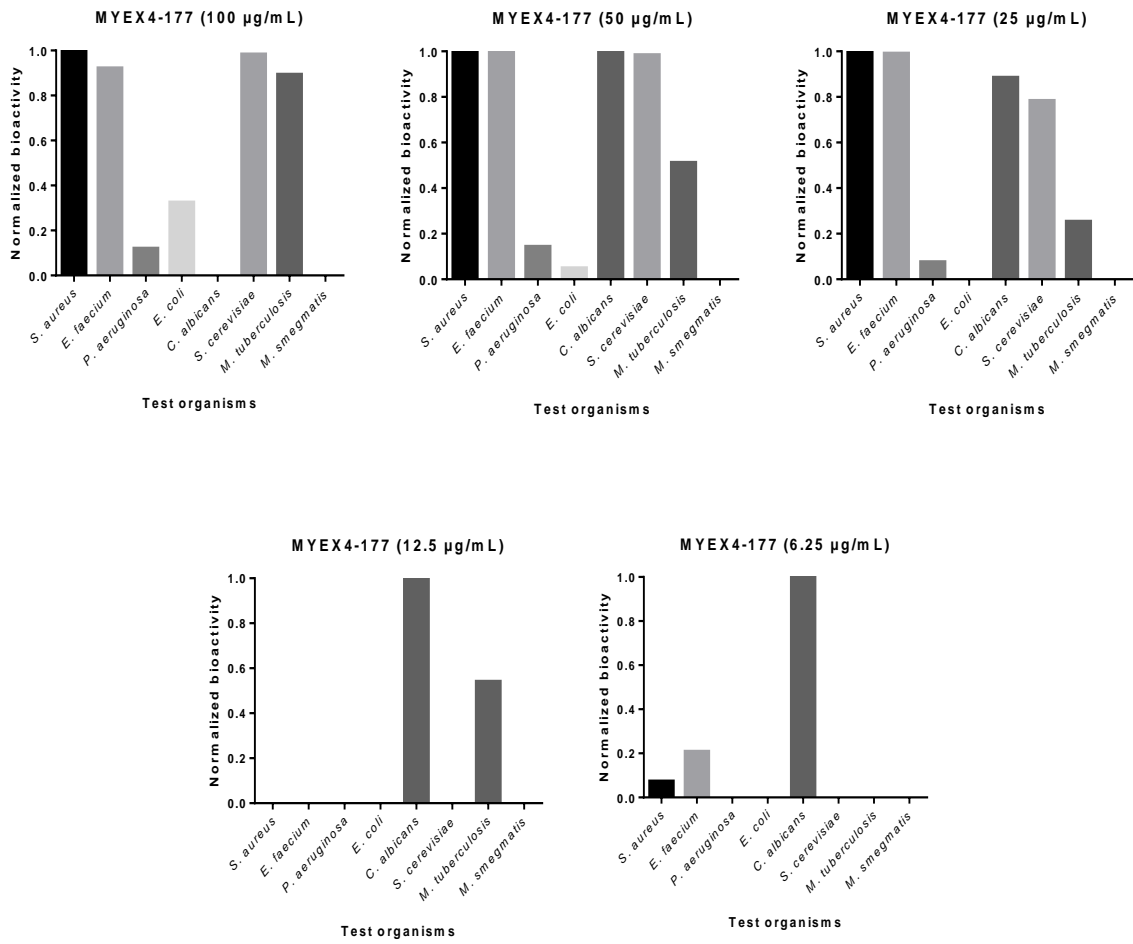


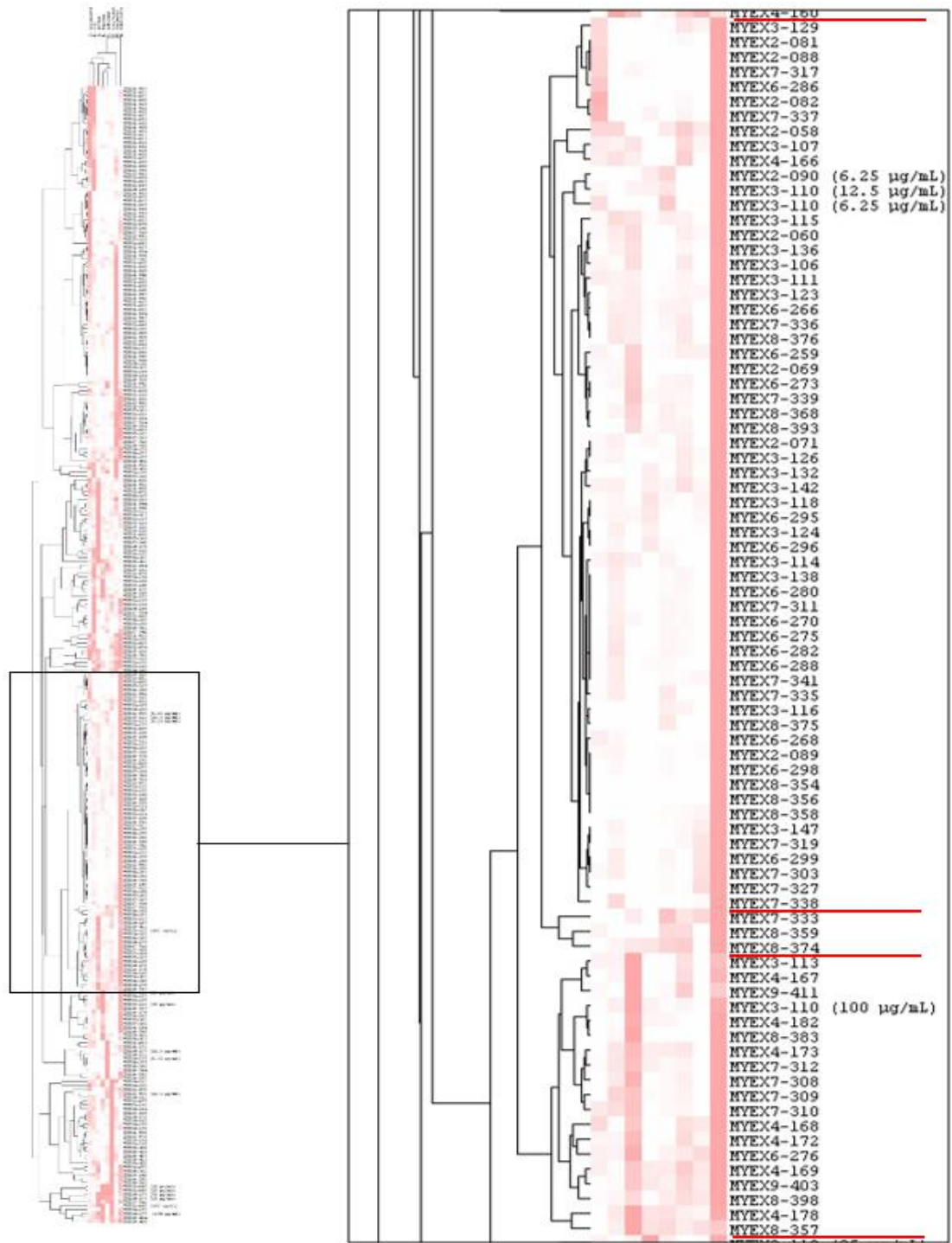
Figure 2.6. Bioactivity profiles from concentration effect testing for MYEX3-110.



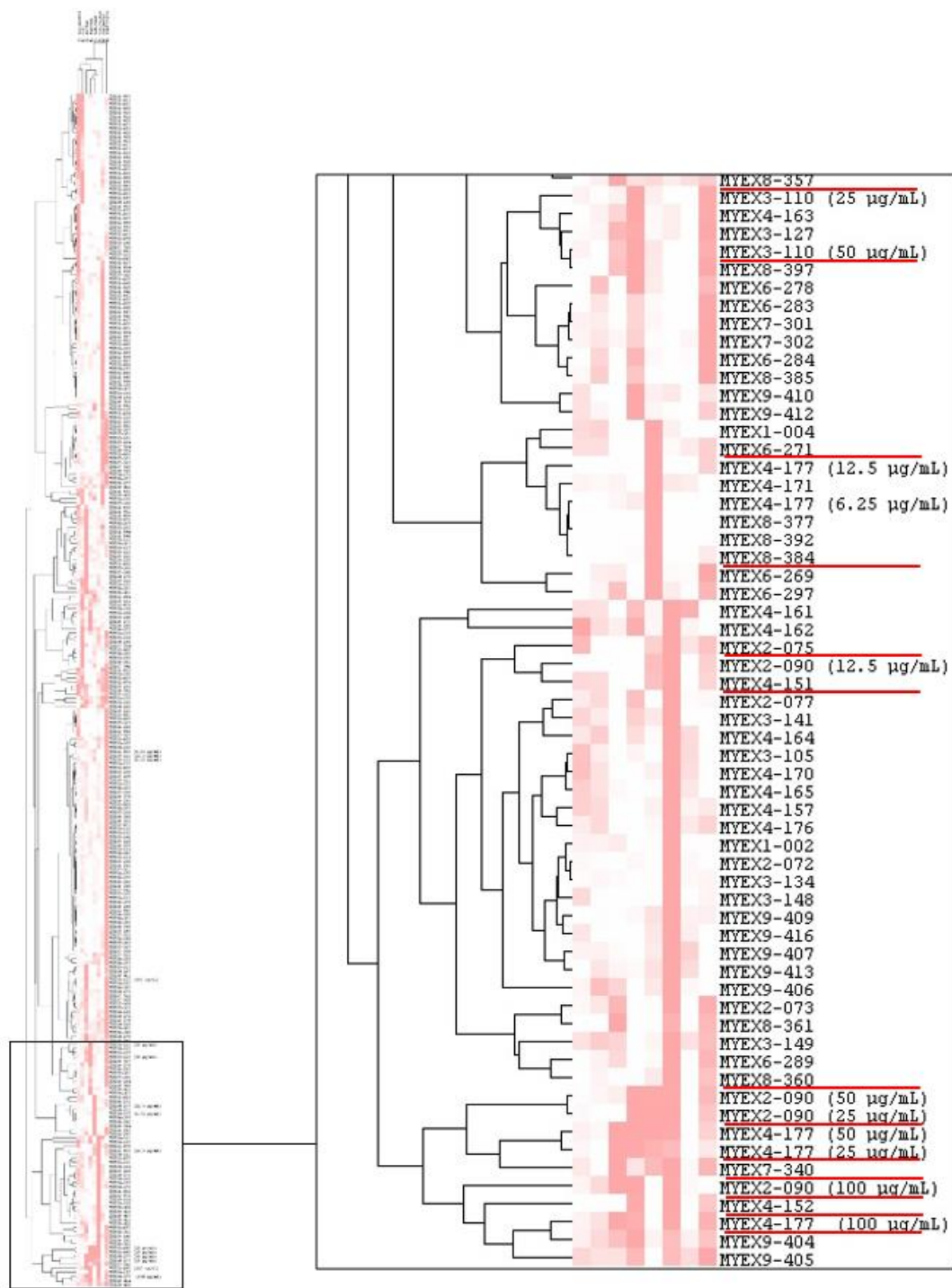
**Figure 2.7.** Bioactivity profiles from concentration effect testing for MYEX4-177.

### **2.3.5 Cluster analysis of bioactivity profiles of extracts in group A of dilution testing experiments**

A hierarchical cluster analysis is used to measure the distance or similarity within a dataset. To test the effect of concentration on bioactivity profiles of extracts from group A, a hierarchical cluster analysis was done to compare the samples to all other extracts in the NPRG collection in a hierarchical cluster analysis (Figure 2.8). MYEX4-177 at 50  $\mu\text{g}/\text{mL}$  and 25  $\mu\text{g}/\text{mL}$ , and at 12.5  $\mu\text{g}/\text{mL}$  and 6.25  $\mu\text{g}/\text{mL}$  grouped together in a cluster showing correlations  $\geq 0.80$ . Furthermore, this clustering pattern was also seen in MYEX3-110, while for MYEX2-090, only 25  $\mu\text{g}/\text{mL}$  and 12.5  $\mu\text{g}/\text{mL}$  grouped together within the same cluster. This further suggests that maximum inhibition concentrations are impacting the bioactivity profiles of extracts as they are not clustering with further dilution, and the hormesis effect is observed at different concentrations in different extracts, changing the bioactivity profiles of extracts at lower concentrations.



**Figure 2.8.** Hierarchical cluster analysis (Euclidean distance, average linkage) of bioactivity profiles of serially diluted extracts in NPRG collection from first sample group half dilution testing in relation to complete extract library. Red lines are separating the labels of clusters that include the investigated test samples, and bioactivity profiles of extracts or antimicrobials within the cluster showed correlations of  $\geq 0.80$ .



**Figure 2.8.** Hierarchical cluster analysis (Euclidean distance, average linkage) of bioactivity profiles of serially diluted extracts in NPRG collection from first sample group half dilution testing in relation to complete extract library. Red lines are separating the labels of clusters that include the investigated test samples, and bioactivity profiles of extracts or antimicrobials within the cluster showed correlations of  $\geq 0.80$  (continued).

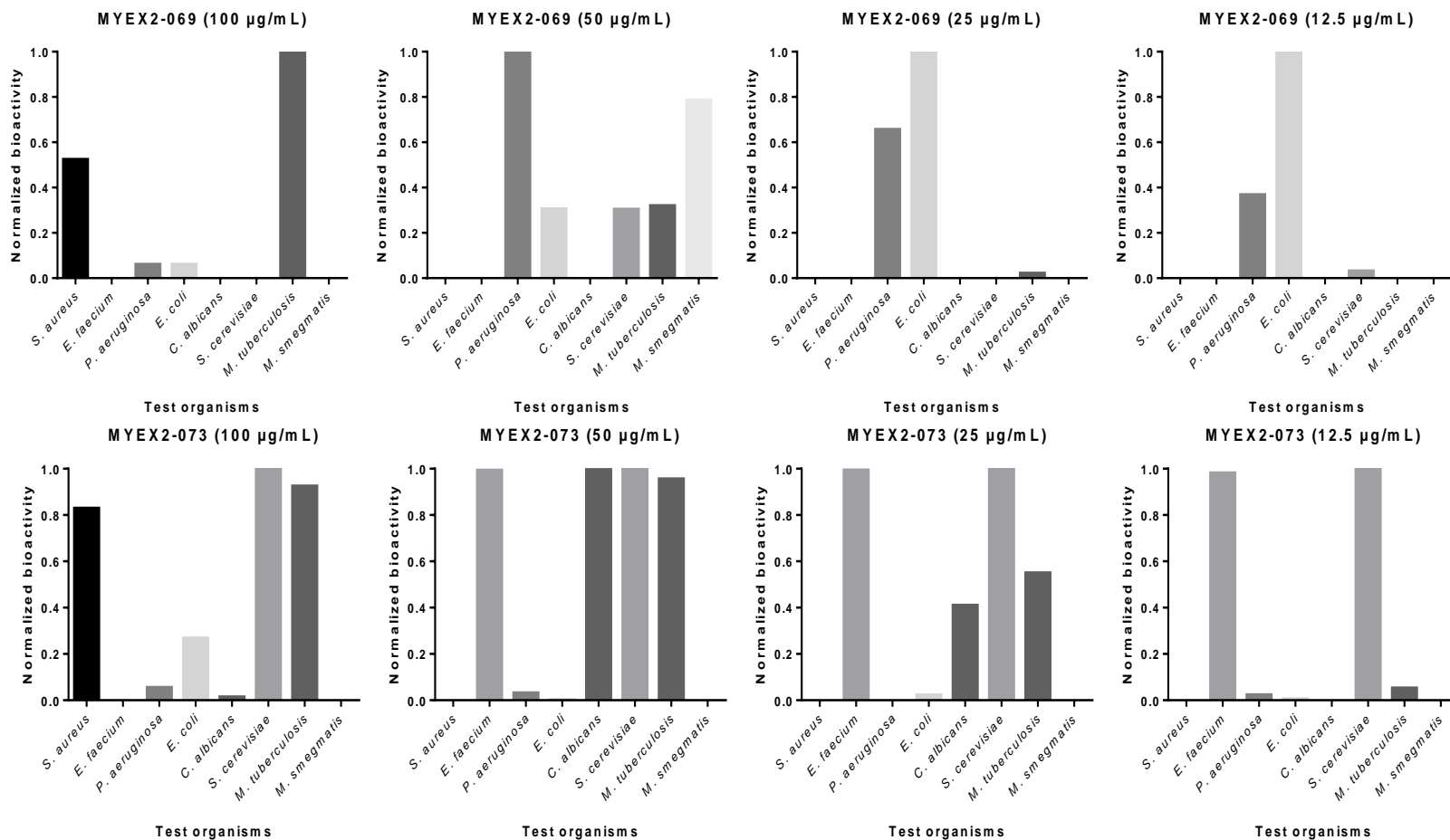
### 2.3.6 Creation of bioactivity profiles for extracts in group B of dilution testing experiments

Samples from group B (Appendix 2; Table A2.4., and Table A2.5.) showed similar results as the samples from the first group of concentration effect testing (Figure 2.9; Figure 2.10; Figure 2.11). Normalized bioactivity profiles for extracts that were tested at 100 µg/mL prior to conducting the concentration testing were visually different to the bioactivity profiles for the other test concentrations (50 µg/mL, 25 µg/mL, and 12.5 µg/mL; Figure 2.9; Figure 2.10; Figure 2.11). Overall, none of the extracts had similar bioactivity profiles across all four different concentrations (Figure 2.9; Figure 2.10; Figure 2.11).

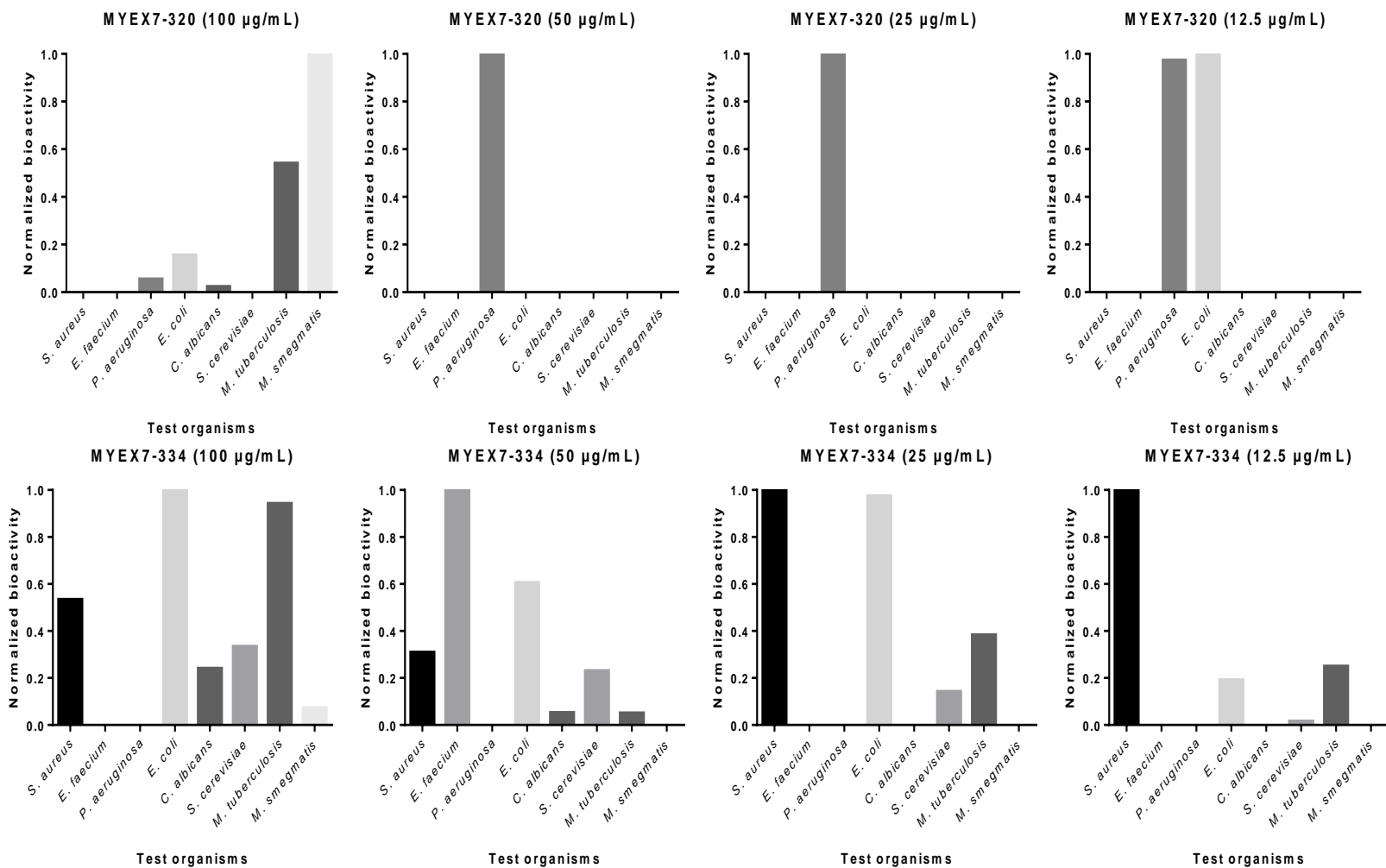
All extracts screened at 12.5 µg/mL had bioactivity profiles that looked very different to those for the other concentrations. However, since the highest percent inhibition (regardless of its true value) is normalized to a value of one, more changes in the bioactivity profiles can be seen at lower concentrations due to the variability of the inhibition values at these concentrations. For example, the bioactivity profile for MYEX7-320 had high activity for both *P. aeruginosa* and *E. coli* at 12.5 µg/mL while it only had *P. aeruginosa* activity at 25 µg/mL. While these results may seem strange, if the percent inhibition values are taken into consideration, it can be seen that both *P. aeruginosa* and *E. coli* have only 3% inhibition at 12.5 µg/mL while at the higher concentration (25 µg/mL) *E. coli* has -1%, slightly promoting growth, another example where small changes from positive to negative inhibition can seem greater in the bioactivity profiles.

Sometimes bioactivity profiles of an extract at different test concentrations looked identical, as was seen in MYEX7-320 at test concentrations of 50 µg/mL and 25 µg/mL (Figure 2.10; Figure 2.11). Sometimes an extract which had very little to no activity against

a specific pathogen at higher concentrations will show activity against this pathogen at more dilute concentrations; this was observed in MYEX7-320 (*Escherichia coli*) and MYEX7-384 (*Candida albicans*; Figure 2.10; Figure 2.11). These occurrences can be attributed to the double-peak effect as described for the samples in group A. In addition, sometimes after each half-dilution less and less bioactivity against the test microorganisms was observed, as shown in MYEX2-073; where when tested at 50 µg/mL it had very high activity against *E. faecium*, *C. albicans*, and *S. cerevisiae*, and at 12.5 µg/mL it only showed high activity against *E. faecium*, and *C. albicans* (Figure 2.11).



**Figure 2.9.** Bioactivity profiles from concentration effect testing for MYEX2-069 and MYEX2-073.



**Figure 2.10.** Bioactivity profiles from concentration effect testing for MYEX7-320 and MYEX7-334.

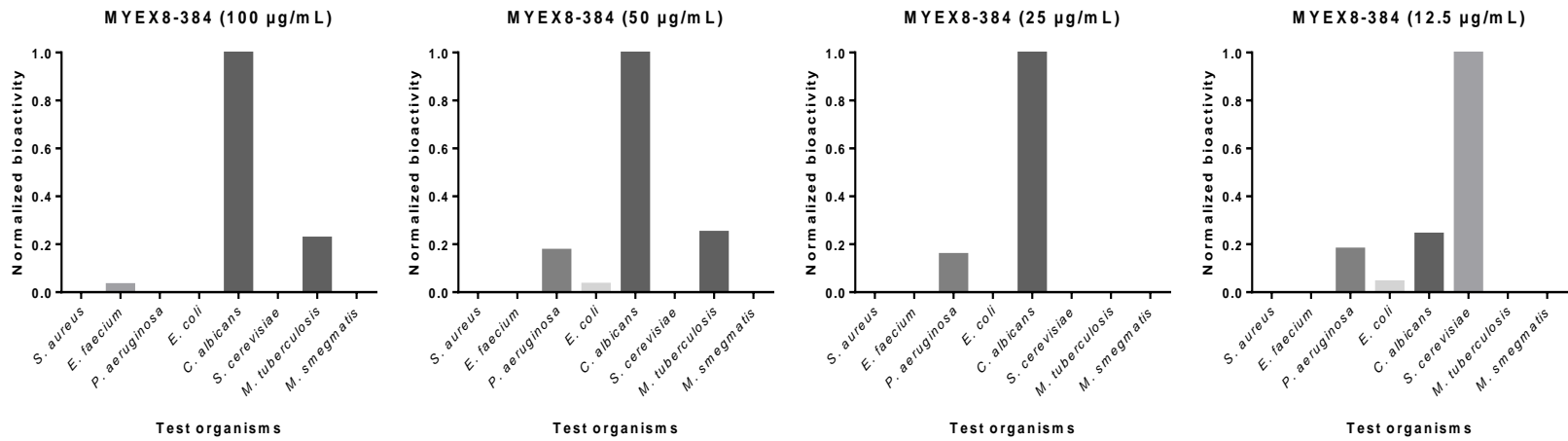
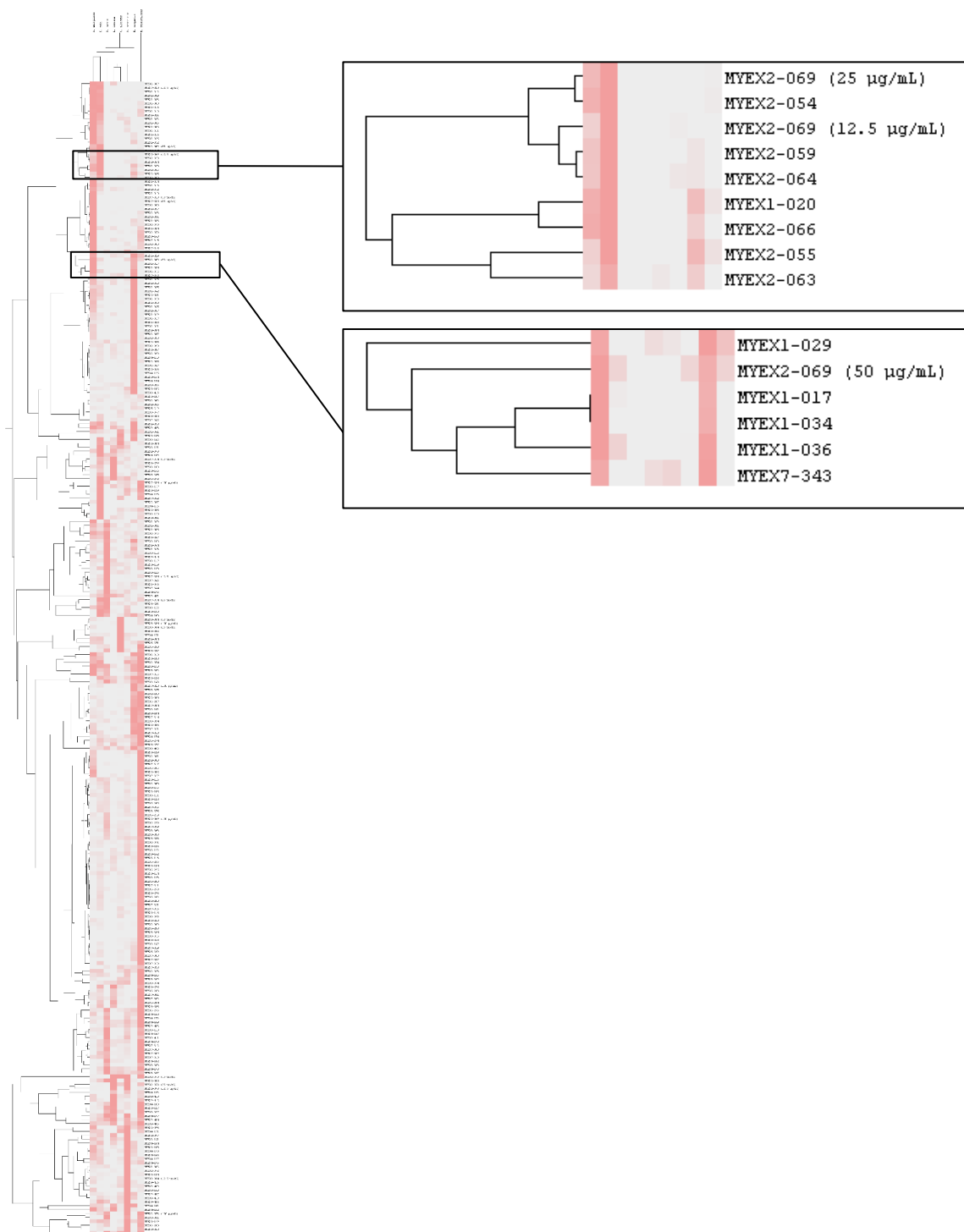


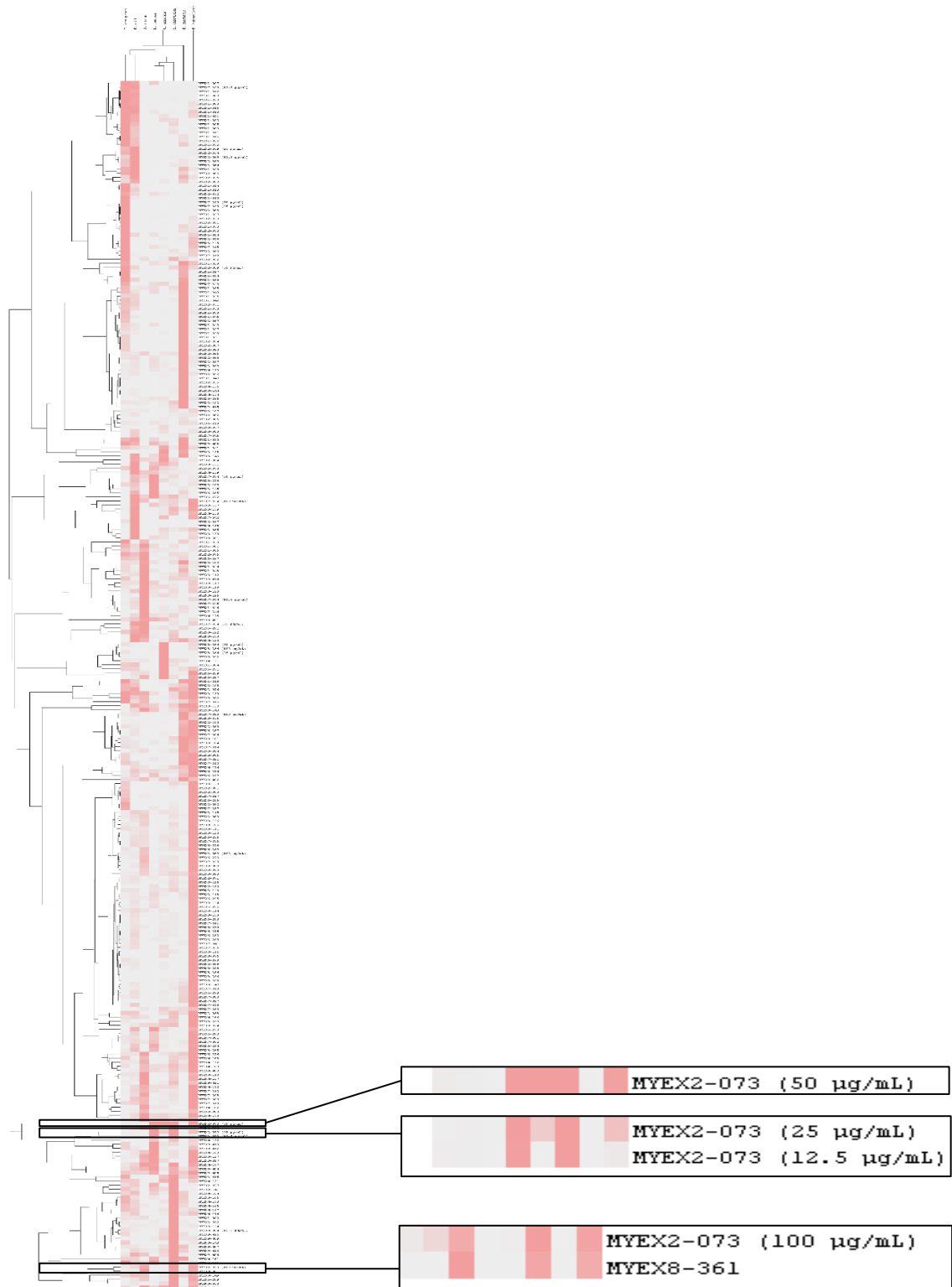
Figure 2.11. Bioactivity profiles from concentration effect testing for MYEX7-384.

### **2.3.7 Cluster analysis of bioactivity profiles for extracts in group B of dilution testing experiments**

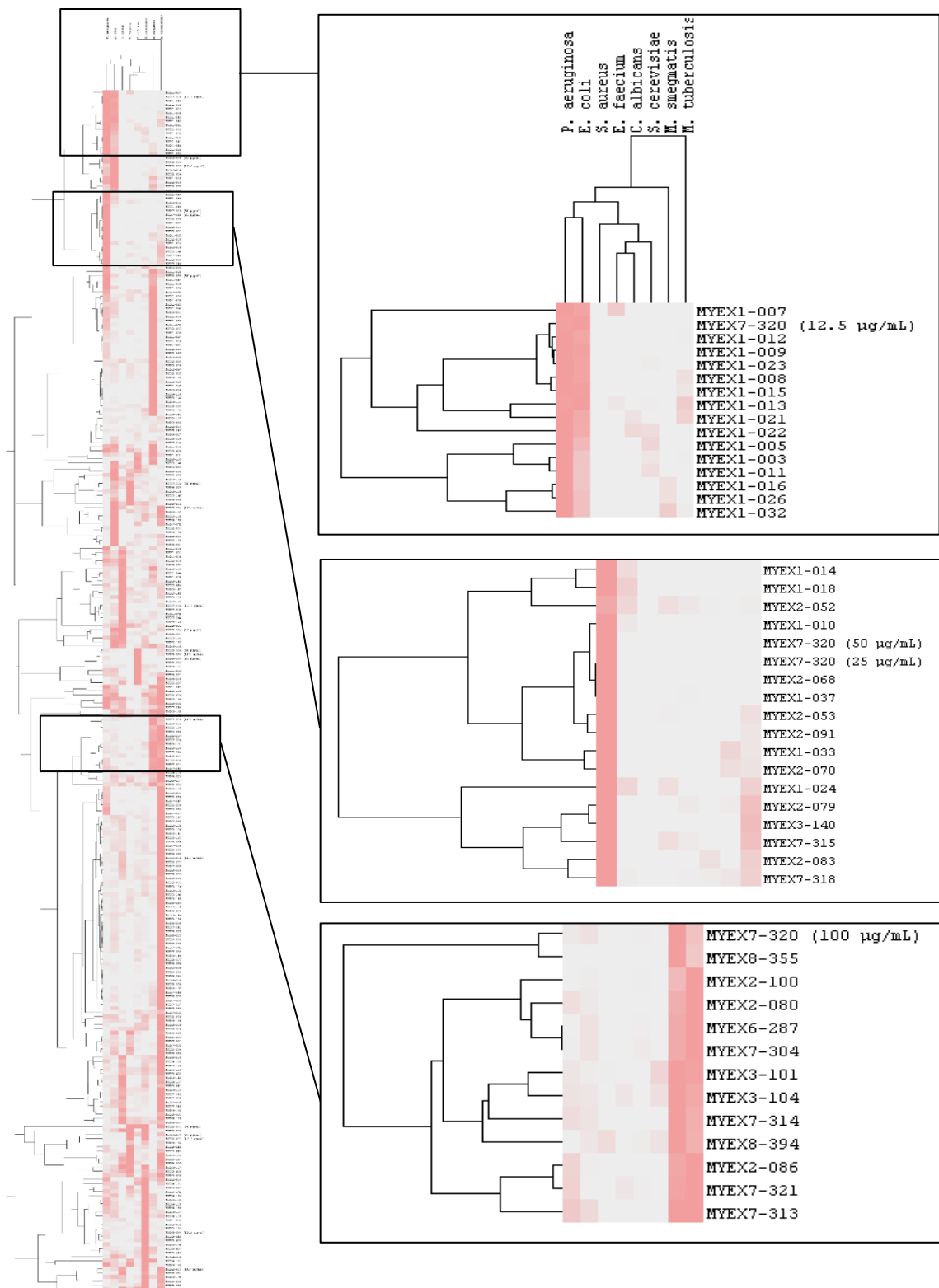
The bioactivity profiles of extracts in group B of dilution testing experiments and all other extracts in the NPRG collection were compared in a hierarchical cluster analysis (Figure 2.12). Some of the extracts only clustered when screened at 25  $\mu\text{g}/\text{mL}$  and 12.5  $\mu\text{g}/\text{mL}$  as seen in MYEX2-069, and MYEX2-073. This indicates that lower concentrations of these extracts are more similar to each other than the higher concentrations potentially due to less overall bioactivity in the lower concentrations. For all extracts except MYEX7-384, the extract screened at 100  $\mu\text{g}/\text{mL}$  did not cluster with any other concentration for that same extract suggesting that maximum inhibition strongly affects the bioactivity profiles. Some extracts like MYEX7-334 had no concentrations clustering together. While concentrations of other extracts such as MYEX8-384 all clustered together with the exception of 12.5  $\mu\text{g}/\text{mL}$ . The variability in these results could be due to hormetic effects present in many of these extractions as well as possible synergism and antagonism with the compounds within the extract contribution in different ways to the expression of bioactivity at the different test concentrations as previously described.



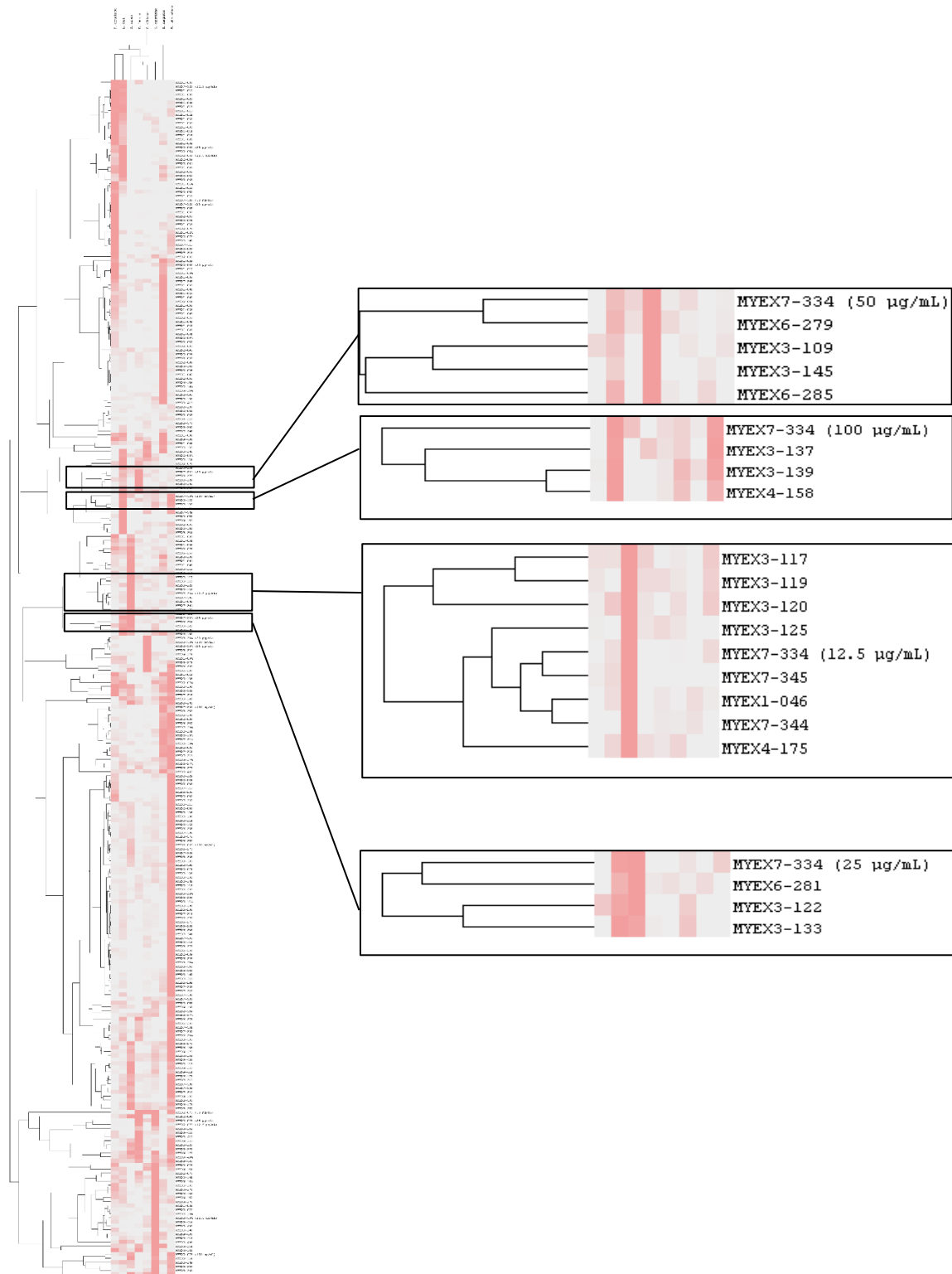
**Figure 2.12.** Hierarchical cluster analysis (Euclidean distance, average linkage) of bioactivity profiles of serially diluted extracts in NPRG collection from second sample group half dilution testing in relation to complete extract library. Boxes are separating the labels of clusters that include the investigated test samples, and bioactivity profiles of extracts or antimicrobials within the cluster showed correlations of  $\geq 0.80$ .



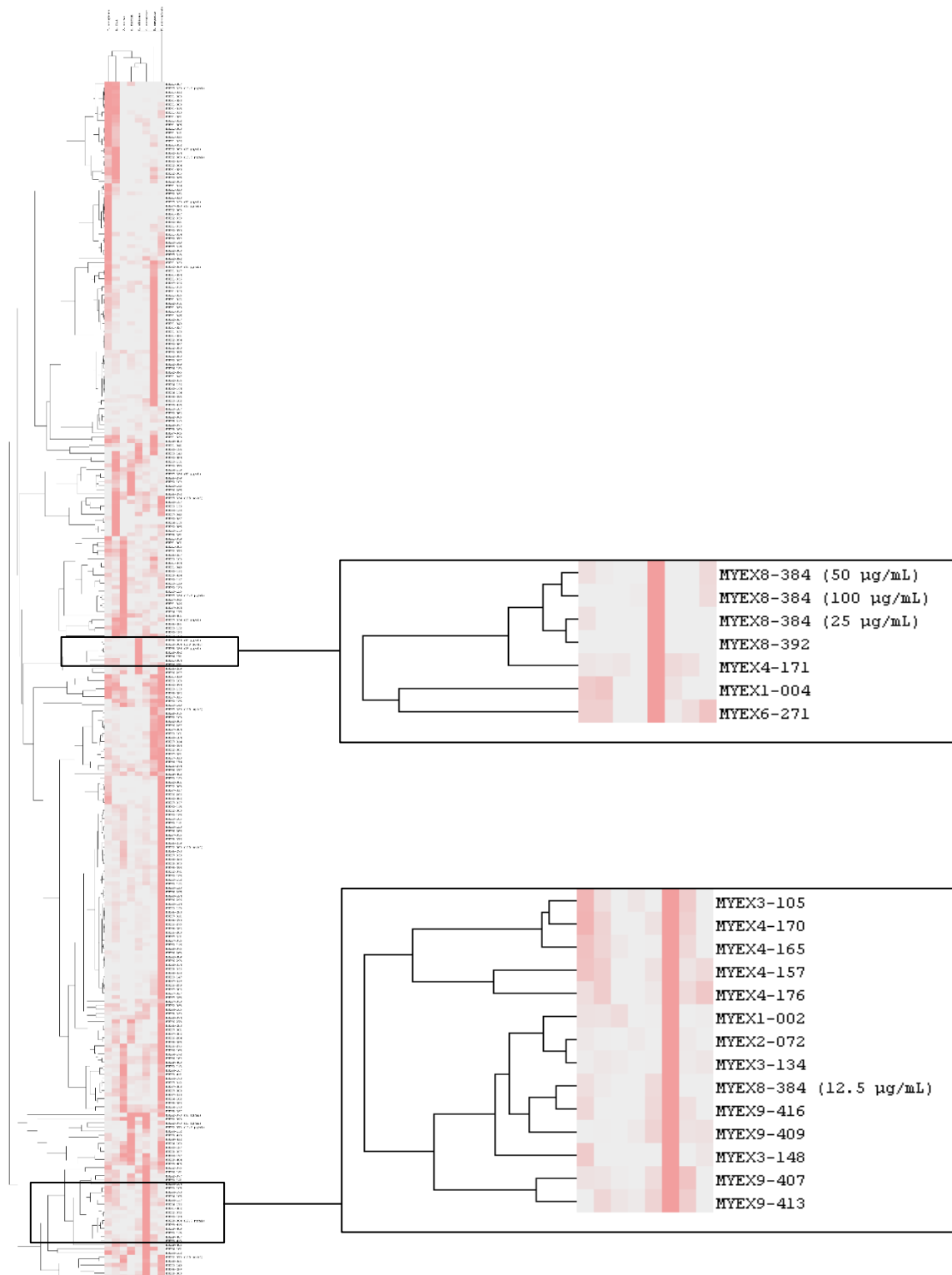
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**Figure 2.12.** Hierarchical cluster analysis (Euclidean distance, average linkage) of bioactivity profiles of serially diluted extracts in NPRG collection from second sample group half dilution testing in relation to complete extract library. Boxes are separating the labels of clusters that include the investigated test samples, and bioactivity profiles of extracts or antimicrobials within the cluster showed correlations of  $\geq 0.80$  (continued).



**Figure 2.12.** Hierarchical cluster analysis (Euclidean distance, average linkage) of bioactivity profiles of serially diluted extracts in NPRG collection from second sample group half dilution testing in relation to complete extract library. Boxes are separating the labels of clusters that include the investigated test samples, and bioactivity profiles of extracts or antimicrobials within the cluster showed correlations of  $\geq 0.80$  (continued).



**Figure 2.12.** Hierarchical cluster analysis (Euclidean distance, average linkage) of bioactivity profiles of serially diluted extracts in NPRG collection from second sample group half dilution testing in relation to complete extract library. Boxes are separating the labels of clusters that include the investigated test samples, and bioactivity profiles of extracts or antimicrobials within the cluster showed correlations of  $\geq 0.80$  (continued).

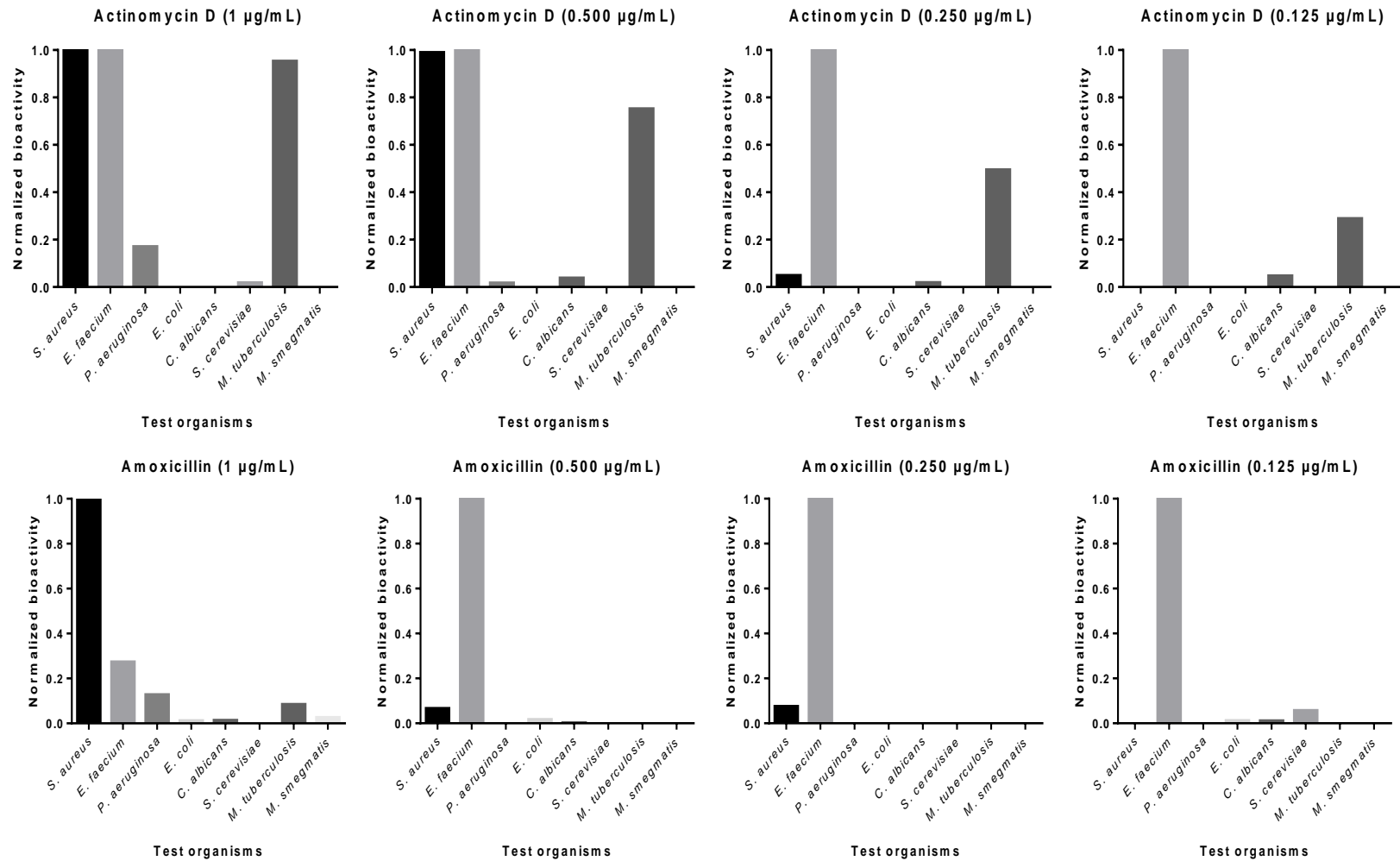
### **2.3.8 Development of bioactivity profiles for commercial antimicrobials from group C**

Commercial antimicrobials with 100% inhibition against test microorganisms used to create bioactivity profiles were serially diluted. Some of the antimicrobials had maximum inhibition activity against multiple pathogens and their bioactivity profiles were different after multiple dilutions such as actinomycin D, ciprofloxacin, and tetracycline (Figure 2.13; Figure 2.14; Figure 2.15). When antimicrobials are screened at a concentration below their MIC, observed bioactivity is most often negative inhibition and not useful in building bioactivity profiles such as with vancomycin (Figure 2.15). These results show that there is a lot of variability in the concentration at which different antimicrobials no longer express maximum inhibition activity against the different pathogens. However, after 100% inhibition, most antimicrobials will cluster together better than seen in the clustering of the extracts. Although, this seems to be showing less bioactivity overall as the antimicrobials are diluted; only showing high activity against a few pathogens due to the increase in negative inhibition that is observed at lower concentrations. Therefore, there is a flip side to diluting antimicrobials to the point where no maximum inhibition occurs against any of the test pathogens and that is increasing the occurrence of negative inhibition which is normalized to zero when normalizing inhibition data.

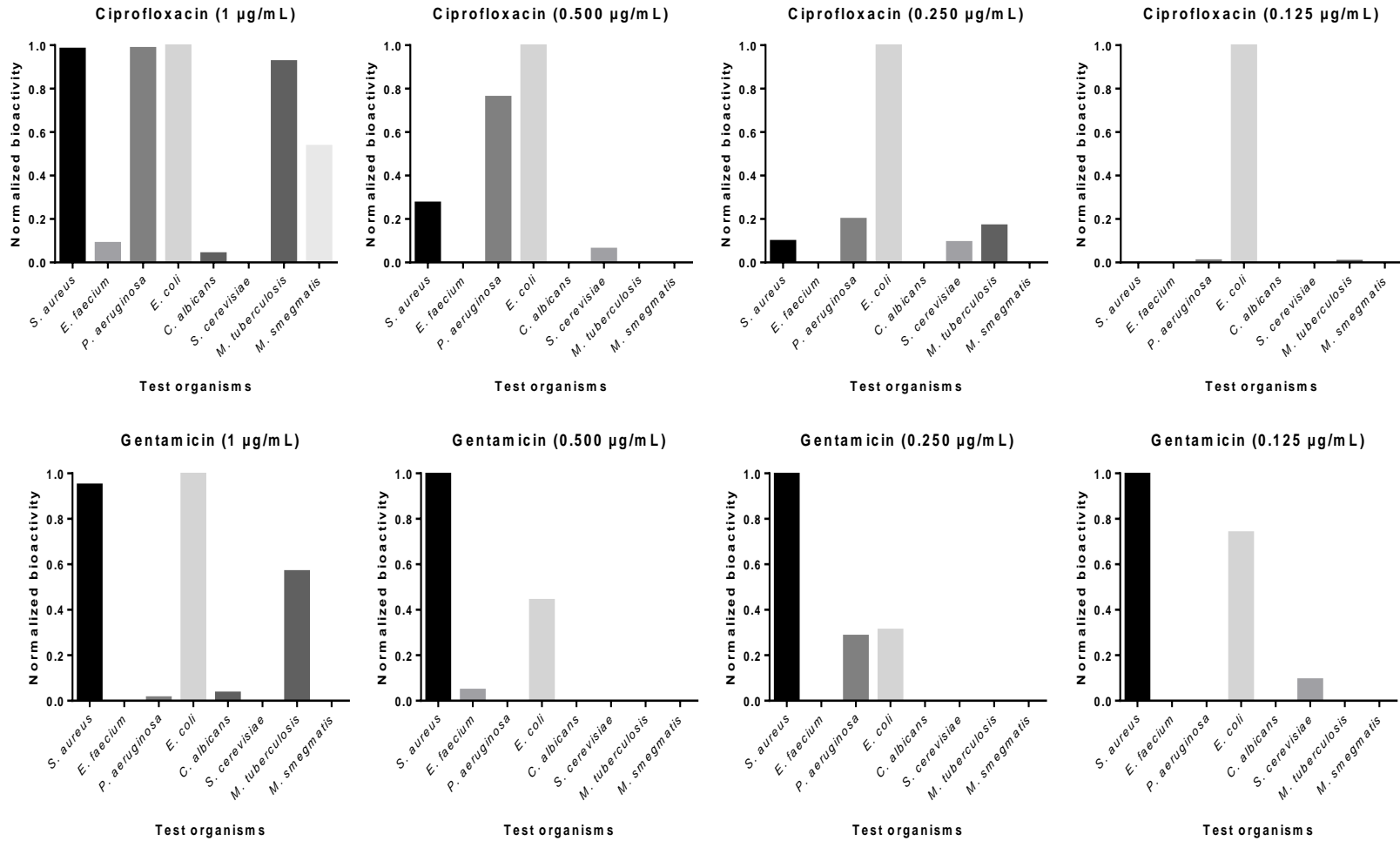
The hormesis effect, described earlier, has been shown to occur in all antimicrobials thus demonstrating that the different phenotypic responses of the antimicrobials are dependent on the concentration of the bioactive compound regardless of their receptors and mode of action (Calabrese, 2004; Davies et al., 2006; Sagan, 1987). Different

antimicrobials have been found to exhibit different effects on various microorganism (Davies et al., 2006). For example, in subinhibitory concentrations, rifampicins reduce shiga-like toxin secretion in *E. coli* while tetracyclines decrease biofilm formation in *Staphylococcus sp.* (Cerca et al., 2005; Matar and Rahal, 2003). It is possible that the hormesis phenomenon is responsible for the different negative inhibition that is observed in the bioactivity profiles at concentrations below 1 µg/mL.

Furthermore, some solvents such as the DMSO used to dissolve the antimicrobials have been shown to inhibit growth of microorganisms such as *Cryptococcus albidus* at levels of DMSO higher than 2.5% (Zgoda and Porter, 2001). However, at levels lower than 2.5% DMSO some compounds have difficulty dissolving (Zgoda and Porter, 2001). Suggesting again, that there has to be an understanding that the solvent used will potentially have an impact on the bioactivity profile by either promoting some inhibition in itself or by not fully dissolving the extract. Antimicrobials such as ciprofloxacin, are very difficult to dissolve and even after they are sonicated for a few minutes, it is difficult to be 100% confident that all of the compound is thoroughly dissolved in the solvent (Yang et al., 2016).



**Figure 2.13.** Bioactivity profiles from concentration effect testing for Actinomycin D and Amoxicillin.



**Figure 2.14.** Bioactivity profiles from concentration effect testing for Ciprofloxacin and Gentamicin.

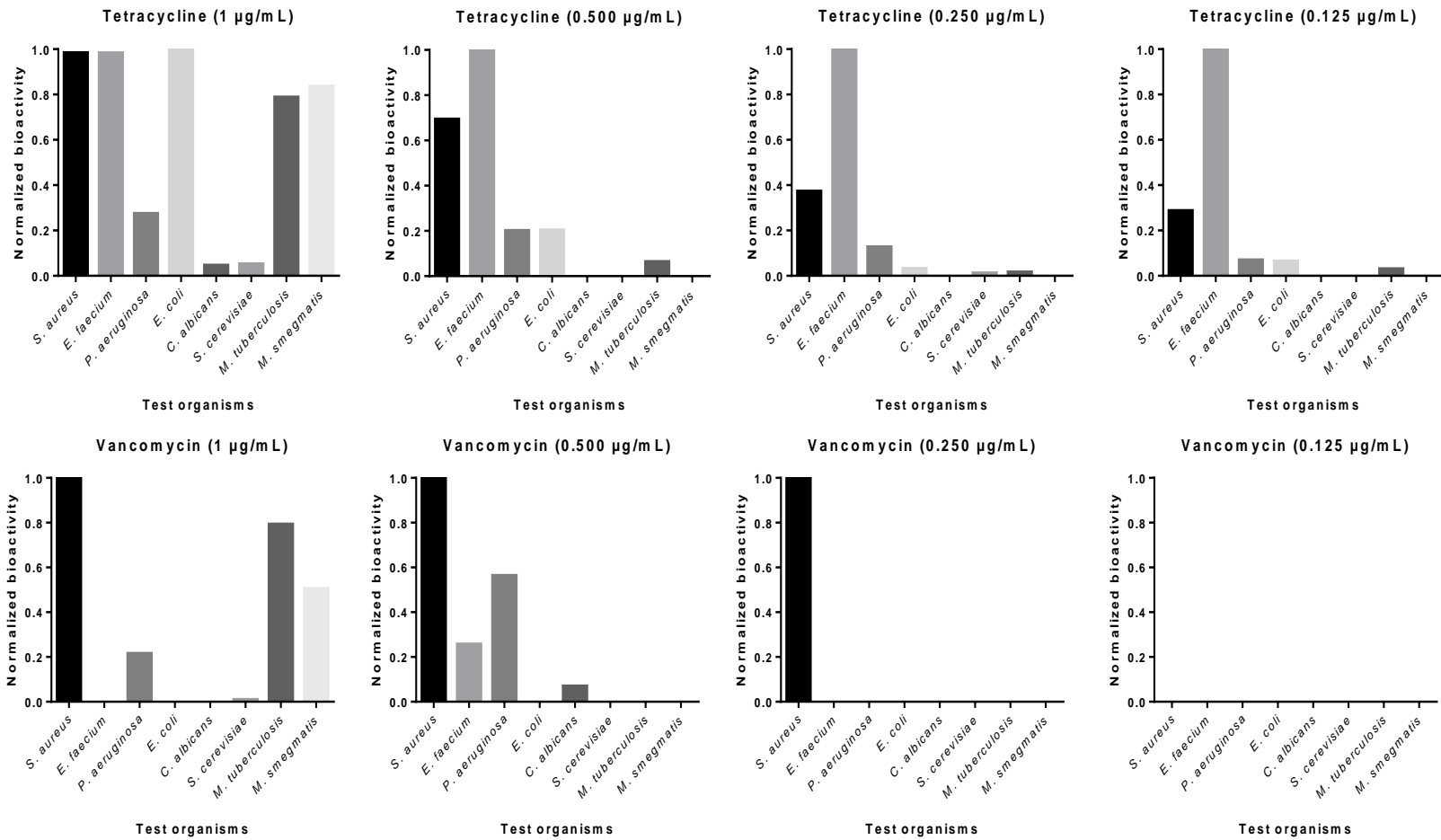
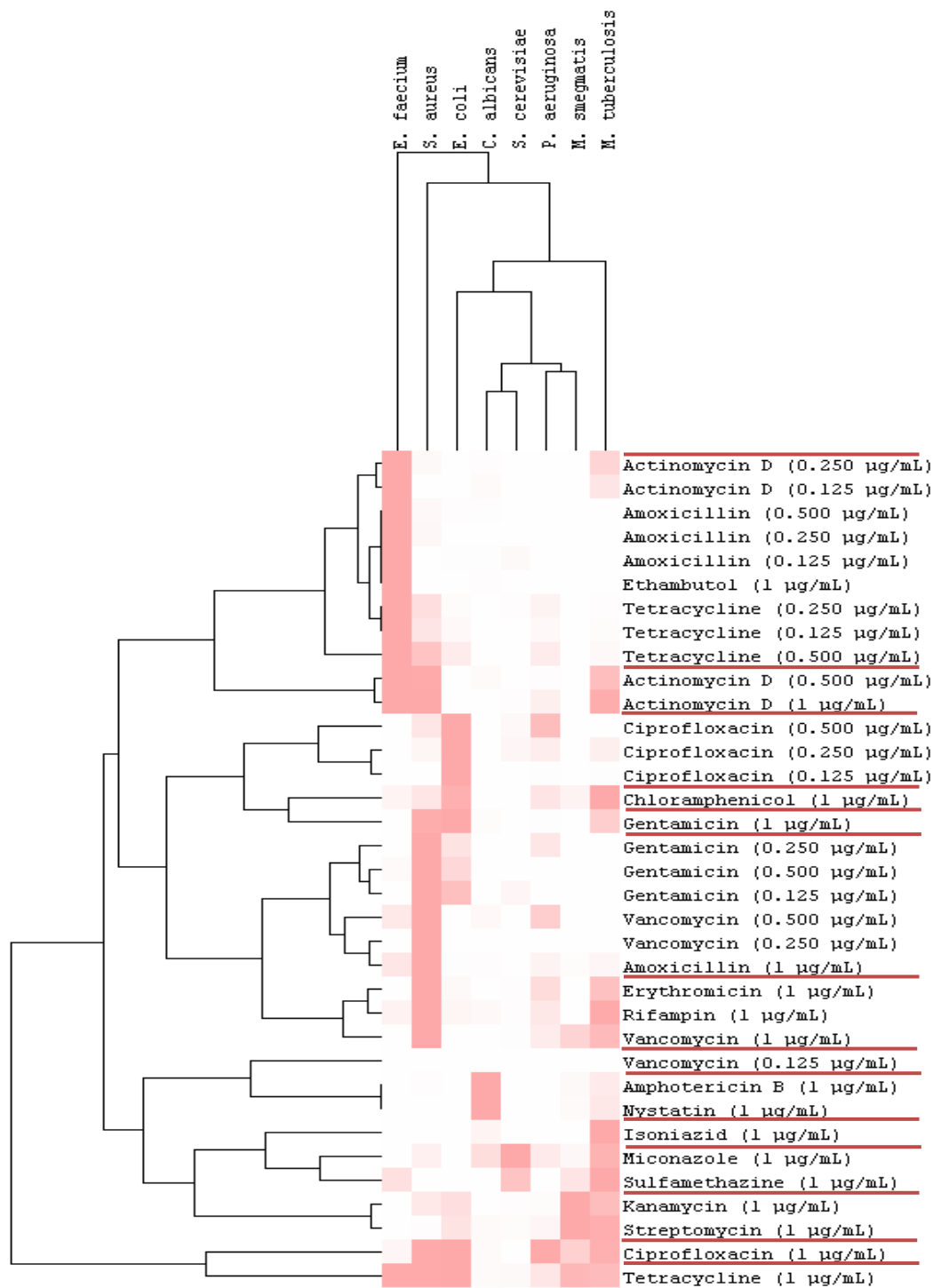


Figure 2.15. Bioactivity profiles from concentration effect testing for Tetracycline and Vancomycin.

### **2.3.9 Cluster analysis of bioactivity profiles for commercial antimicrobial compounds from group C**

Fourteen clusters were formed from the hierarchical cluster analysis of commercial antimicrobials used in the test of the effect of concentration on bioactivity profiles (Figure 2.16). However, in the hierarchical cluster analysis none of the commercial antimicrobials screened at 1  $\mu\text{g}/\text{mL}$  clustered with the remaining concentrations and instead, most antimicrobials clustered near other antimicrobials screened at 1  $\mu\text{g}/\text{mL}$ . These results provide evidence supporting the idea that by diluting samples to remove maximum inhibition concentrations (or 100% inhibition values) which are observed at 1  $\mu\text{g}/\text{mL}$ , the bioactivity profile will exhibit a disproportionate number of negative inhibition in other microorganisms, making the antimicrobials concentrations at 1  $\mu\text{g}/\text{mL}$  cluster together only because they are the ones showing multiple high bioactivity against the different test pathogens. This indicates that bioactivity profiles for antimicrobials can be variable, however, they can still be useful if their limitations are understood and if conditions such as the screening concentration are the same for all antimicrobials.



**Figure 2.16.** Hierarchical cluster analysis (Euclidean distance, average linkage) of bioactivity profiles of serially diluted antimicrobials in NPRG collection from third sample group half dilution testing. Fourteen clusters were formed (identified by red lines separating the labels), and the bioactivity profiles of the antimicrobial standards within the cluster showed correlations of  $\geq 0.80$ .

## 2.4 Summary

A collection of 280 fungal extracts was prioritized using the bioactivity profiling technique as described by Flewelling (2018) and 70 extracts were identified as potential projects with unique bioactivity worth further investigating. However, before the projects could be worked on, some potential limitations in Flewelling's technique had to be addressed. One of these limitations addressed the screening concentration of both extracts and commercial antimicrobials. Since Flewelling observed 100% inhibition when screening extracts and commercial antimicrobials at a single concentration, tests were done to determine if and how bioactivity profiles of samples change after being diluted. Some commercial antimicrobials and extracts showed similar bioactivity profiles at some of the concentrations. However, for other commercial antimicrobials and extracts many differences were seen across bioactivity profiles of extracts and commercial antimicrobials for the different concentrations most often due to 100% inhibition being observed. Frequently, the middle concentrations were most similar to each other for both extracts and antimicrobials. These findings suggest that 100% inhibition will influence bioactivity profiles, along with hormesis effects which influence bioactivity expression at very low screening concentrations. Regardless of these discrepancies, the bioactivity profiling approach as described by Flewelling could still be an effective and simple method to prioritize extracts based on their unique bioactivities. As long as these limitations are considered, and when 100% inhibition is observed in an extract or antimicrobial it is further diluted, a true bioactivity profile can be determined. By applying this modification to Flewelling's bioactivity profiling technique, this simple and effective prioritization

technique can be used in future natural product research to find compounds with new antimicrobial activity within extracts.

**Chapter 3: Investigating the ability of an antimicrobial compound to be detected in  
a natural product extract in order to determine the effect of the extract matrix in  
bioactivity profiling**

### 3.1 Introduction

The purpose of the experiments in this chapter was to test how well potential antimicrobial compounds within an extract are being detected in bioactivity profiling. In Chapter 2 it was found that the concentration affected the bioactivity profile of both extracts and antimicrobials. Subsequently, it was important to determine whether the matrix of an extract affects the bioactivity of different compounds, as it is unknown whether the extract matrix is masking the antimicrobial activity of compounds in an extract. Similarities between bioactivity profiles can be compared in a hierarchical cluster analysis, where samples in the same cluster would have similar bioactivity profiles. If the extract matrix is changing the bioactivity profiles such that potential antimicrobial compounds within an extract are not clustering with the pure compounds in a cluster analysis, then this provides evidence suggesting that there are limitations to identifying known bioactive compounds from natural product extract mixtures in the bioactivity profiling technique developed by Flewelling (2018). By comparing the bioactivity profile of a known antimicrobial compound to the the bioactivity profile of that same antimicrobial compound dissolved in a natural product extract, the effect of the extract matrix on the antimicrobial can be investigated. However, to determine whether the antimicrobial alone and the antimicrobial mixed with an extract are clustering together, extracts with low or negative inhibition should be selected in an attempt to control for the commercial antimicrobial being masked by the activity of the extract. For example, if extracts with high activity (>50% inhibition) are selected, it may not be as easy to notice any difference in the bioactivity profile after the antimicrobial compound is added to the extract if the antimicrobial alone has lower inhibition activity.

The objective of this chapter was to compare bioactivity profiles for all seventeen commercial antimicrobial compounds used in Flewelling's bioactivity profiling approach to the bioactivity profiles of those same antimicrobial compounds individually dissolved in a natural product extract matrix. This objective would allow the researcher to determine if the extract matrix is affecting bioactivity profiles of pure compounds within an extract causing them to not cluster with pure compounds which would then indicate limitations in the bioactivity profiling technique developed by Flewelling (2018).

## **3.2 Experimental**

### **3.2.1 Extract selection**

To test the effect of an extract matrix on a known antimicrobial compound, two extracts from the NPRG collection of 280 natural product extracts were selected for further investigation (Appendix 1; Table A1.1.). Before any extracts were selected, the percent inhibition values for all 280 extracts against the test microorganisms used to create bioactivity profiles (*S. aureus*, *E. faecium*, *P. aeruginosa*, *E. coli*, *C. albicans*, *S. cerevisiae*, *M. tuberculosis* and *M. smegmatis*) were compared to identify extracts with low or no activity against most of the test microorganisms (Appendix 2; Table A2.1.). Two extracts (MYEX7-311 and MYEX7-318) were selected for extract matrix testing as they had no more than 25 % inhibition against at least seven of the eight microorganisms.

### **3.2.2 Preparation of antimicrobial compounds**

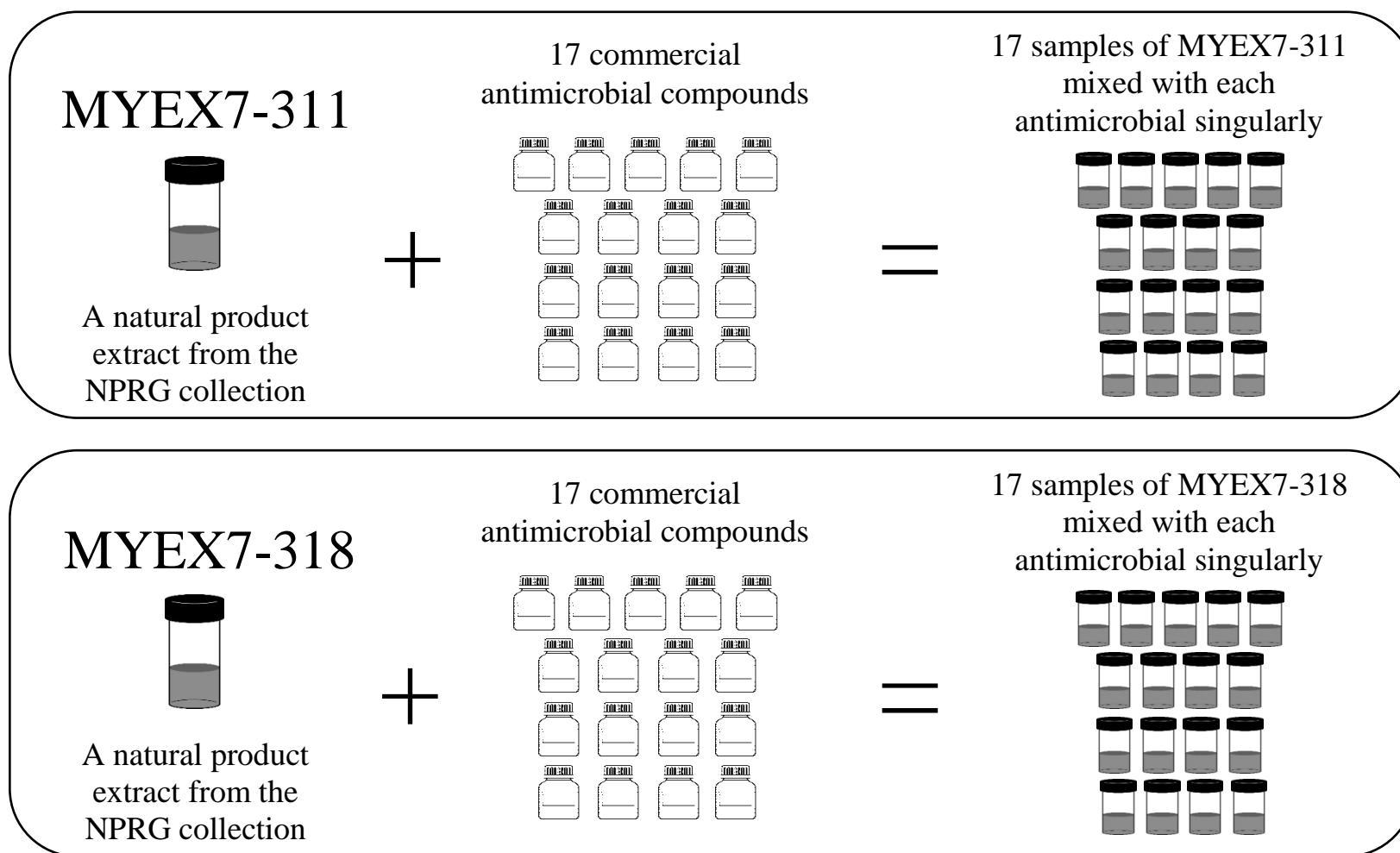
All seventeen standard antimicrobial compounds from Flewelling's bioactivity profiling approach were used in the extract matrix effect tests. These antimicrobial compounds were actinomycin D, amoxicillin, amphotericin B, chloramphenicol, ciprofloxacin, erythromycin, ethambutol, gentamicin, isoniazid, kanamycin, miconazole, nystatin, rifampin, streptomycin, sulfamethazine, tetracycline, and vancomycin. Prior to dissolving an antimicrobial compound in an extract, a stock solution of the antimicrobial was prepared. Stock solutions of 1 mg/mL for each antimicrobial were prepared in the same broth medium as used for each biological assay (Table 3.1) and stored at 4 °C prior to use.

**Table 3.1.** Broth media used for each test microorganism in biological assay screening.

Biological assay	Test microorganism	Broth media
Antifungal	<i>C. albicans</i>	Difco™ Sabouraud dextrose broth
	<i>S. cerevisiae</i>	yeast mold broth
Antibiotic	<i>P. aeruginosa, E. coli</i> and <i>S. aureus</i>	BBL™ Mueller Hinton II cation- adjusted broth
	<i>E. faecium</i>	BBL™ Brain Heart Infusion broth
Antimycobacterial	<i>M. tuberculosis</i> and <i>M.</i> <i>smegmatis</i>	BBL™ modified Middlebrook 7H9 broth

### 3.2.3 Sample preparation

In this chapter, the term “sample” will be used to refer to a commercial antimicrobial compound dissolved in an extract. All samples used in the experiments for this chapter were prepared on the same day as the bioassays for these samples were performed (Figure 3.1). All extracts (MYEX7-311 and MYEX7-318) were dissolved in dimethyl sulfoxide (DMSO) and all commercial antimicrobial compounds were dissolved in the appropriate broth as per each bioassay (Table 3.1) before each commercial antimicrobial compound and extract were dissolved together. The resulting test solutions for bioassay screening consisted of a 4% DMSO in broth solution with an extract at 100 µg/mL, and a single commercial antimicrobial at 1 µg/mL. For this experiment, there were two extracts that were each spiked singularly with each one of the seventeen antimicrobials. This resulted in thirty-four samples that were prepared and tested against all eight microorganisms in bioassay screening (*S. aureus*, *E. faecium*, *P. aeruginosa*, *E. coli*, *S. cerevisiae*, *C. albicans*, *M. tuberculosis* and *M. smegmatis*).



**Figure 3.1.** Preparation of samples for testing the effect of an extract matrix on an antimicrobial compound. A sample consists of a single commercial antimicrobial compound dissolved in an extract from the NPRG collection. Two extracts from the NPRG collection, MYEX7-311 and MYEX7-318, were each dissolved with an antimicrobial compound from the 17 antimicrobials used in Flewelling’s bioactivity profiling approach. All 17 antimicrobials used in Flewelling’s bioactivity profiling approach were dissolved singularly in each extract. In total, 34 samples were prepared for bioassay screening.

### **3.2.4 Source of antimicrobial standards**

All commercial antimicrobial standards used to prepare samples for extract matrix effect tests, and as positive controls in antimicrobial susceptibility tests were obtained from the suppliers described in chapter two.

### **3.2.5 Antifungal, antibiotic, and antimycobacterial activity assays**

All procedures of the antimicrobial susceptibility tests were as described in chapter two.

### **3.2.6 Creation of bioactivity profiles**

The percentage inhibition values obtained for each commercial antimicrobial compound, natural product extract, and antimicrobial compound dissolved in an extract were normalized to create bioactivity profiles as described in chapter two.

### **3.2.7 Identification of clusters using hierarchical cluster analysis**

Hierarchical cluster analysis using Cluster 3.0 (de Hoon et al. 2004) with a Euclidean distance measure and average linkage clustering method was performed to compare the bioactivity profiles of the commercial antimicrobial compounds to those antimicrobials dissolved in an extract. The hierarchical cluster analysis dendrogram was visualised using Java Treeview (Saldanha 2004) whereby clusters were identified as clades where bioactivity profiles of extracts and/or antimicrobial standards possessed correlations  $\geq 0.8$

### 3.3 Results and discussion

Bioactivity profiles were compiled for all seventeen antimicrobial compounds standards used in Flewelling's bioactivity profiling approach, those antimicrobials dissolved singularly in two extracts (MYEX7-311 and MYEX7-318), and the two extracts alone (Figure 3.2, Figure 3.3).

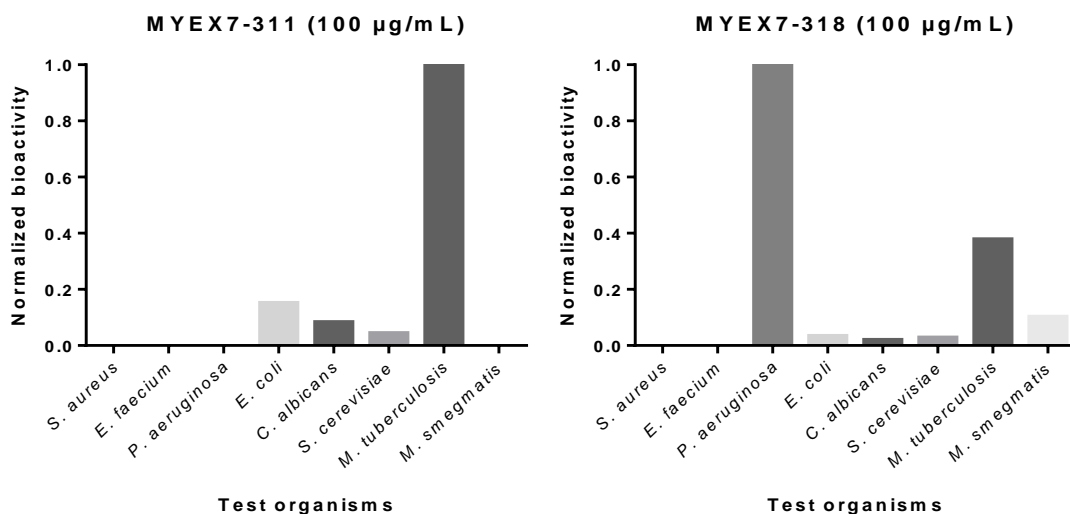


Figure 3.2. Bioactivity profiles of extracts used in matrix effect testing.

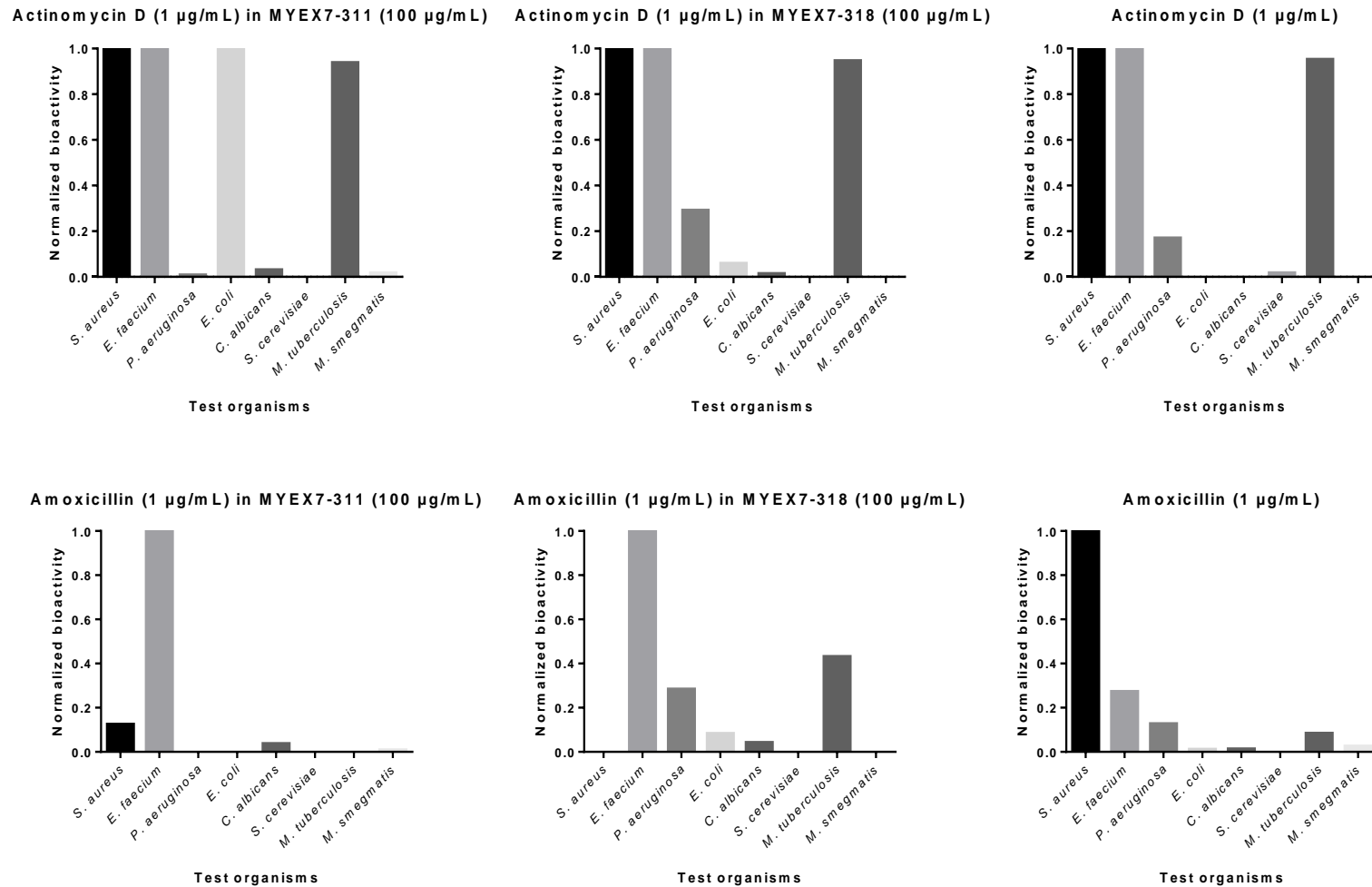


Figure 3.3. Bioactivity profiles of samples from matrix effect testing.

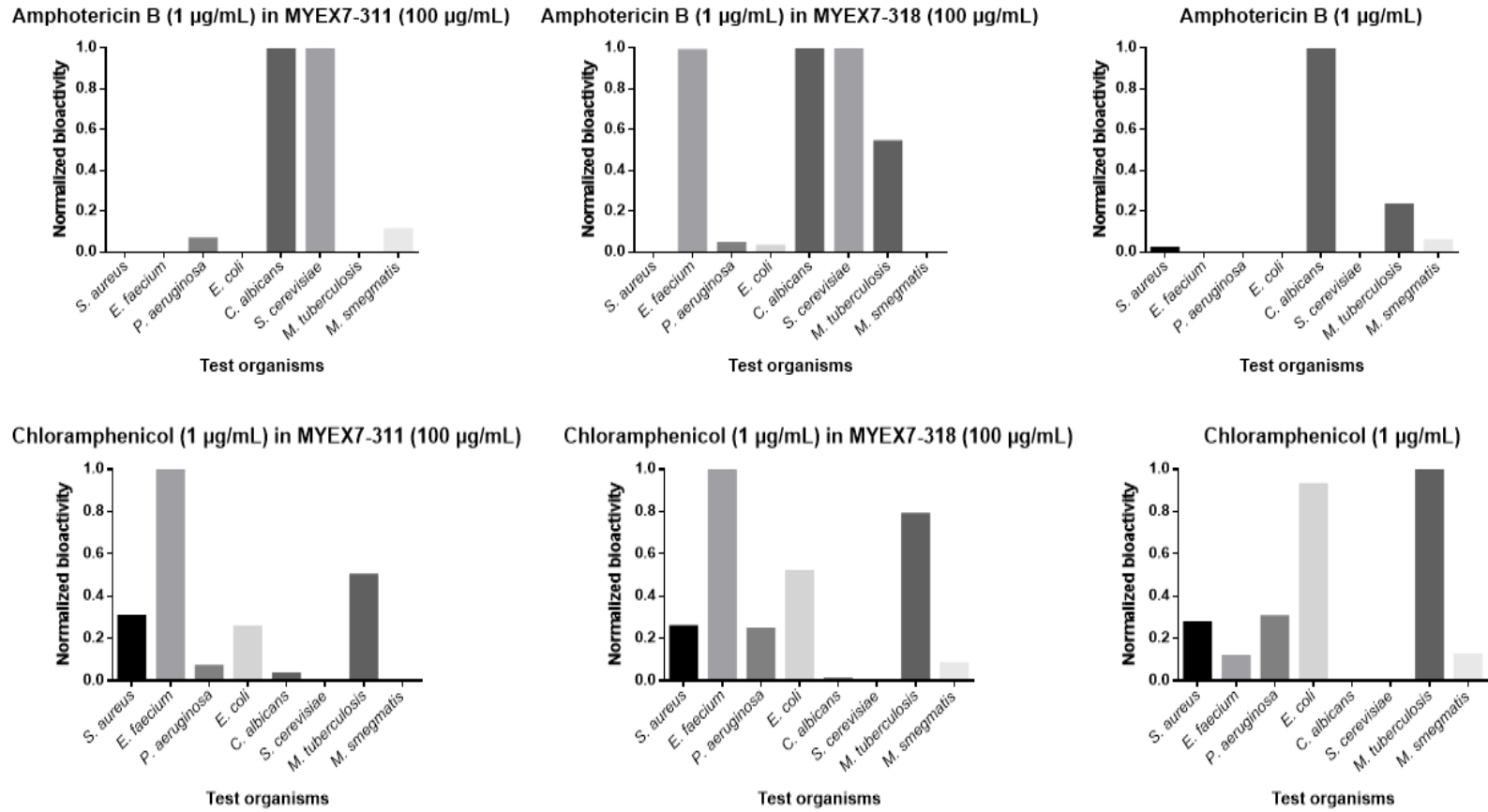


Figure 3.3. Bioactivity profiles of samples from matrix effect testing (continued).

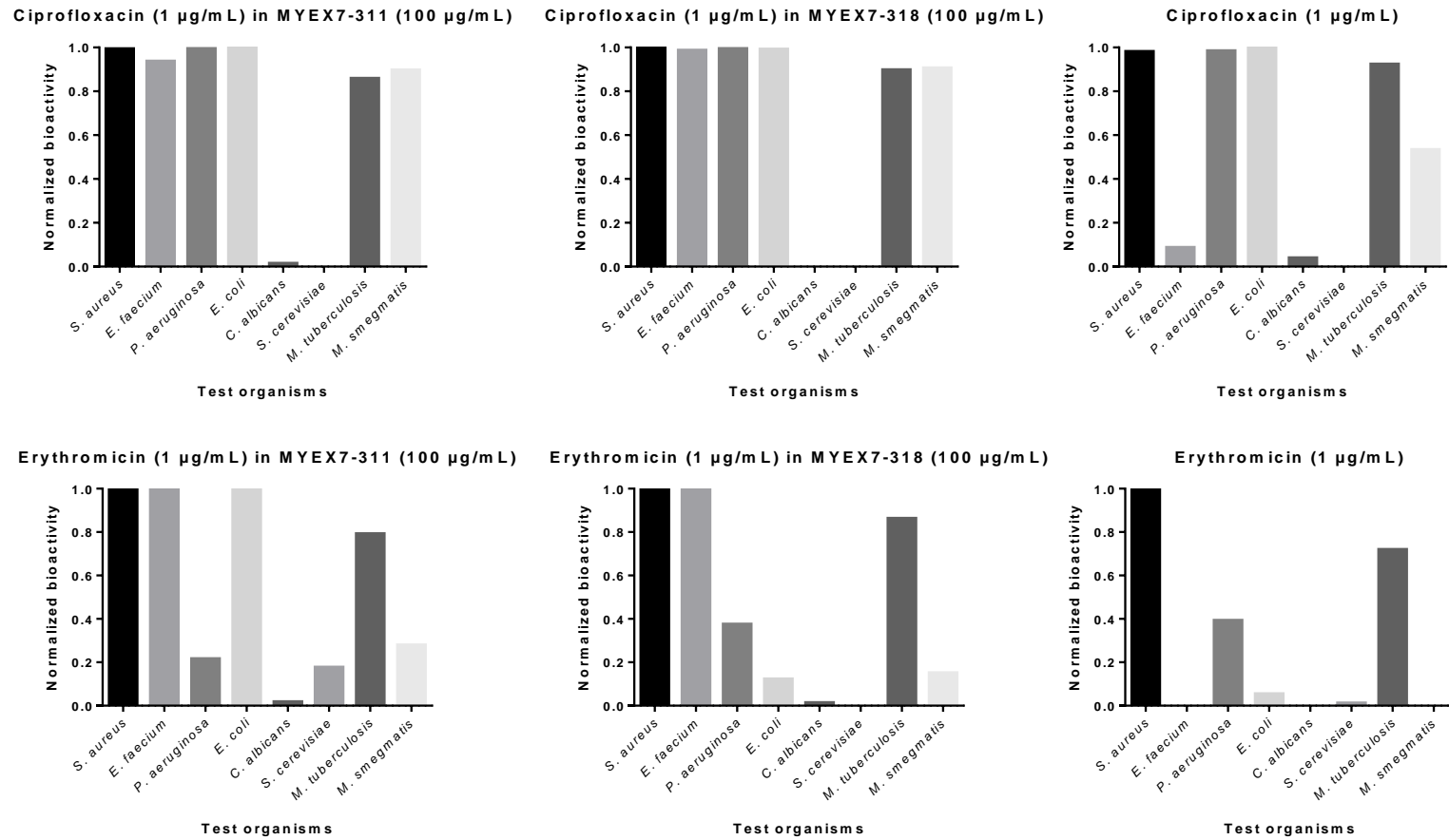
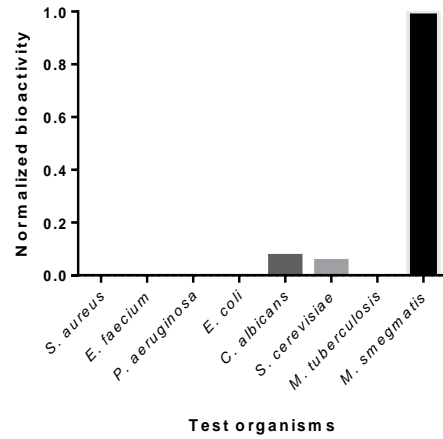
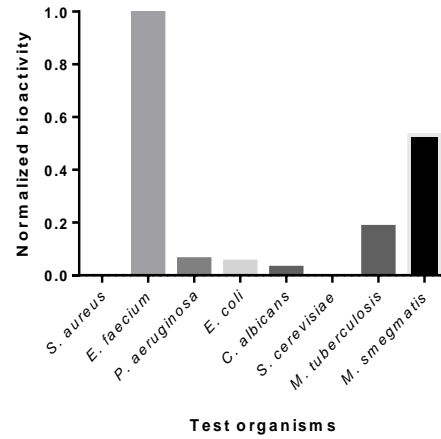


Figure 3.3. Bioactivity profiles of samples from matrix effect testing (continued).

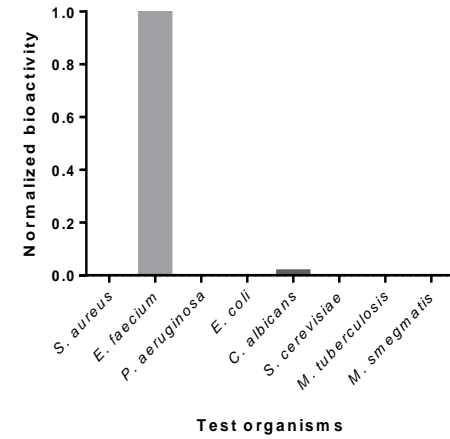
Ethambutol (1 µg/mL) in MYEX7-311 (100 µg/mL)



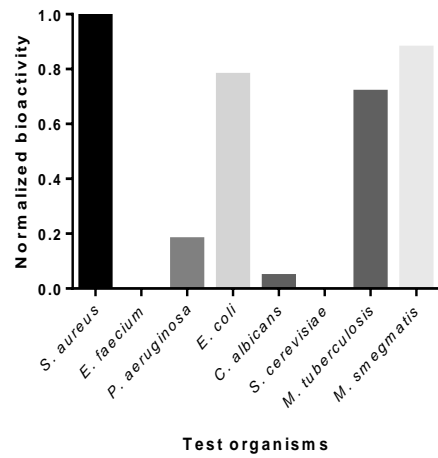
Ethambutol (1 µg/mL) in MYEX7-318 (100 µg/mL)



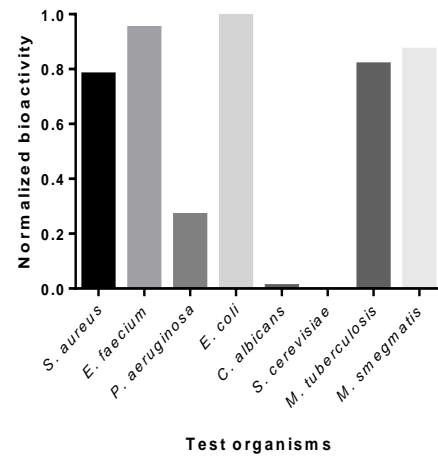
Ethambutol (1 µg/mL)



Gentamicin (1 µg/mL) in MYEX7-311 (100 µg/mL)



Gentamicin (1 µg/mL) in MYEX7-318 (100 µg/mL)



Gentamicin (1 µg/mL)

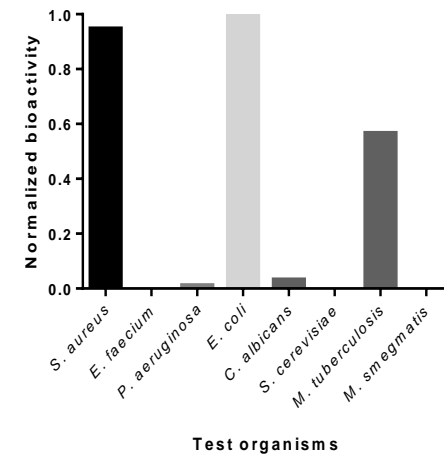
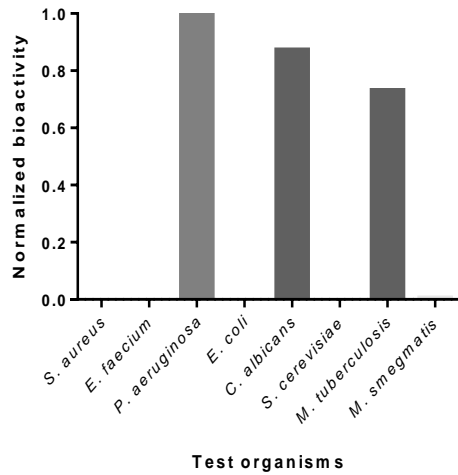
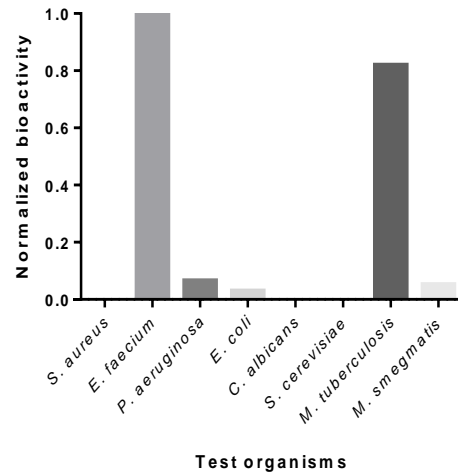


Figure 3.3. Bioactivity profiles of samples from matrix effect testing (continued).

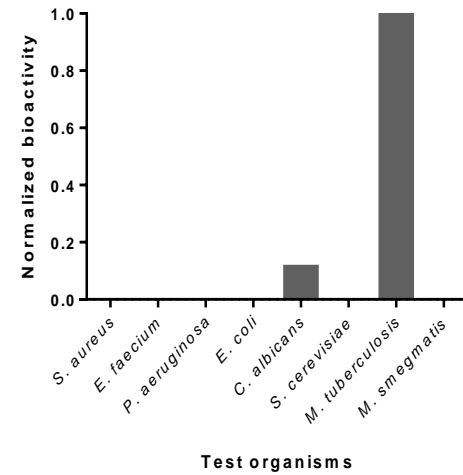
Isoniazid (1 µg/mL) in MYEX7-311 (100 µg/mL)



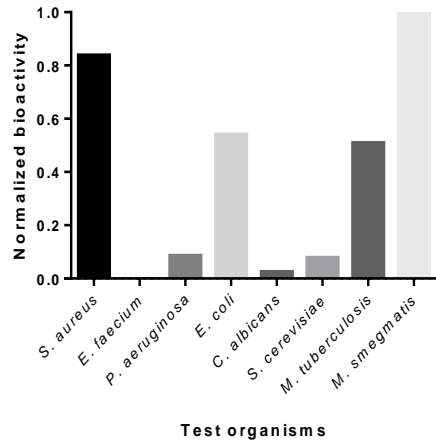
Isoniazid (1 µg/mL) in MYEX7-318 (100 µg/mL)



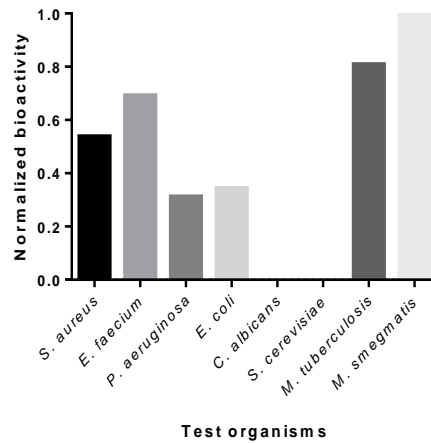
Isoniazid (1 µg/mL)



Kanamycin (1 µg/mL) in MYEX7-311 (100 µg/mL)



Kanamycin (1 µg/mL) in MYEX7-318 (100 µg/mL)



Kanamycin (1 µg/mL)

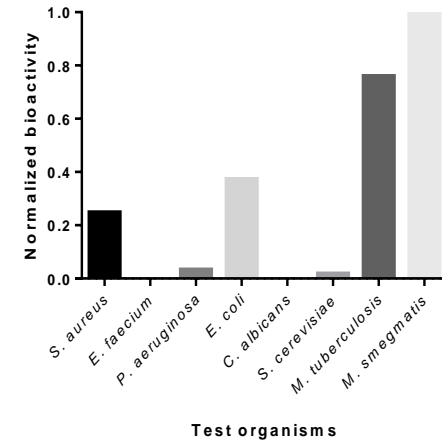
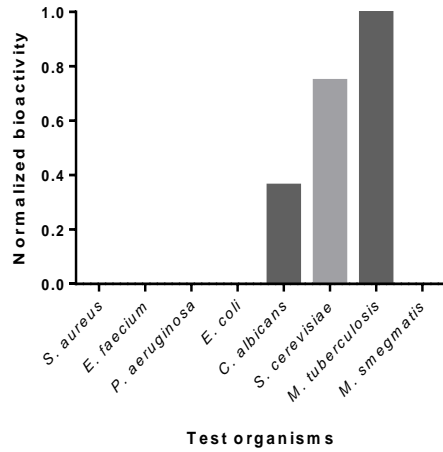
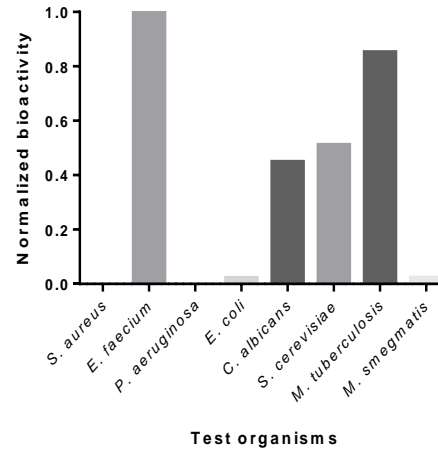


Figure 3.3. Bioactivity profiles of samples from matrix effect testing (continued).

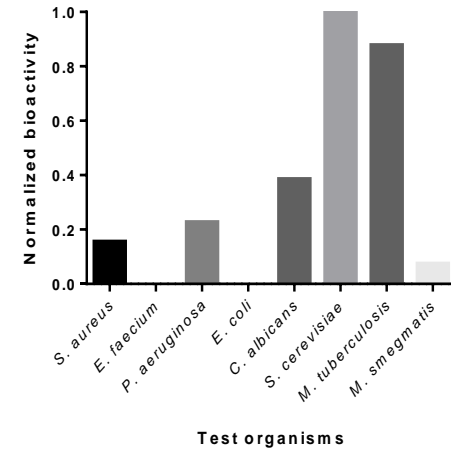
Miconazole (1 µg/mL) in MYEX7-311 (100 µg/mL)



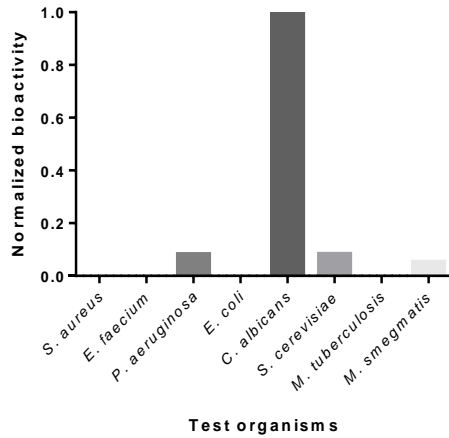
Miconazole (1 µg/mL) in MYEX7-318 (100 µg/mL)



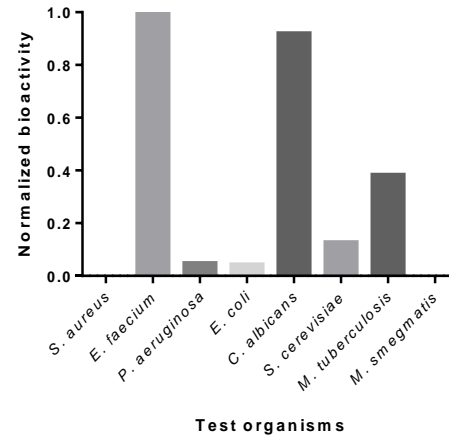
Miconazole (1 µg/mL)



Nystatin (1 µg/mL) in MYEX7-311 (100 µg/mL)



Nystatin (1 µg/mL) in MYEX7-318 (100 µg/mL)



Nystatin (1 µg/mL)

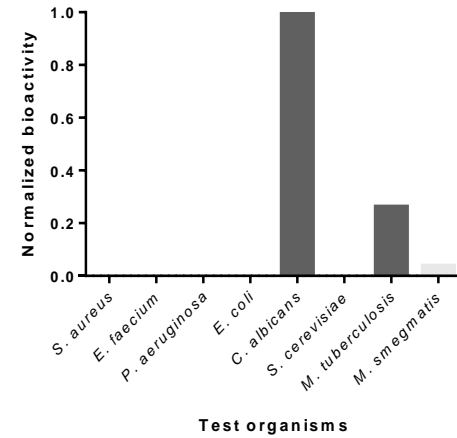
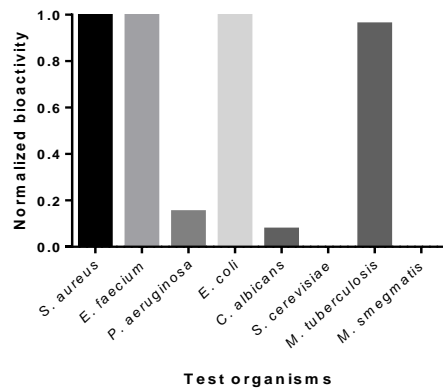
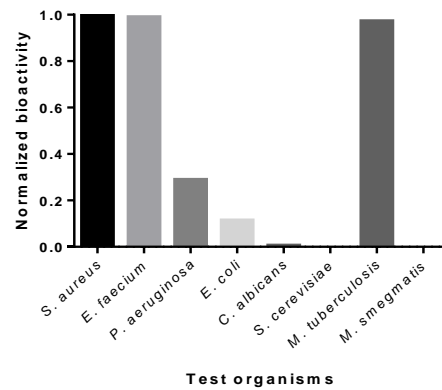


Figure 3.3. Bioactivity profiles of samples from matrix effect testing (continued).

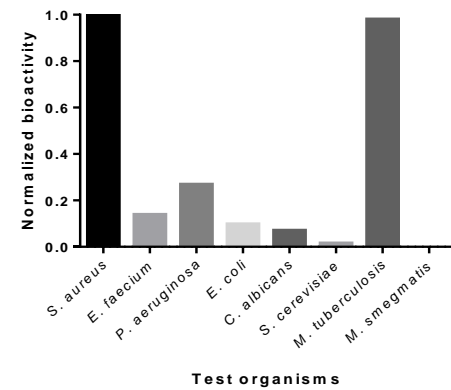
Rifampin (1 µg/mL) in MYEX7-311 (100 µg/mL)



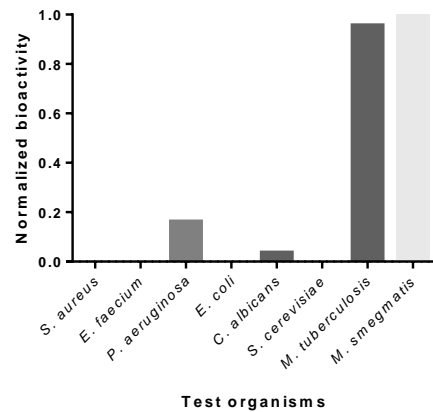
Rifampin (1 µg/mL) in MYEX7-318 (100 µg/mL)



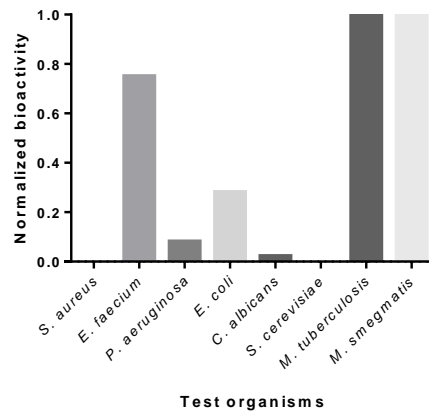
Rifampin (1 µg/mL)



Streptomycin (1 µg/mL) in MYEX7-311 (100 µg/mL)



Streptomycin (1 µg/mL) in MYEX7-318 (100 µg/mL)



Streptomycin (1 µg/mL)

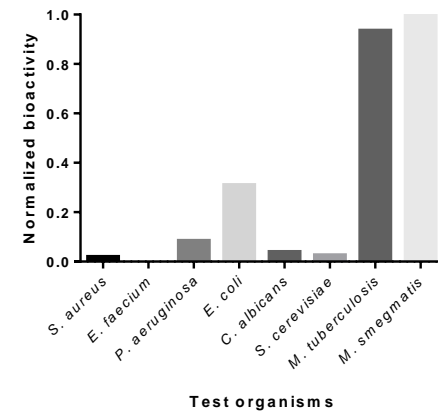
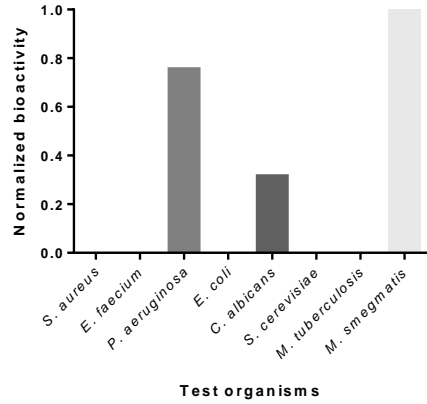
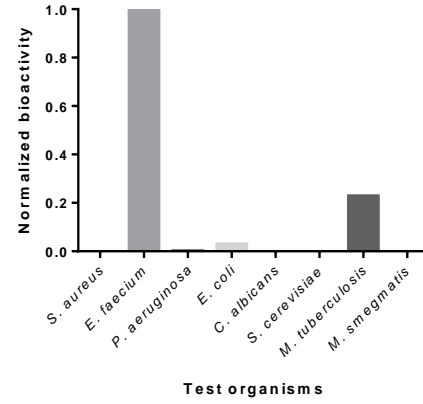


Figure 3.3. Bioactivity profiles of samples from matrix effect testing (continued).

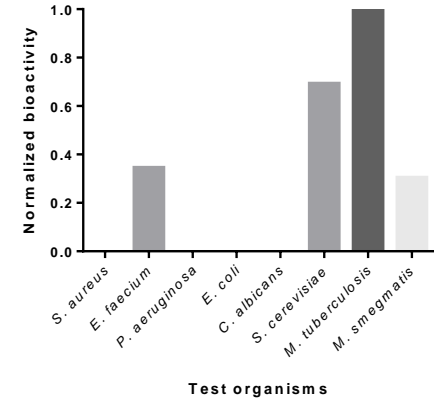
Sulfamethazine (1 µg/mL) in MYEX7-311 (100 µg/mL)



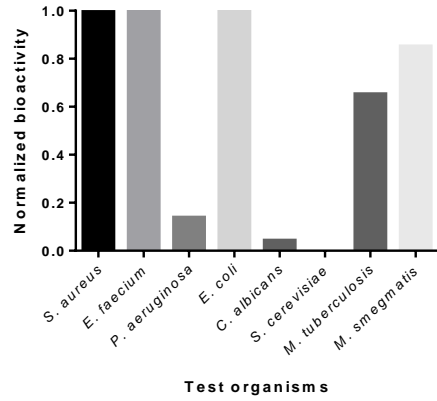
Sulfamethazine (1 µg/mL) in MYEX7-318 (100 µg/mL)



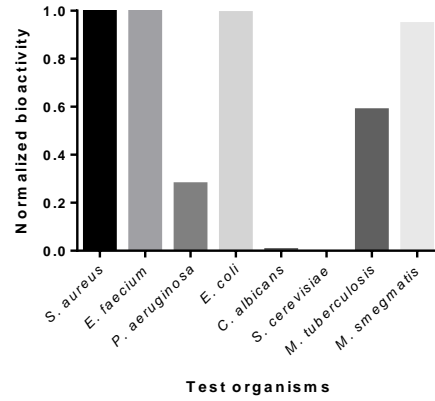
Sulfamethazine (1 µg/mL)



Tetracycline (1 µg/mL) in MYEX7-311 (100 µg/mL)



Tetracycline (1 µg/mL) in MYEX7-318 (100 µg/mL)



Tetracycline (1 µg/mL)

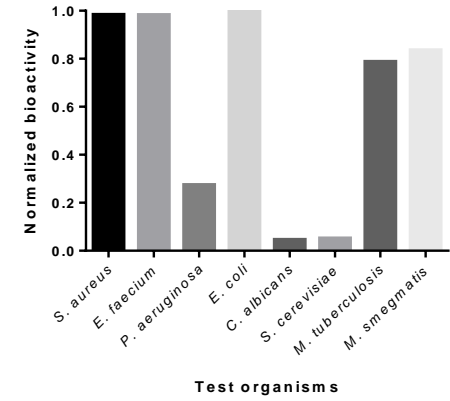


Figure 3.3. Bioactivity profiles of samples from matrix effect testing (continued).

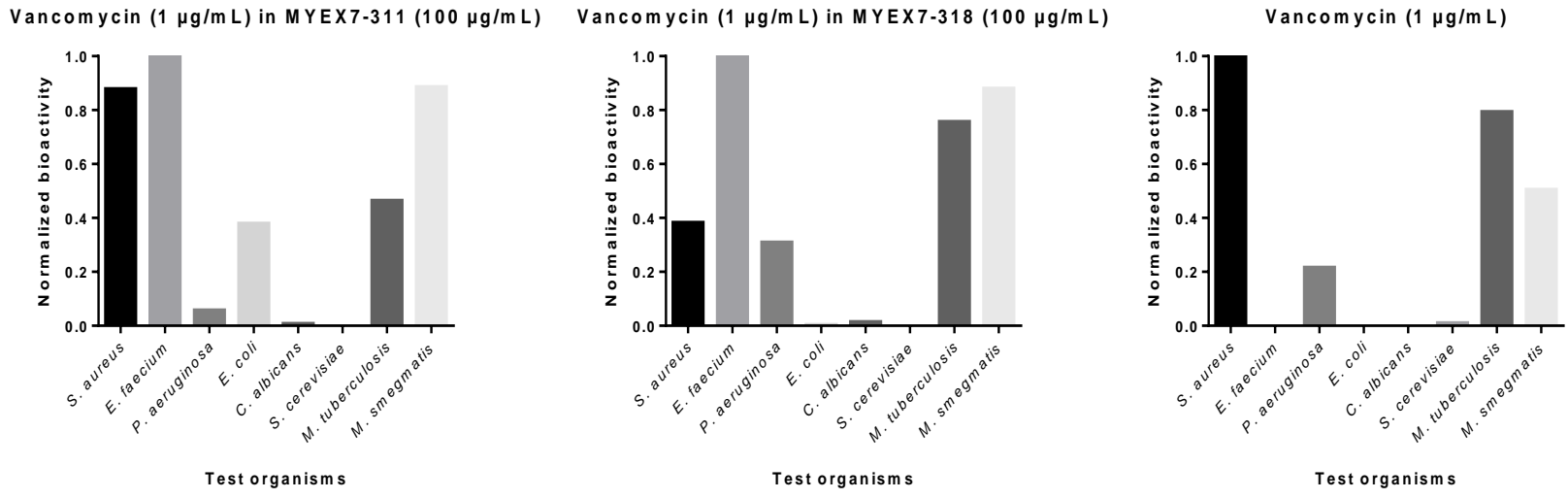
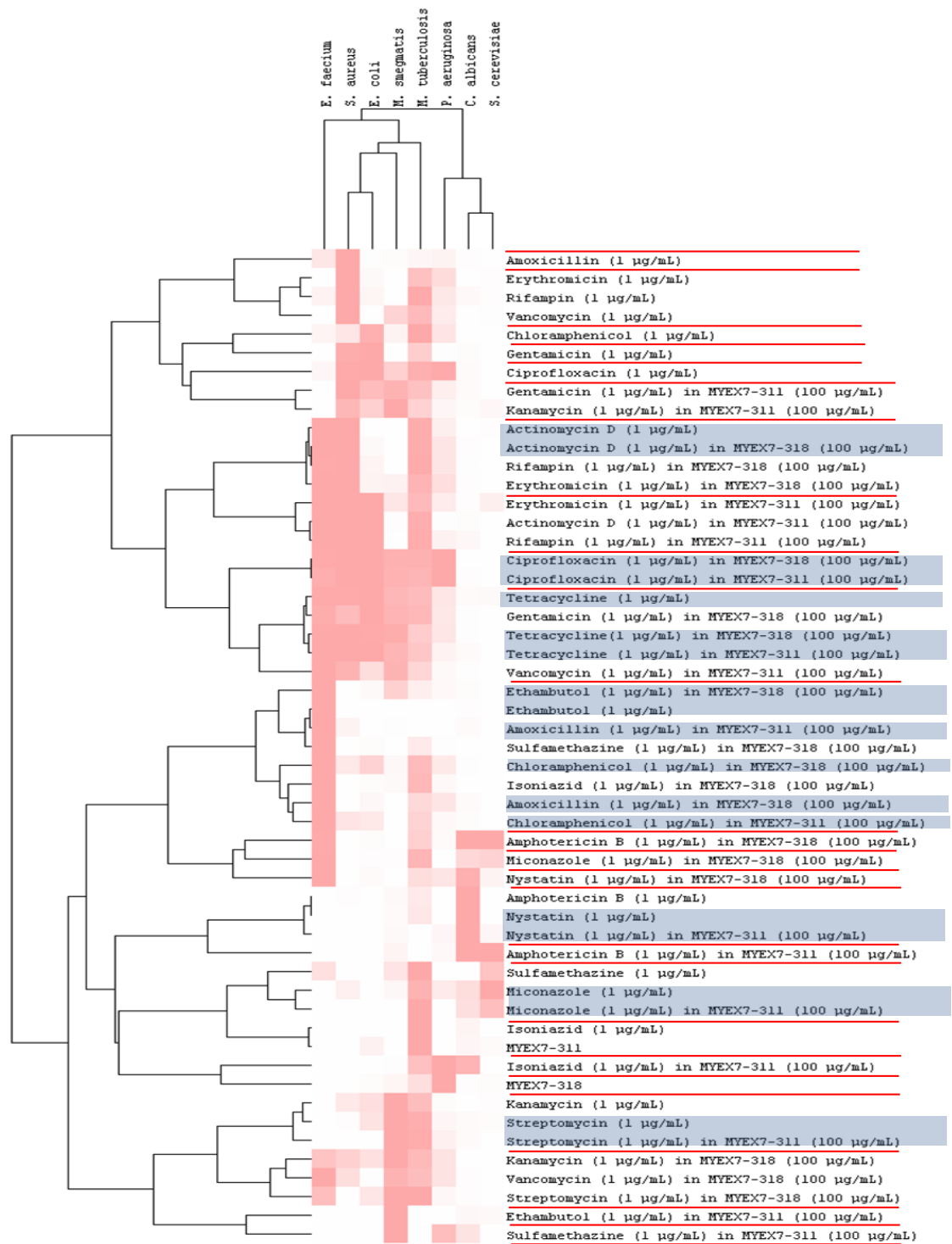


Figure 3.3. Bioactivity profiles of samples from matrix effect testing (continued).

The bioactivity profiles of pure antimicrobial compounds and those antimicrobial compounds dissolved in an extract matrix (Appendix 2; Table A2.8., and Table A2.9.) were often different given the bioactivity profile of the extract before the antimicrobial was added. The similarity between bioactivity profiles was assessed using a hierarchical clustering method (Figure 3.4). In some instances the bioactivity profiles of commercial antimicrobial compounds clustered with those same antimicrobials dissolved in an extract matrix, and in other instances they did not. For example, the bioactivity profiles for rifampin and tetracycline clustered with those antimicrobials spiked in an extract, while the bioactivity profiles of amoxicillin and sulfamethazine did not cluster with those antimicrobials spiked in an extract (Figure 3.2, Figure 3.3, Figure 3.4). A closer look at the bioactivity profiles can help to better understand these results.



**Figure 3.4.** Hierarchical cluster analysis (Euclidean distance, average linkage) of bioactivity profiles of extracts (MYEX7-311 and MYEX7-318), those extracts spiked with antimicrobial from the NPRG training set, and the antimicrobials alone. Twenty-four clusters were formed (identified by red lines separating the labels), and the bioactivity profiles of the samples within the cluster showed correlations of  $\geq 0.80$ .

A hierarchical cluster analysis (Figure 3.4; Euclidean distance measure with average linkage) of bioactivity profiles of natural product extracts (MYEX7-311 and MYEX7-318), commercial antimicrobial compounds (actinomycin D, amoxicillin, amphotericin B, chloramphenicol, ciprofloxacin, erythromycin, ethambutol, gentamicin, isoniazid, kanamycin, miconazole, nystatin, rifampin, streptomycin, sulfamethazine, tetracycline, and vancomycin), and those antimicrobial compounds dissolved in an extract was performed to assess the similarity of the bioactivity profiles. While different statistical analyses could have been performed (e.g. k-means clustering), the hierarchical cluster analysis was performed in order to use the same methodology as Flewelling's bioactivity profiling approach to better compare the results (Flewelling, 2012). The Euclidean distance measure is the best distance measure for continuous data and for this data set as the values (normalized percent inhibition data from 0 to 1) are on a continuous scale (Almeida et al. 2007; Flewelling, 2018).

In order to determine which commercial antimicrobial compounds possessed bioactivity profiles that were similar to those of the same antimicrobials mixed with an extract, clusters were identified in the dendrogram where bioactivity profiles of extracts, antimicrobials and/or extracts spiked with an antimicrobial possessed correlations  $\geq 0.80$  (Flewelling, 2018). Previous research has described data with very strong correlations using correlation coefficient values between 0.7 and 0.9, therefore, Flewelling (2018) used  $\geq 0.80$  as a cut-off point for determining clusters as it was half way between the limits of this range (Chan, 2003; Flewelling, 2018; Kozak, 2009; Mukaka, 2012; Ratner, 2009). As the research described in this thesis is further investigating Flewelling's approach to

prioritizing extracts, the same correlation coefficient of 0.8 will be used to determine clusters in this hierarchical cluster analysis.

Through the use of the hierarchical clustering method, 24 clusters were formed, and 6 of the clusters included a commercial antimicrobial compound that clustered with that same antimicrobial compound dissolved in an extract matrix. Four antimicrobials clustered with those same antimicrobials in MYEX7-311 (miconazole, nystatin, streptomycin, and tetracycline) and three antimicrobials clustered with those same antimicrobials in MYEX7-318 (actinomycin D, ethambutol, tetracycline). Overall, approximately 20% of antimicrobial compounds were found to cluster with antimicrobials dissolved in an extract.

Some of the antimicrobials that did not cluster with the same antimicrobials in spiked extracts showed different bioactivity profiles due to synergism (Chaudhry et al., 2017; Klastersky and Zinner, 1982; Monzon et al., 2001). It is important to remember natural product extracts are complicated mixtures composed of many different chemical compounds in different concentrations (Anochie et al., 2018; Chemat et al., 2017; David et al., 2015; Harvey et al., 2015; Singh et al., 2018; Wolfender et al., 2015). Many different metabolites within these extracts target the same biological receptors in bioassays. However, due to the interaction of all these potentially bioactive chemical compounds working against or with each other, various expressions of inhibition activity can be observed as a result due to a complex phenomenon known as ‘chemical combination effects’ or more commonly, synergistic effects (Calvo et al., 2015; Harvey et al., 2015; Kiyohara et al., 2004; Tiwari et al., 2005).

From a pharmacological perspective, the proposed explanations by Berenbaum with respect to defining synergy seem to be well accepted and widely used (David et al, 2015; Merzenich et al., 2010; Wagner, 2011). According to Berenbaum, the effect of different dose combinations of two substances (a and b) can have different interactions and produce different effects (Berenbaum, 1977; Berenbaum, 1981; Berenbaum, 1989). The overall effect is said to be an additive interaction when the effect of the combination of two substances (a and b) is a pure summation effect (equation 1). When the interaction of the combination of two substances (a and b) is less than expected from the summation of each individual effect then the overall effect is antagonistic (equation 2). Finally, real synergism with potentiated or over-additive effects occurs when the overall effect of two substances (a and b) is greater than expected from the summation of each individual effect (equation 3).

$$\text{Equation 1: } E(da, db) = E(da) + E(db)$$

$$\text{Equation 2: } E(da, db) \leq E(da) + E(db)$$

$$\text{Equation 3: } E(da, db) \geq E(da) + E(db)$$

where E stands for the observed effect; da and db are the doses of agents a and b.

(Berenbaum, 1977; Berenbaum, 1981; Berenbaum, 1989)

There were many examples of synergy seen in the samples tested for extract matrix effects. For example, the bioactivity of amoxicillin, chloramphenicol, ciprofloxacin, and rifampin against *E. faecium* was much greater when the antimicrobials were dissolved singularly in an extract (Figure 3.2, Figure 3.3). Furthermore, erythromycin, ethambutol, and gentamicin all had activity against *M. smegmatis* in extracts spiked with those antimicrobials singularly, however, when the antimicrobials were tested alone they

showed no activity against *M. smegmatis*. While MYEX7-318 had some activity against *M. smegmatis*, MYEX7-311 had none, and even the activity for MYEX7-318 spiked with either erythromycin, ethambutol, or gentamicin was greater than the activity of MYEX7-318 alone.

There were some interesting examples of synergy worth noting. Some antimicrobials such as erythromycin and vancomycin, which are known to have activity against *E. faecium*, only had inhibition activity when the antimicrobial was dissolved in an extract (Figure 3.2, Figure 3.3). While other antimicrobials, such as gentamicin, and streptomycin, which are not expected to inhibit *E. faecium*, had inhibition activity when they were dissolved in MYEX7-318.

Some examples of antagonistic effect were observed. For example, ethambutol and sulfamethazine; when these antimicrobials were mixed with MYEX7-311 there was less inhibition activity against *E. faecium* than when they were screened alone. In addition, when vancomycin was mixed with MYEX7-311, it showed less inhibition activity against *P. aeruginosa* than when the antimicrobial was tested alone bioactivity of the antibiotic alone.

These results give a sense of the complexity and variability in bioactivity expression that was seen from performing extract matrix effect testing. Overall, the findings concur with research that has investigated different mixtures of antibiotics shown to have synergistic activity (Chaudhry et al., 2017; Klastersky and Zinner, 1982; Monzon et al., 2001). Furthermore, studies have shown that mixtures of bioactive compounds have shown some microorganisms to have more synergistic activity than other such as *E. faecium* (Pankey, 2005; Spera and Farber, 1994). These results suggest that the bioactivity

profile for an antimicrobial inside of an extract could be different to that of the antimicrobial alone and that the extract matrix is affecting the expression of bioactivity of antimicrobials.

Flewelling (2018) observed some of the antimicrobial standards cluster together due to their similar modes of action (Flewelling, 2018). However, only some of the antimicrobial standards from the same class were observed to cluster together due to their similar modes of action, in the experiments of this chapter (Flewelling, 2018). For example, amphotericin B, miconazole and nystatin are all antifungal agents and have a similar mode of action against ergosterol in fungi (Georgopapadakou and Walsh 1996; Hammond et al. 1974). However, only amphotericin B and nystatin had similar bioactivity profiles that clustered together (Georgopapadakou and Walsh 1996; Hammond et al. 1974). Furthermore, gentamicin, kanamycin and streptomycin are all aminoglycosides and have a similar mode of action, however, only kanamycin and streptomycin had similar bioactivity profiles that clustered together (Coates et al., 2011; Davies and Davies, 2010; Lewis, 2013). This clustering of commercial antimicrobials further suggests the bioactivity profiling technique developed by Flewelling (2018) is not a reliable approach to grouping antimicrobials by their similar modes of action. To better observe clustering of the antibiotics by biological pathways or even by class, the data set could be increased to include more antibiotics from the same class.

As previously noted, in some cases activity against a pathogen was present in an extract spiked with a commercial antimicrobial, but was not seen in the bioactivity profile of the antibiotic alone; as seen in gentamicin (1 µg/mL) and gentamicin (1 µg/mL) and MYEX7-318 (100 µg/mL) with activity against *P. aeruginosa* (Figure 3.2, Figure 3.3).

However, this activity can be seen in the bioactivity profile of the extract alone (Figure 3.2, Figure 3.3) and from Berenbaum's definition there is an understanding that synergistic effects are occurring to increase inhibition activity in the spiked extracts. Most interaction effects observed from the matrix effect tests were a result of synergism. However, there were instances, as previously described, where different components would work against each other masking activity (Harvey et al., 2015; Merzenich et al., 2010; Wagner, 2011), but these occurrences were less common. This observation would make sense as, most often, therapeutic effects are greater when multiple compounds in extracts or plants are combined and are working together or interacting with each other to affect the different biological targets (Harvey et al., 2015; Kiyohara et al., 2004; Thomford et al., 2018). There are many examples of synergism in herbal treatments; one example is a treatment used for asthma. Extracts from *Ganoderma lucidum*, *Glycyrrhiza uralensis* and *Sophora flavescens* are all required to achieve the long-lasting effects of treatment and alleviate bronchoconstriction in asthma (Thomford et al., 2018; Wen et al., 2005). Additionally, there are now more and more examples of natural product extracts interacting in synergism with commercial antibiotics such as the research conducted by Chusri and others that investigated bioactivity from plants of the *Apocynaceae* family and antibiotics against clinical isolates of *Acinetobacter baumannii* (Adwan and Mhanna, 2008; Calvo et al., 2015; Chusri et al., 2014; Sharma et al., 2004; Tiwari et al., 2005; Thomford et al., 2018).

Both extracts (MYEX7-311 and MYEX7-318) had some activity against one the test microorganisms and this could have skewed the bioactivity profiles of antimicrobial compounds dissolved in the extracts. The research conducted by Wong and colleagues (2012) demonstrated that when there were multiple bioactive constituents

present in a single extract, those extracts did not cluster well with the different standard antimicrobial compounds (Wong et al., 2012). This limitation was due to “mixed-mode” effects or effects from multiple bioactive constituents in a single matrix. To avoid “mixed-mode” BioMAP profiles, they suggested that extracts be prefractionated before screening to avoid having multiple bioactive compounds in the same extracts (Wong et al., 2012). However, they suggest that even crude extracts can still be used in the BioMAP approach as long as this limitation is kept in mind and other information such as chemical peak library data is considered during the prioritization process.

Many researchers have investigated interaction effects such as synergistic, additive, and antagonistic effects (Anochie et al., 2018; Calvo et Al., 2015; Cheesman et al., 2017; David et al., 2015; Harvey et al., 2015; Merzenich et al., 2010; Wagner, 2011). Some of these effects can explain the discrepancies seen in bioactivity profiles of antimicrobials and extracts spiked with those same antimicrobials individually. However, more research is still needed for the combination effects of chemicals within plants (Thomford et al., 2018; Kiyohara et al., 2004). Mechanistically supported evidence for synergy in natural product extracts has been lacking (Duke and Bogenschutz-Godwin, 1998; Stermitz et al., 2000). Particularly, research is needed to better understand and predict when processes are occurring to potentiate or prolong the various bioactivities of compounds within an extract, in a synergistic or antagonistic manner (Thomford et al., 2018; Kiyohara et al., 2004). In the meantime, some antimicrobials were showing similar inhibition activity even when they were spiked in an extract that previously had some inhibition activity. With the exception of some organisms such as *E. faecium*, activity present in an antimicrobial alone was also seen in an extract spiked with that antimicrobial. Although the strength of

inhibition was not always the same between the antimicrobials and the antimicrobials in an extract, the general patterns of activity were similar and changing the concentration of the antimicrobial could help better detect activity. The bioactivity profiles of extracts, antimicrobial compounds, and antimicrobials dissolved in an extract were analysed using statistical analyses (a heat-map hierarchical cluster analysis) are used to further investigate the bioactivity of commercial antimicrobial compounds dissolved in an extract matrix.

### **3.4 Summary**

The bioactivity profiles of the seventeen standard antimicrobial compounds from Flewelling's (2018) bioactivity profiling approach were compared to the bioactivity profiles of those same antimicrobials dissolved singularly in two different extracts (MYEX7-311 and MYEX7-318). These bioactivity profiles were compared to determine whether the extract matrix affects the bioactivity profile of antimicrobial compounds. The results of the hierarchical cluster analysis showed that most antimicrobial compounds did not cluster with the same antimicrobial in an extract matrix. Of the 17 commercial antimicrobial compounds that were individually dissolved in the two extracts, only 6 antimicrobials clustered with the same antimicrobial dissolved in either MYEX7-311 or MYEX7-318. Multiple factors including synergism, antagonism, and 'mixed-mode' effects are occurring when antimicrobial compounds are dissolved in an extract matrix and are affecting the the bioactivity that would be expressed by the antimicrobial compound alone. The results show that the extract matrix is a concern in bioactivity profiling and there are limitations to identifying potential antimicrobial compound within an extract using the bioactivity profiling technique developed by Flewelling (2018). Therefore, the bioactivity profiling approach developed by Flewelling should not be used as a prioritization technique

to identify potential antimicrobial compounds with unknown modes of action without further considering these effects. For example, more standards could be used to compare against the bioactivity profiles of extract collection in cluster analysis. Bioactivity profiles for the different antimicrobial compounds dissolved in a natural product extract matrix could be used as standards in addition to bioactivity profiles of pure antimicrobial compounds. Modifying this technique, in an attempt to control for synergy, could improve this bioactivity profiling technique, making it a useful prioritization tool in natural product discovery.

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## **APPENDICES**

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## Appendix 1: Host plant of isolate extracts in NPRG library

**Table A1.1.** Taxonomic name of host plants for all two hundred and eighty endophytic fungal isolates in the Natural Products Research library.

<b>Extract</b>	<b>Endophyte</b>	<b>Host plant</b>
MYEX1-001	TC2-078	<i>Heracleum maximum</i>
MYEX1-002	TC2-073	<i>Populus tremuloides</i>
MYEX1-003	TC2-074	<i>Populus tremuloides</i>
MYEX1-004	TC2-062	<i>Symplocarpus foetidus</i>
MYEX1-005	TC2-044	<i>Juniperis communis</i>
MYEX1-006	TC2-086	<i>Geum macrophyllum</i>
MYEX1-007	TC2-058	<i>Fragaria virginiana</i>
MYEX1-008	TC2-057	<i>Fragaria virginiana</i>
MYEX1-009	TC2-069	<i>Populus tremuloides</i>
MYEX1-010	TC2-035	<i>Hypericum perforatum</i>
MYEX1-011	TC2-041	<i>Empetrum nigrum</i>
MYEX1-012	TC2-011	<i>Nuphar lutea</i>
MYEX1-013	TC2-014	<i>Nuphar lutea</i>
MYEX1-014	TC2-034	<i>Hypericum perforatum</i>
MYEX1-015	TC2-030	<i>Hypericum perforatum</i>
MYEX1-016	TC2-016	<i>Nuphar lutea</i>
MYEX1-017	TC2-026	<i>Hypericum perforatum</i>
MYEX1-018	TC2-018	<i>Nuphar lutea</i>
MYEX1-019	TC2-033	<i>Hypericum perforatum</i>
MYEX1-020	TC2-063	<i>Symplocarpus foetidus</i>
MYEX1-021	TC2-061	<i>Symplocarpus foetidus</i>
MYEX1-022	TC2-045	<i>Hypericum perforatum</i>
MYEX1-023	TC2-032	<i>Hypericum perforatum</i>
MYEX1-024	TC2-048	<i>Populus tremuloides</i>
MYEX1-025	TC2-036	<i>Hypericum perforatum</i>
MYEX1-026	TC2-042	<i>Empetrum nigrum</i>
MYEX1-027	TC2-072	<i>Populus tremuloides</i>
MYEX1-028	TC2-067	<i>Populus tremuloides</i>
MYEX1-029	TC2-054	<i>Fragaria virginiana</i>
MYEX1-030	TC2-039	<i>Nuphar lutea</i>
MYEX1-031	TC2-027	<i>Hypericum perforatum</i>
MYEX1-032	TC2-053	<i>Fragaria virginiana</i>
MYEX1-033	TC3-009	<i>Moneses uniflora</i>
MYEX1-034	TC2-089	<i>Moneses uniflora</i>
MYEX1-035	TC2-091	<i>Acorus calamus</i>

**Table A1.1.** Taxonomic name of host plants for all two hundred and eighty endophytic fungal isolates in the Natural Products Research Group library.

<b>Extract</b>	<b>Endophyte</b>	<b>Host plant</b>
<b>MYEX1-036</b>	TC3-002	<i>Acorus calamus</i>
<b>MYEX1-037</b>	TC3-004	<i>Acorus calamus</i>
<b>MYEX1-038</b>	TC2-025	<i>Aralia nudicaulis</i>
<b>MYEX1-039</b>	TC2-013	<i>Juniperus communis</i>
<b>MYEX1-040</b>	TC2-052	<i>Fragaria virginiana</i>
<b>MYEX1-041</b>	TC2-084	<i>Geum macrophyllum</i>
<b>MYEX1-042</b>	TC2-031	<i>Hypericum perforatum</i>
<b>MYEX1-044</b>	TC2-028	<i>Hypericum perforatum</i>
<b>MYEX1-045</b>	TC2-051	<i>Fragaria virginiana</i>
<b>MYEX1-046</b>	TC3-006	<i>Moneses uniflora</i>
<b>MYEX1-047</b>	TC2-050	<i>Fragaria virginiana</i>
<b>MYEX1-048</b>	TC2-020	<i>Aralia nudicaulis</i>
<b>MYEX1-049</b>	TC2-082	<i>Symplocarpus foetidus</i>
<b>MYEX1-050</b>	TC2-092	<i>Acorus calamus</i>
<b>MYEX1-051</b>	TC3-003	<i>Acorus calamus</i>
<b>MYEX1-052</b>	TC2-088	<i>Moneses uniflora</i>
<b>MYEX1-053</b>	TC2-076	<i>Symplocarpus foetidus</i>
<b>MYEX1-054</b>	TC3-005	<i>Moneses uniflora</i>
<b>MYEX1-055</b>	TC2-043	<i>Juniperus communis</i>
<b>MYEX1-056</b>	TC2-022	<i>Aralia nudicaulis</i>
<b>MYEX1-057</b>	TC2-017	<i>Juniperus communis</i>
<b>MYEX1-058</b>	TC2-012	<i>Juniperus communis</i>
<b>MYEX1-059</b>	TC2-049	<i>Fragaria virginiana</i>
<b>MYEX1-060</b>	TC2-075	<i>Heracleum maximum</i>
<b>MYEX1-062</b>	TC2-071	<i>Populus tremuloides</i>
<b>MYEX1-063</b>	TC2-090	<i>Moneses uniflora</i>
<b>MYEX1-064</b>	TC3-012	<i>Moneses uniflora</i>
<b>MYEX1-065</b>	TC2-065	<i>Symplocarpus foetidus</i>
<b>MYEX1-066</b>	TC2-080	<i>Heracleum maximum</i>
<b>MYEX1-067</b>	TC2-021	<i>Aralia nudicaulis</i>
<b>MYEX1-068</b>	TC2-059	<i>Symplocarpus foetidus</i>
<b>MYEX1-069</b>	TC2-015	<i>Nuphar lutea</i>
<b>MYEX1-070</b>	TC2-060	<i>Symplocarpus foetidus</i>
<b>MYEX1-071</b>	TC2-038	<i>Nuphar lutea</i>
<b>MYEX1-072</b>	TC2-064	<i>Symplocarpus foetidus</i>

**Table A1.1.** Taxonomic name of host plants for all two hundred and eighty endophytic fungal isolates in the Natural Products Research Group library.

<b>Extract</b>	<b>Endophyte</b>	<b>Host plant</b>
<b>MYEX1-073</b>	TC2-029	<i>Hypericum perforatum</i>
<b>MYEX1-074</b>	TC2-077	<i>Heracleum maximum</i>
<b>MYEX1-075</b>	TC2-019	<i>Aralia nudicaulis</i>
<b>MYEX1-076</b>	TC2-046	<i>Juniperus communis</i>
<b>MYEX1-077</b>	TC2-055	<i>Populus tremuloides</i>
<b>MYEX1-078</b>	KP2-005A	<i>Fucus vesiculosus</i>
<b>MYEX1-079</b>	KP2-029E	<i>Fucus spiralis</i>
<b>MYEX1-080</b>	KP2-029F	<i>Fucus spiralis</i>
<b>MYEX1-081</b>	KP1-009A	<i>Porphyra sp.</i>
<b>MYEX1-082</b>	KP2-013G	<i>Petalonia fascia</i>
<b>MYEX1-083</b>	KP1-063E	<i>Mastocarpus stellatus</i>
<b>MYEX1-084</b>	KP1-063F	<i>Mastocarpus stellatus</i>
<b>MYEX1-085</b>	KP1-063L	<i>Mastocarpus stellatus</i>
<b>MYEX1-086</b>	KP1-063M	<i>Mastocarpus stellatus</i>
<b>MYEX1-087</b>	KP1-063N	<i>Mastocarpus stellatus</i>
<b>MYEX1-088</b>	KP1-063P	<i>Mastocarpus stellatus</i>
<b>MYEX1-089</b>	KP1-095A	<i>Ulva lactuca</i>
<b>MYEX1-090</b>	KP1-123A	<i>Spongomorpha arcta</i>
<b>MYEX1-091</b>	KP1-135E	<i>Petalonia fascia</i>
<b>MYEX1-092</b>	KP1-135E2	<i>Petalonia fascia</i>
<b>MYEX1-093</b>	KP1-135F	<i>Petalonia fascia</i>
<b>MYEX1-094</b>	KP1-139A	<i>Ulva lactuca</i>
<b>MYEX1-095</b>	KP1-139B	<i>Ulva lactuca</i>
<b>MYEX1-096</b>	KP2-001A	<i>Polysiphonia lanosa</i>
<b>MYEX1-097</b>	KP2-001B	<i>Polysiphonia lanosa</i>
<b>MYEX1-098</b>	KP2-013A	<i>Petalonia fascia</i>
<b>MYEX1-099</b>	KP2-013E	<i>Petalonia fascia</i>
<b>MYEX1-100</b>	KP2-013F	<i>Petalonia fascia</i>
<b>MYEX1-101</b>	KP1-131M	<i>Scytosiphon lomentaria</i>
<b>MYEX1-102</b>	KP1-131N	<i>Scytosiphon lomentaria</i>
<b>MYEX1-103</b>	KP1-131Q	<i>Scytosiphon lomentaria</i>
<b>MYEX1-104</b>	KP1-131U	<i>Scytosiphon lomentaria</i>
<b>MYEX1-105</b>	KP1-131Y	<i>Scytosiphon lomentaria</i>
<b>MYEX1-106</b>	KP1-131AA	<i>Scytosiphon lomentaria</i>
<b>MYEX1-107</b>	KP1-131BB	<i>Scytosiphon lomentaria</i>

**Table A1.1.** Taxonomic name of host plants for all two hundred and eighty endophytic fungal isolates in the Natural Products Research Group library.

<b>Extract</b>	<b>Endophyte</b>	<b>Host plant</b>
<b>MYEX1-108</b>	KP1-175D	<i>Spongomorpha arcta</i>
<b>MYEX1-109</b>	KP1-175K	<i>Spongomorpha arcta</i>
<b>MYEX1-111</b>	KP1-131B	<i>Scytosiphon lomentaria</i>
<b>MYEX1-112</b>	KP1-131DA	<i>Scytosiphon lomentaria</i>
<b>MYEX1-113</b>	KP1-131C	<i>Scytosiphon lomentaria</i>
<b>MYEX1-114</b>	KP1-175G	<i>Spongomorpha arcta</i>
<b>MYEX1-115</b>	KP2-025C	<i>Ulva intestinalis</i>
<b>MYEX1-116</b>	KP2-025D	<i>Ulva intestinalis</i>
<b>MYEX1-117</b>	KP1-017C	<i>Scytosiphon lomentaria</i>
<b>MYEX1-118</b>	KP1-075A	<i>Porphyra sp.</i>
<b>MYEX1-119</b>	KP1-075B	<i>Porphyra sp.</i>
<b>MYEX1-120</b>	KP1-131G	<i>Scytosiphon lomentaria</i>
<b>MYEX1-122</b>	KP1-131I	<i>Scytosiphon lomentaria</i>
<b>MYEX1-123</b>	KP1-131L	<i>Scytosiphon lomentaria</i>
<b>MYEX1-124</b>	KP2-001C	<i>Polysiphonia lanosa</i>
<b>MYEX3-125</b>	KP2-001F	<i>Polysiphonia lanosa</i>
<b>MYEX3-126</b>	KP2-001G	<i>Polysiphonia lanosa</i>
<b>MYEX3-127</b>	KP2-029D	<i>Fucus spiralis</i>
<b>MYEX3-128</b>	KP1-045C	<i>Ascophyllum nodosum</i>
<b>MYEX3-129</b>	KP1-045I	<i>Ascophyllum nodosum</i>
<b>MYEX3-130</b>	KP2-033B	<i>Desmarestia viridis</i>
<b>MYEX3-131</b>	KP1-045J	<i>Ascophyllum nodosum</i>
<b>MYEX3-132</b>	KP2-033E	<i>Desmarestia viridis</i>
<b>MYEX3-133</b>	KP1-045A	<i>Ascophyllum nodosum</i>
<b>MYEX3-134</b>	KP2-033A	<i>Desmarestia viridis</i>
<b>MYEX3-135</b>	KP2-033D	<i>Desmarestia viridis</i>
<b>MYEX3-136</b>	KP1-119C	<i>Devaleraea ramentacea</i>
<b>MYEX3-137</b>	KP1-115A	<i>Chondrus crispus</i>
<b>MYEX3-138</b>	KP1-081A	<i>Devaleraea ramentacea</i>
<b>MYEX3-139</b>	KP2-029B	<i>Fucus spiralis</i>
<b>MYEX3-140</b>	KP1-045G	<i>Ascophyllum nodosum</i>
<b>MYEX3-141</b>	KP2-033F	<i>Desmarestia viridis</i>
<b>MYEX3-142</b>	KP1-179C	<i>Dumontia contorta</i>
<b>MYEX3-143</b>	KP1-119E	<i>Devaleraea ramentacea</i>
<b>MYEX3-144</b>	KP1-119A	<i>Devaleraea ramentacea</i>

**Table A1.1.** Taxonomic name of host plants for all two hundred and eighty endophytic fungal isolates in the Natural Products Research Group library.

<b>Extract</b>	<b>Endophyte</b>	<b>Host plant</b>
<b>MYEX3-145</b>	KP2-033C	<i>Desmarestia viridis</i>
<b>MYEX3-146</b>	KP1-119B	<i>Devaleraea ramentacea</i>
<b>MYEX3-147</b>	KP1-179A	<i>Dumontia contorta</i>
<b>MYEX3-148</b>	KP1-021A	<i>Fucus distichus</i> supsp. <i>evanescens</i>
<b>MYEX3-149</b>	KP1-025B	<i>Mastocarpus stellatus</i>
<b>MYEX3-150</b>	KP1-025C	<i>Mastocarpus stellatus</i>
<b>MYEX4-151</b>	KP1-063A	<i>Mastocarpus stellatus</i>
<b>MYEX4-152</b>	KP1-063B	<i>Mastocarpus stellatus</i>
<b>MYEX4-153</b>	KP1-063C	<i>Mastocarpus stellatus</i>
<b>MYEX4-154</b>	KP1-069A	<i>Fucus distichus</i> supsp. <i>evanescens</i>
<b>MYEX4-155</b>	KP1-069B	<i>Fucus distichus</i> supsp. <i>evanescens</i>
<b>MYEX4-156</b>	KP1-069C	<i>Fucus distichus</i> supsp. <i>evanescens</i>
<b>MYEX4-157</b>	KP1-069D	<i>Fucus distichus</i> supsp. <i>evanescens</i>
<b>MYEX4-158</b>	KP1-069E	<i>Fucus distichus</i> supsp. <i>evanescens</i>
<b>MYEX4-159</b>	KP1-099A	<i>Fucus distichus</i> supsp. <i>edentatus</i>
<b>MYEX4-160</b>	KP1-143A	<i>Fucus distichus</i> supsp. <i>edentatus</i>
<b>MYEX4-161</b>	KP1-143B	<i>Fucus distichus</i> supsp. <i>edentatus</i>
<b>MYEX4-162</b>	KP1-143C	<i>Fucus distichus</i> supsp. <i>edentatus</i>
<b>MYEX4-163</b>	KP1-143D	<i>Fucus distichus</i> supsp. <i>edentatus</i>
<b>MYEX4-164</b>	KP1-017A	<i>Scytosiphon lomentaria</i>
<b>MYEX4-165</b>	KP1-123C	<i>spongomorpha arcta</i>
<b>MYEX4-166</b>	KP1-045D	<i>Ascophyllum nodosum</i>
<b>MYEX4-167</b>	KP1-131W	<i>Scytosiphon lomentaria</i>
<b>MYEX4-168</b>	KP1-009B	<i>Porphyra</i> sp.
<b>MYEX4-169</b>	KP1-013B	<i>Fucus spiralis</i>
<b>MYEX4-170</b>	KP1-131K	<i>Scytosiphon lomentaria</i>
<b>MYEX4-171</b>	KP1-089A	<i>Desmarestia viridis</i>
<b>MYEX4-172</b>	KP1-135C	<i>Petalonia fascio</i>
<b>MYEX4-173</b>	KP1-045B	<i>Ascophyllum nodosum</i>
<b>MYEX4-174</b>	KP1-135D	<i>Petalonia fascio</i>
<b>MYEX4-175</b>	KP1-045K	<i>Ascophyllum nodosum</i>
<b>MYEX4-176</b>	KP1-119D	<i>Devaleraea ramentacea</i>
<b>MYEX4-177</b>	KP1-123B	<i>Spongomorpha arcta</i>
<b>MYEX4-178</b>	KP1-135B	<i>Petalonia fascio</i>
<b>MYEX4-182</b>	CT1-002A	<i>Carissa Macrocorpa</i>

**Table A1.1.** Taxonomic name of host plants for all two hundred and eighty endophytic fungal isolates in the Natural Products Research Group library.

<b>Extract</b>	<b>Endophyte</b>	<b>Host plant</b>
<b>MYEX5-219</b>	CT1-005A	<i>Carissa Macrocorpa</i>
<b>MYEX6-259</b>	CT1-006A	<i>Pelagonium tomentosum</i>
<b>MYEX6-266</b>	KP1-063J	<i>Mastocarpus stellatus</i>
<b>MYEX6-267</b>	KP1-131DD	<i>Scytosiphon lomentaria</i>
<b>MYEX6-268</b>	KP1-131O	<i>Scytosiphon lomentaria</i>
<b>MYEX6-269</b>	KP1-131S	<i>Scytosiphon lomentaria</i>
<b>MYEX6-270</b>	KP1-131T	<i>Scytosiphon lomentaria</i>
<b>MYEX6-271</b>	KP1-175E	<i>Spongomorpha arcta</i>
<b>MYEX6-272</b>	KP1-175L	<i>Spongomorpha arcta</i>
<b>MYEX6-273</b>	KP1-175M	<i>Spongomorpha arcta</i>
<b>MYEX6-274</b>	KP2-001D	<i>Polysiphonia lanosa</i>
<b>MYEX6-275</b>	KP2-009B	<i>Ulva intestinalis</i>
<b>MYEX6-276</b>	KP2-025B	<i>Ulva intestinalis</i>
<b>MYEX6-277</b>	KP1-131DB	<i>Scytosiphon lomentaria</i>
<b>MYEX6-278</b>	KP2-033G	<i>Desmarestia viridis</i>
<b>MYEX6-279</b>	KP1-131V	<i>Scytosiphon lomentaria</i>
<b>MYEX6-280</b>	KP2-025E	<i>Ulva intestinalis</i>
<b>MYEX6-281</b>	KP1-131R	<i>Scytosiphon lomentaria</i>
<b>MYEX6-282</b>	KP2-001E	<i>Polysiphonia lanosa</i>
<b>MYEX6-283</b>	KP2-029C	<i>Fucus spiralis</i>
<b>MYEX6-284</b>	KP1-017D	<i>Scytosiphon lomentaria</i>
<b>MYEX6-285</b>	TC2-047	<i>Populus tremuloides</i>
<b>MYEX6-286</b>	KP1-063O	<i>Mastocarpus stellatus</i>
<b>MYEX6-287</b>	KP1-171A	<i>Saccharina latissima</i>
<b>MYEX6-288</b>	KP2-017A	<i>Fucus distichus subsp. Edentatus</i>
<b>MYEX6-289</b>	TC2-070	<i>Populus tremuloides</i>
<b>MYEX6-295</b>	JAJ1-033	<i>Picea pungens</i>
<b>MYEX6-296</b>	JAJ1-089	<i>Thuja occidentalis</i>
<b>MYEX6-297</b>	TC3-014	<i>Moness uniflora</i>
<b>MYEX6-298</b>	KP2-009A	<i>Ulva intestinalis</i>
<b>MYEX6-299</b>	KP2-025A	<i>Ulva intestinalis</i>
<b>MYEX7-301</b>	JAJ1-141	<i>Thuja occidentalis</i>
<b>MYEX7-302</b>	JAJ1-105	<i>Thuja occidentalis</i>
<b>MYEX7-303</b>	KP1-009C	<i>Porphyra sp.</i>
<b>MYEX7-304</b>	KP1-175A	<i>Spongomorpha arcta</i>

**Table A1.1.** Taxonomic name of host plants for all two hundred and eighty endophytic fungal isolates in the Natural Products Research Group library.

<b>Extract</b>	<b>Endophyte</b>	<b>Host plant</b>
<b>MYEX7-308</b>	KP1-175J	<i>Spongomorpha arcta</i>
<b>MYEX7-309</b>	KP1-091A	<i>Fucus vesiculosus</i>
<b>MYEX7-310</b>	KP1-017E	<i>Scytosiphon lomentaria</i>
<b>MYEX7-311</b>	KP1-131CC	<i>Scytosiphon lomentaria</i>
<b>MYEX7-312</b>	KP2-033H	<i>Desmarestia viridis</i>
<b>MYEX7-313</b>	KP1-175F	<i>Spongomorpha arcta</i>
<b>MYEX7-314</b>	KP1-175C	<i>Spongomorpha arcta</i>
<b>MYEX7-315</b>	JAJ1-057	<i>Thuja occidentalis</i>
<b>MYEX7-316</b>	JAJ1-113	<i>Thuja occidentalis</i>
<b>MYEX7-317</b>	JAJ1-149	<i>Thuja occidentalis</i>
<b>MYEX7-318</b>	JAJ1-093	<i>Thuja occidentalis</i>
<b>MYEX7-319</b>	JAJ1-001	<i>Picea pungens</i>
<b>MYEX7-320</b>	KP1-175H	<i>Spongomorpha arcta</i>
<b>MYEX7-321</b>	KP1-179B	<i>Dumontia contorta</i>
<b>MYEX7-327</b>	KP1-131E	<i>Scytosiphon lomentaria</i>
<b>MYEX7-333</b>	JAJ1-025	<i>Picea pungens</i>
<b>MYEX7-334</b>	JAJ1-081	<i>Thuja occidentalis</i>
<b>MYEX7-335</b>	JAJ1-009	<i>Picea pungens</i>
<b>MYEX7-336</b>	JAJ1-077	<i>Thuja occidentalis</i>
<b>MYEX7-337</b>	JAJ1-153	<i>Thuja occidentalis</i>
<b>MYEX7-338</b>	JAJ1-137	<i>Thuja occidentalis</i>
<b>MYEX7-339</b>	JAJ1-021	<i>Picea pungens</i>
<b>MYEX7-340</b>	JAJ1-073	<i>Thuja occidentalis</i>
<b>MYEX7-341</b>	JAJ1-157	<i>Thuja occidentalis</i>
<b>MYEX7-342</b>	JAJ1-097	<i>Thuja occidentalis</i>
<b>MYEX7-343</b>	JAJ1-029	<i>Picea pungens</i>
<b>MYEX7-344</b>	JAJ1-069	<i>Thuja occidentalis</i>
<b>MYEX7-345</b>	JAJ1-049	<i>Thuja occidentalis</i>
<b>MYEX7-346</b>	JAJ1-045	<i>Picea pungens</i>
<b>MYEX8-354</b>	JAJ1-017	<i>Picea pungens</i>
<b>MYEX8-355</b>	JAJ1-053	<i>Thuja occidentalis</i>
<b>MYEX8-356</b>	JAJ1-061	<i>Thuja occidentalis</i>
<b>MYEX8-357</b>	JAJ1-065	<i>Thuja occidentalis</i>
<b>MYEX8-358</b>	JAJ1-121	<i>Thuja occidentalis</i>
<b>MYEX8-359</b>	JAJ1-133	<i>Thuja occidentalis</i>

**Table A1.1.** Taxonomic name of host plants for all two hundred and eighty endophytic fungal isolates in the Natural Products Research Group library.

<b>Extract</b>	<b>Endophyte</b>	<b>Host plant</b>
<b>MYEX8-360</b>	JAJ1-145	<i>Thuja occidentalis</i>
<b>MYEX8-361</b>	JAJ1-161	<i>Thuja occidentalis</i>
<b>MYEX8-368</b>	CT1-007A	<i>Cotyledon orbiculata</i>
<b>MYEX8-374</b>	JAJ1-037	<i>Picea pungens</i>
<b>MYEX8-375</b>	SC1-079H	Seafoam
<b>MYEX8-376</b>	SC1-085G	Seafoam
<b>MYEX8-377</b>	SC1-085H	Seafoam
<b>MYEX8-380</b>	JAJ1-129	<i>Thuja occidentalis</i>
<b>MYEX8-381</b>	SC1-077C	Seafoam
<b>MYEX8-382</b>	SC1-079G	Seafoam
<b>MYEX8-383</b>	SC1-085P	Seafoam
<b>MYEX8-384</b>	JAJ1-005	<i>Picea pungens</i>
<b>MYEX8-385</b>	JAJ1-041	<i>Picea pungens</i>
<b>MYEX8-392</b>	SC1-063C	Seafoam
<b>MYEX8-393</b>	SC1-079D	Seafoam
<b>MYEX8-394</b>	SC1-085N	Seafoam
<b>MYEX8-395</b>	TC2-068	<i>Populus tremuloides</i>
<b>MYEX8-397</b>	SC1-065E	Seafoam
<b>MYEX8-398</b>	SC1-067J	Seafoam
<b>MYEX9-401</b>	SC1-077G	Seafoam
<b>MYEX9-402</b>	CT1-006B	<i>Pelagonium tomentosum</i>
<b>MYEX9-403</b>	CT1-010B	<i>Monella cordufolia</i>
<b>MYEX9-404</b>	CT1-011A	Not recorded
<b>MYEX9-405</b>	CT1-012A	Not recorded
<b>MYEX9-406</b>	CT1-013A	Not recorded
<b>MYEX9-407</b>	CT1-016A	Not recorded
<b>MYEX9-408</b>	KP1-131J	<i>Scytosiphon lomentaria</i>
<b>MYEX9-409</b>	SC1-065G	Seafoam
<b>MYEX9-410</b>	JAJ1-117	<i>Thuja occidentalis</i>
<b>MYEX9-411</b>	SC1-067B	Seafoam
<b>MYEX9-412</b>	JAJ1-013	<i>Picea pungens</i>
<b>MYEX9-413</b>	CT1-008A	<i>Carpobrotus edulis</i>
<b>MYEX9-414</b>	CT1-012B	Not recorded
<b>MYEX9-415</b>	CT1-017A	Not recorded
<b>MYEX9-416</b>	JAJ1-109	<i>Thuja occidentalis</i>

## Appendix 2: Extract antimicrobial raw data

**Table A2.1.** Antimicrobial activity of extracts in the NPRG collection before normalization<sup>a</sup>.

Extract	SA	EF	PA	EC	CA	SC	MT	MS
MYEX1-001	29.7	7.3	20.3	8.3	0.0	4.6	3.3	0.0
MYEX1-002	22.1	0.0	14.5	13.5	1.0	99.0	1.9	0.0
MYEX1-003	0.0	0.0	18.7	9.6	0.0	2.1	0.2	0.0
MYEX1-004	0.0	0.0	13.9	16.5	32.9	5.2	0.0	0.0
MYEX1-005	0.0	0.0	21.5	14.4	0.0	6.7	0.0	0.0
MYEX1-006	29.8	3.7	18.7	8.0	3.3	2.2	3.3	0.0
MYEX1-007	0.0	7.0	16.6	15.4	0.0	0.0	0.0	0.0
MYEX1-008	0.0	0.0	17.5	16.3	0.0	0.0	3.0	0.0
MYEX1-009	0.0	0.0	17.3	16.5	0.0	0.0	0.0	0.0
MYEX1-010	0.0	0.0	14.6	1.2	0.0	0.0	0.0	0.0
MYEX1-011	0.0	0.0	21.5	10.8	0.0	4.2	0.0	0.0
MYEX1-012	0.0	0.0	18.4	16.3	0.0	0.0	0.2	0.0
MYEX1-013	0.0	1.4	16.9	17.4	0.0	0.2	7.3	0.0
MYEX1-014	0.0	0.0	18.5	4.5	0.0	0.0	0.0	0.0
MYEX1-015	0.0	0.0	14.7	14.3	0.0	0.0	1.6	0.0
MYEX1-016	0.0	0.0	13.9	7.0	0.0	0.0	0.0	2.5
MYEX1-017	0.0	0.0	15.5	0.6	0.0	0.0	0.0	11.8
MYEX1-018	0.0	0.0	12.4	4.8	0.0	0.0	0.0	0.0
MYEX1-019	0.0	0.0	11.4	5.0	0.6	0.0	10.7	10.7
MYEX1-020	0.0	0.0	10.2	13.1	0.0	0.0	1.6	7.8

<sup>a</sup> Mean percentage inhibition (n=3); All negative values removed and represented as zeros. SA: *Staphylococcus aureus*; EF: *Enterococcus faecium*; PA: *Pseudomonas aeruginosa*; EC: *Escherichia coli*; CA: *Candida albicans*; SC: *Saccharomyces cerevisiae*; MT: *Mycobacterium tuberculosis*; MS: *Mycobacterium smegmatis*

**Table A2.1.** Antimicrobial activity of extracts in the NPRG collection before normalization<sup>a</sup>.

Extract	SA	EF	PA	EC	CA	SC	MT	MS
MYEX1-021	0.0	0.0	9.8	8.2	1.3	0.0	3.4	0.0
MYEX1-022	0.0	0.0	15.3	12.3	4.0	3.3	0.0	0.0
MYEX1-023	0.0	0.0	12.4	11.5	0.0	0.4	0.0	0.0
MYEX1-024	0.0	2.3	12.3	3.5	0.0	0.0	4.6	0.0
MYEX1-025	0.0	0.0	9.1	9.4	0.0	0.0	0.0	26.7
MYEX1-026	0.0	0.0	9.5	5.4	0.0	0.0	0.0	1.6
MYEX1-027	0.0	0.0	9.9	3.2	0.0	0.0	0.3	36.0
MYEX1-028	0.0	0.0	11.5	5.1	3.1	0.0	1.1	72.9
MYEX1-029	0.0	2.8	11.9	0.0	1.5	0.0	7.4	13.6
MYEX1-030	0.0	7.5	17.0	21.9	0.0	0.0	0.0	24.5
MYEX1-031	0.0	0.0	12.2	0.0	0.0	0.0	0.0	32.4
MYEX1-032	0.0	0.0	14.4	8.4	0.0	0.0	0.5	6.0
MYEX1-033	0.0	0.0	12.0	0.0	0.0	0.0	1.3	3.6
MYEX1-034	0.0	0.0	1.8	0.0	0.0	0.0	0.0	1.4
MYEX1-035	2.7	4.7	13.7	1.7	0.0	4.9	0.0	21.6
MYEX1-036	0.0	0.0	22.8	7.7	0.0	0.1	0.0	21.9
MYEX1-037	0.0	0.0	14.4	0.0	0.1	0.0	0.0	0.0
MYEX1-038	0.0	0.0	10.7	0.0	0.3	0.0	1.8	16.6
MYEX1-039	15.5	0.0	23.8	0.0	0.5	0.0	1.8	9.7
MYEX1-040	0.0	8.7	20.8	6.3	0.0	2.0	0.5	34.0

<sup>a</sup> Mean percentage inhibition (n=3); All negative values removed and represented as zeros. SA: *Staphylococcus aureus*; EF: *Enterococcus faecium*; PA: *Pseudomonas aeruginosa*; EC: *Escherichia coli*; CA: *Candida albicans*; SC: *Saccharomyces cerevisiae*; MT: *Mycobacterium tuberculosis*; MS: *Mycobacterium smegmatis*

**Table A2.1.** Antimicrobial activity of extracts in the NPRG collection before normalization<sup>a</sup>.

Extract	SA	EF	PA	EC	CA	SC	MT	MS
MYEX1-041	3.5	0.7	18.0	10.0	35.1	5.0	11.5	41.5
MYEX1-042	0.0	0.0	18.7	10.1	0.0	1.7	0.0	31.7
MYEX1-044	69.3	0.0	17.0	1.8	0.0	0.0	5.1	30.5
MYEX1-045	0.0	0.0	24.2	3.3	0.0	0.0	0.0	41.8
MYEX1-046	74.3	0.0	0.0	16.2	7.2	1.3	2.9	16.4
MYEX1-047	0.0	0.0	2.2	0.0	0.4	0.0	0.0	33.2
MYEX1-048	69.7	7.3	17.9	4.2	0.0	1.8	1.2	48.0
MYEX1-049	0.0	9.0	16.5	6.9	0.0	0.0	0.5	52.2
MYEX1-050	0.0	0.0	19.2	8.4	0.0	0.0	0.0	35.4
MYEX2-051	0.0	0.0	20.1	13.8	0.0	0.0	0.0	35.9
MYEX2-052	0.0	8.0	36.8	12.1	3.6	0.5	1.2	0.0
MYEX2-053	0.0	0.3	21.3	0.0	0.0	0.0	3.9	0.0
MYEX2-054	0.0	0.0	8.2	10.8	0.0	0.0	0.5	0.0
MYEX2-055	0.0	0.0	3.4	9.3	0.0	0.0	1.5	6.2
MYEX2-056	0.0	0.0	0.0	0.0	0.0	0.0	0.0	15.5
MYEX2-057	0.0	0.0	1.2	6.7	0.0	0.0	0.0	0.0
MYEX2-058	0.0	0.0	8.9	8.8	4.6	11.2	20.1	4.5
MYEX2-059	0.0	0.0	7.6	15.0	0.0	0.0	0.0	1.8
MYEX2-060	23.9	0.0	3.8	13.1	0.0	10.3	59.6	2.9
MYEX2-062	0.0	0.0	2.3	9.0	0.0	2.5	8.2	5.9

<sup>a</sup> Mean percentage inhibition (n=3); All negative values removed and represented as zeros. SA: *Staphylococcus aureus*; EF: *Enterococcus faecium*; PA: *Pseudomonas aeruginosa*; EC: *Escherichia coli*; CA: *Candida albicans*; SC: *Saccharomyces cerevisiae*; MT: *Mycobacterium tuberculosis*; MS: *Mycobacterium smegmatis*

**Table A2.1.** Antimicrobial activity of extracts in the NPRG collection before normalization<sup>a</sup>.

Extract	SA	EF	PA	EC	CA	SC	MT	MS
MYEX2-064	0.0	0.0	4.3	8.7	0.0	0.9	0.0	1.0
MYEX2-065	0.0	0.0	0.0	0.0	3.7	0.0	0.0	9.4
MYEX2-066	0.0	0.0	4.4	5.4	0.0	0.0	0.0	2.0
MYEX2-067	0.0	0.0	1.2	0.0	0.0	0.0	0.0	2.2
MYEX2-068	0.0	0.0	2.5	0.0	0.0	0.0	0.0	0.0
MYEX2-069	52.9	0.0	6.1	6.1	0.0	0.0	100.0	0.0
MYEX2-070	0.0	0.0	5.2	0.0	0.0	0.0	0.7	1.0
MYEX2-071	2.2	7.2	6.3	14.3	0.0	16.9	92.8	0.0
MYEX2-072	0.0	6.3	7.1	0.8	0.0	99.8	0.0	5.9
MYEX2-073	89.0	0.0	5.9	28.7	1.5	100.0	99.3	0.0
MYEX2-074	0.0	0.0	12.2	15.5	0.0	13.6	17.4	10.7
MYEX2-075	0.0	0.0	10.0	0.0	6.6	12.5	8.6	3.7
MYEX2-076	50.4	2.2	41.8	29.4	0.0	11.7	0.8	9.9
MYEX2-077	0.0	8.3	2.5	4.8	0.0	11.3	0.6	0.4
MYEX2-078	0.0	9.1	5.1	12.5	3.5	1.8	0.0	0.0
MYEX2-079	0.0	0.0	20.2	0.0	1.1	0.0	11.7	0.8
MYEX2-080	0.0	0.0	15.9	5.0	0.0	0.0	88.9	70.3
MYEX2-081	0.0	0.0	24.3	0.0	0.0	3.6	60.2	0.0
MYEX2-082	0.0	0.0	23.9	0.0	0.0	1.8	27.1	0.0
MYEX2-083	0.0	0.0	25.0	0.0	0.0	4.0	9.6	0.0

<sup>a</sup> Mean percentage inhibition (n=3); All negative values removed and represented as zeros. SA: *Staphylococcus aureus*; EF: *Enterococcus faecium*; PA: *Pseudomonas aeruginosa*; EC: *Escherichia coli*; CA: *Candida albicans*; SC: *Saccharomyces cerevisiae*; MT: *Mycobacterium tuberculosis*; MS: *Mycobacterium smegmatis*

**Table A2.1.** Antimicrobial activity of extracts in the NPRG collection before normalization<sup>a</sup>.

Extract	SA	EF	PA	EC	CA	SC	MT	MS
MYEX2-084	2.9	1.9	0.0	7.8	8.9	2.1	0.9	0.0
MYEX2-085	0.0	0.0	0.0	15.2	4.1	1.4	7.4	3.3
MYEX2-086	0.0	0.0	25.8	1.2	0.0	0.0	78.8	69.0
MYEX2-087	0.0	0.0	26.3	0.0	0.0	0.0	4.2	71.3
MYEX2-088	0.0	0.0	22.2	0.0	0.0	0.0	44.7	0.0
MYEX2-089	0.0	0.0	3.9	7.0	3.1	0.0	68.4	1.6
MYEX2-090	99.8	100.0	11.4	42.3	0.0	98.6	0.0	6.4
MYEX2-091	0.0	0.0	22.7	0.0	0.0	0.0	2.1	0.0
MYEX2-092	2.9	0.0	13.2	0.0	0.8	8.6	0.0	2.8
MYEX2-093	0.0	0.0	23.7	0.0	0.0	0.0	5.3	63.3
MYEX2-094	0.0	0.0	24.3	0.0	0.6	0.0	0.0	70.9
MYEX2-095	27.2	0.0	10.2	8.0	8.8	12.5	0.0	72.1
MYEX2-096	0.0	0.0	3.0	10.3	2.3	0.0	10.4	73.8
MYEX2-097	0.0	16.7	1.8	9.9	2.3	0.0	7.9	76.1
MYEX2-098	0.0	13.8	11.2	15.0	1.9	0.0	7.2	72.6
MYEX2-099	0.0	13.2	4.6	11.2	5.5	0.0	3.0	72.7
MYEX2-100	0.0	0.0	0.0	2.9	0.4	0.0	92.1	58.7
MYEX3-101	0.2	0.0	6.5	1.9	0.0	18.8	56.3	59.5
MYEX3-102	4.0	0.0	10.3	8.5	4.4	32.8	1.1	73.8
MYEX3-103	74.7	14.2	17.6	14.1	0.0	17.9	8.9	78.9

<sup>a</sup> Mean percentage inhibition (n=3); All negative values removed and represented as zeros. SA: *Staphylococcus aureus*; EF: *Enterococcus faecium*; PA: *Pseudomonas aeruginosa*; EC: *Escherichia coli*; CA: *Candida albicans*; SC: *Saccharomyces cerevisiae*; MT: *Mycobacterium tuberculosis*; MS: *Mycobacterium smegmatis*

**Table A2.1.** Antimicrobial activity of extracts in the NPRG collection before normalization<sup>a</sup>.

Extract	SA	EF	PA	EC	CA	SC	MT	MS
MYEX3-104	0.0	8.0	11.1	8.0	0.0	13.9	67.9	79.8
MYEX3-105	0.0	2.3	14.6	4.0	0.0	20.9	0.0	8.8
MYEX3-106	32.8	0.0	9.4	3.1	0.0	21.5	92.2	0.0
MYEX3-107	8.9	0.0	6.4	3.9	0.0	13.2	31.1	3.2
MYEX3-108	0.0	0.0	29.6	23.1	1.1	10.4	42.1	19.2
MYEX3-109	0.0	22.0	6.1	11.4	0.0	2.3	3.3	0.0
MYEX3-110	100.0	12.0	1.3	15.5	0.1	9.4	93.3	0.0
MYEX3-111	9.4	0.0	11.6	7.3	0.3	6.3	61.3	0.0
MYEX3-112	70.3	0.0	7.4	13.5	4.1	13.4	5.5	30.3
MYEX3-113	74.3	0.0	9.7	10.5	0.8	23.8	53.6	0.0
MYEX3-114	5.5	0.0	3.7	9.4	4.6	0.0	34.6	0.0
MYEX3-115	16.3	1.4	3.6	21.7	10.3	7.1	56.1	0.0
MYEX3-116	0.0	0.0	0.0	0.0	4.5	3.2	27.6	0.0
MYEX3-117	73.1	26.7	13.4	12.3	0.4	5.4	31.4	0.0
MYEX3-118	0.0	20.8	2.5	6.6	3.5	0.0	71.4	9.2
MYEX3-119	27.2	6.4	3.2	0.0	6.5	3.0	10.2	0.0
MYEX3-120	62.4	8.5	2.7	28.3	0.0	12.7	26.4	0.0
MYEX3-122	6.7	0.0	3.0	5.9	0.0	2.7	0.0	0.0
MYEX3-123	10.6	0.0	0.0	6.3	2.0	5.2	55.5	4.7
MYEX3-124	0.0	3.6	0.0	4.2	0.0	0.0	24.7	0.5

<sup>a</sup> Mean percentage inhibition (n=3); All negative values removed and represented as zeros. SA: *Staphylococcus aureus*; EF: *Enterococcus faecium*; PA: *Pseudomonas aeruginosa*; EC: *Escherichia coli*; CA: *Candida albicans*; SC: *Saccharomyces cerevisiae*; MT: *Mycobacterium tuberculosis*; MS: *Mycobacterium smegmatis*

**Table A2.1.** Antimicrobial activity of extracts in the NPRG collection before normalization<sup>a</sup>.

Extract	SA	EF	PA	EC	CA	SC	MT	MS
MYEX3-125	20.6	2.5	0.6	0.0	5.3	2.6	0.0	0.0
MYEX3-126	0.0	0.0	4.3	7.3	2.3	15.9	68.4	3.6
MYEX3-127	19.1	22.1	0.7	2.2	1.0	3.9	22.9	0.0
MYEX3-128	9.4	6.6	0.0	13.3	1.2	6.6	17.6	16.8
MYEX3-129	0.0	0.0	18.5	0.0	0.4	11.7	42.5	5.5
MYEX3-130	77.6	17.8	96.7	81.8	0.0	24.5	95.7	79.2
MYEX3-131	0.0	3.8	0.0	6.9	5.3	2.7	0.0	0.0
MYEX3-132	0.0	5.7	0.0	0.0	0.0	7.5	32.3	0.0
MYEX3-133	15.1	0.7	0.0	16.7	0.0	8.3	0.0	0.0
MYEX3-134	4.5	0.0	2.6	7.7	0.4	99.1	9.4	4.7
MYEX3-135	0.0	0.0	0.0	0.1	4.5	0.0	0.0	4.0
MYEX3-136	28.1	0.0	0.0	10.9	0.0	6.2	82.7	0.0
MYEX3-137	0.0	7.7	0.0	15.9	2.8	5.8	16.5	0.0
MYEX3-138	0.0	0.0	0.0	15.9	2.1	6.8	78.9	0.0
MYEX3-139	0.0	0.0	0.4	12.3	1.2	7.9	11.3	2.7
MYEX3-140	0.0	0.0	19.4	0.0	0.0	0.0	12.2	0.0
MYEX3-141	0.0	12.7	11.8	5.5	5.9	23.9	0.0	0.0
MYEX3-142	0.0	12.8	11.0	12.1	8.0	27.3	79.9	6.0
MYEX3-143	99.6	48.5	0.0	26.1	4.5	18.8	94.3	75.4
MYEX3-144	0.0	0.0	0.0	4.1	0.0	3.7	0.0	75.5

<sup>a</sup> Mean percentage inhibition (n=3); All negative values removed and represented as zeros. SA: *Staphylococcus aureus*; EF: *Enterococcus faecium*; PA: *Pseudomonas aeruginosa*; EC: *Escherichia coli*; CA: *Candida albicans*; SC: *Saccharomyces cerevisiae*; MT: *Mycobacterium tuberculosis*; MS: *Mycobacterium smegmatis*

**Table A2.1.** Antimicrobial activity of extracts in the NPRG collection before normalization<sup>a</sup>.

Extract	SA	EF	PA	EC	CA	SC	MT	MS
MYEX3-145	0.0	22.2	0.0	8.7	0.0	0.0	0.0	2.6
MYEX3-146	0.0	4.8	0.0	16.7	16.5	0.0	0.0	18.3
MYEX3-147	0.0	0.0	0.0	12.2	1.8	6.6	100.0	20.1
MYEX3-148	0.0	0.0	11.7	0.0	0.0	27.7	2.6	0.0
MYEX3-149	16.1	0.0	11.2	19.3	3.3	32.8	24.8	11.2
MYEX3-150	0.0	0.0	0.0	8.4	1.3	0.0	2.0	0.0
MYEX4-151	0.0	0.0	3.8	3.4	6.4	8.8	4.1	0.0
MYEX4-152	0.0	8.2	0.0	0.0	0.0	8.4	5.2	0.0
MYEX4-153	0.0	9.5	4.5	9.5	0.5	6.3	0.8	82.2
MYEX4-154	0.0	0.0	0.0	6.0	0.0	1.9	0.0	87.9
MYEX4-155	0.0	0.0	0.0	13.4	0.2	0.0	0.0	0.0
MYEX4-156	0.0	0.0	0.0	0.0	0.5	0.6	0.0	70.8
MYEX4-157	0.0	0.0	14.5	11.3	3.9	27.8	7.0	3.3
MYEX4-158	0.0	0.0	0.0	11.7	1.3	6.5	8.4	0.0
MYEX4-159	8.6	9.2	0.7	18.6	2.5	0.8	0.0	1.2
MYEX4-160	8.2	0.0	0.0	11.8	1.4	6.5	4.0	9.6
MYEX4-161	0.0	19.8	9.6	8.6	2.4	26.9	0.0	24.8
MYEX4-162	3.1	18.4	19.5	7.4	1.6	19.6	0.0	2.1
MYEX4-163	47.0	99.7	0.0	15.0	4.8	16.8	97.3	0.0
MYEX4-164	0.0	10.1	7.4	14.8	1.2	26.5	0.0	10.6

<sup>a</sup> Mean percentage inhibition (n=3); All negative values removed and represented as zeros. SA: *Staphylococcus aureus*; EF: *Enterococcus faecium*; PA: *Pseudomonas aeruginosa*; EC: *Escherichia coli*; CA: *Candida albicans*; SC: *Saccharomyces cerevisiae*; MT: *Mycobacterium tuberculosis*; MS: *Mycobacterium smegmatis*

**Table A2.1.** Antimicrobial activity of extracts in the NPRG collection before normalization<sup>a</sup>.

Extract	SA	EF	PA	EC	CA	SC	MT	MS
MYEX4-165	1.5	0.0	12.2	6.9	0.0	22.2	0.0	5.8
MYEX4-166	10.0	0.0	10.2	18.1	1.3	29.1	55.7	0.0
MYEX4-167	74.9	0.0	0.0	6.5	0.0	29.4	62.1	0.0
MYEX4-168	45.1	0.0	30.4	0.0	0.0	25.1	71.9	17.5
MYEX4-169	34.3	11.5	10.3	0.1	11.8	31.2	54.6	13.5
MYEX4-170	0.0	0.0	17.0	7.6	3.4	23.2	0.0	8.3
MYEX4-171	11.1	0.0	11.0	6.8	98.9	24.0	0.0	16.7
MYEX4-172	76.7	0.0	8.7	8.4	0.8	39.6	97.0	9.2
MYEX4-173	44.9	9.8	0.0	15.8	11.3	15.5	57.1	0.0
MYEX4-174	5.1	0.0	0.0	10.1	3.2	15.1	41.4	22.6
MYEX4-175	47.5	10.2	0.0	13.7	4.3	14.7	0.0	0.0
MYEX4-176	0.0	0.0	5.6	11.5	1.9	28.0	13.4	6.4
MYEX4-177	100.0	92.6	12.1	32.7	0.0	98.8	89.7	0.0
MYEX4-178	44.5	12.6	0.0	11.5	15.3	7.9	42.1	4.5
MYEX4-182	74.1	8.2	0.0	0.8	7.8	0.0	78.9	0.0
MYEX5-219	0.0	11.7	9.5	5.0	5.6	0.1	16.3	25.2
MYEX6-259	51.0	0.8	13.0	0.9	5.8	16.4	99.2	15.8
MYEX6-266	19.9	2.3	0.6	16.8	11.1	11.0	91.6	0.0
MYEX6-267	76.3	0.0	39.9	31.8	4.8	6.6	0.0	24.0
MYEX6-268	0.0	0.0	6.8	2.4	0.0	0.0	39.1	0.0

<sup>a</sup> Mean percentage inhibition (n=3); All negative values removed and represented as zeros. SA: *Staphylococcus aureus*; EF: *Enterococcus faecium*; PA: *Pseudomonas aeruginosa*; EC: *Escherichia coli*; CA: *Candida albicans*; SC: *Saccharomyces cerevisiae*; MT: *Mycobacterium tuberculosis*; MS: *Mycobacterium smegmatis*

**Table A2.1.** Antimicrobial activity of extracts in the NPRG collection before normalization<sup>a</sup>.

Extract	SA	EF	PA	EC	CA	SC	MT	MS
MYEX6-269	21.1	0.0	0.0	15.6	94.2	4.2	93.9	3.0
MYEX6-270	7.3	0.0	0.0	15.8	6.9	6.1	92.5	0.0
MYEX6-271	0.0	0.0	34.6	34.0	91.6	3.9	48.7	19.2
MYEX6-272	7.3	19.2	0.0	18.9	7.1	11.4	0.0	2.9
MYEX6-273	49.1	0.0	1.9	7.3	7.4	10.0	83.5	0.0
MYEX6-274	4.7	7.1	1.6	11.2	3.2	2.6	23.4	10.5
MYEX6-275	0.0	0.0	0.5	18.1	7.2	3.3	77.7	0.0
MYEX6-276	38.4	0.0	6.6	5.1	4.9	10.4	41.0	16.7
MYEX6-277	0.0	24.8	2.8	16.2	1.8	0.1	46.2	27.0
MYEX6-278	0.0	25.5	0.0	17.9	5.1	0.0	20.3	0.0
MYEX6-279	3.8	20.4	0.0	14.0	5.0	0.9	1.5	0.0
MYEX6-280	0.0	0.0	0.0	7.2	2.1	2.4	47.1	0.0
MYEX6-281	24.6	2.0	0.0	18.1	4.2	1.1	0.0	5.9
MYEX6-282	0.0	0.0	0.0	15.0	4.8	2.7	50.1	0.0
MYEX6-283	0.0	21.7	0.2	13.7	2.2	0.0	46.8	0.0
MYEX6-284	0.0	37.7	0.0	20.8	2.6	0.0	44.8	1.3
MYEX6-285	0.0	20.2	0.0	14.8	1.8	0.0	0.0	5.8
MYEX6-286	0.0	0.0	13.0	0.0	0.0	0.0	20.3	0.0
MYEX6-287	0.0	0.0	0.0	11.3	1.8	0.0	77.7	65.2
MYEX6-288	0.0	3.5	2.4	17.6	4.9	0.0	83.8	0.0

<sup>a</sup> Mean percentage inhibition (n=3); All negative values removed and represented as zeros. SA: *Staphylococcus aureus*; EF: *Enterococcus faecium*; PA: *Pseudomonas aeruginosa*; EC: *Escherichia coli*; CA: *Candida albicans*; SC: *Saccharomyces cerevisiae*; MT: *Mycobacterium tuberculosis*; MS: *Mycobacterium smegmatis*

**Table A2.1.** Antimicrobial activity of extracts in the NPRG collection before normalization<sup>a</sup>.

Extract	SA	EF	PA	EC	CA	SC	MT	MS
MYEX6-289	42.3	0.0	0.0	13.9	2.7	99.7	83.6	0.0
MYEX6-295	5.9	19.6	2.2	8.1	0.0	0.0	82.4	6.6
MYEX6-296	0.0	18.7	1.6	7.6	2.7	0.0	83.3	0.0
MYEX6-297	70.5	1.3	0.0	9.6	99.4	19.2	84.1	0.1
MYEX6-298	0.0	0.0	0.0	5.2	3.9	0.0	86.1	0.0
MYEX6-299	0.0	0.0	0.0	16.7	7.4	0.0	85.3	25.6
MYEX7-301	0.0	23.0	7.1	9.5	3.8	0.0	49.3	0.0
MYEX7-302	3.9	20.0	4.3	10.6	0.8	0.0	38.3	4.0
MYEX7-303	0.0	0.0	5.5	15.5	3.5	0.0	92.4	30.8
MYEX7-304	0.0	0.0	0.4	9.7	3.0	0.0	65.3	54.9
MYEX7-308	73.5	0.0	0.0	19.5	4.5	12.9	83.1	0.0
MYEX7-309	64.2	2.8	0.0	45.9	5.0	12.8	96.9	0.0
MYEX7-310	67.8	0.0	22.8	36.2	5.5	7.0	95.8	0.0
MYEX7-311	0.0	0.0	0.0	11.7	6.4	3.3	77.1	0.0
MYEX7-312	60.0	13.2	2.0	24.2	4.7	8.1	82.3	0.0
MYEX7-313	0.0	0.0	33.5	18.3	0.0	0.0	74.7	75.0
MYEX7-314	0.0	0.0	20.6	12.2	0.0	0.0	77.4	99.1
MYEX7-315	0.0	4.7	26.0	0.0	1.0	0.0	14.8	0.0
MYEX7-316	64.6	0.0	98.8	26.0	0.0	9.3	79.4	31.7
MYEX7-317	4.1	0.6	27.0	0.0	0.0	0.0	54.5	0.0

<sup>a</sup> Mean percentage inhibition (n=3); All negative values removed and represented as zeros. SA: *Staphylococcus aureus*; EF: *Enterococcus faecium*; PA: *Pseudomonas aeruginosa*; EC: *Escherichia coli*; CA: *Candida albicans*; SC: *Saccharomyces cerevisiae*; MT: *Mycobacterium tuberculosis*; MS: *Mycobacterium smegmatis*

**Table A2.1.** Antimicrobial activity of extracts in the NPRG collection before normalization<sup>a</sup>.

Extract	SA	EF	PA	EC	CA	SC	MT	MS
MYEX7-318	0.0	0.0	25.1	0.8	0.5	0.7	9.5	2.6
MYEX7-319	0.0	0.0	0.0	2.3	0.0	2.9	37.1	11.2
MYEX7-320	0.0	0.0	5.5	15.7	2.3	0.0	54.7	100.0
MYEX7-321	0.0	7.9	28.5	0.0	1.6	0.0	81.0	74.2
MYEX7-327	0.0	0.0	0.0	0.0	2.2	0.5	46.9	18.3
MYEX7-333	4.3	0.0	0.0	0.0	13.4	5.3	19.5	8.6
MYEX7-334	53.3	0.0	0.0	100.0	24.0	33.3	94.2	7.3
MYEX7-335	0.0	0.0	1.6	7.9	10.2	0.0	40.3	0.0
MYEX7-336	18.9	0.0	0.0	23.4	8.8	20.0	98.0	0.0
MYEX7-337	0.0	3.9	28.4	0.0	0.0	3.9	35.3	0.0
MYEX7-338	0.0	0.0	0.0	10.8	0.0	0.0	25.9	0.8
MYEX7-339	55.0	4.4	0.0	0.8	10.0	8.1	86.9	0.0
MYEX7-340	99.6	29.4	24.7	0.0	79.3	99.5	85.1	4.5
MYEX7-341	0.0	0.0	3.3	21.7	3.6	2.4	98.0	8.5
MYEX7-342	0.0	0.0	0.0	6.8	3.9	0.1	5.6	0.0
MYEX7-343	0.0	3.1	12.4	0.0	4.6	0.0	0.0	14.5
MYEX7-344	55.6	0.0	0.0	0.0	4.9	2.9	0.0	9.1
MYEX7-345	78.3	0.0	3.5	18.9	0.0	0.0	0.0	0.0
MYEX7-346	0.0	0.0	0.0	16.8	1.2	0.7	0.0	19.1
MYEX8-354	0.0	0.0	0.0	0.0	0.0	0.0	93.7	0.0

<sup>a</sup> Mean percentage inhibition (n=3); All negative values removed and represented as zeros. SA: *Staphylococcus aureus*; EF: *Enterococcus faecium*; PA: *Pseudomonas aeruginosa*; EC: *Escherichia coli*; CA: *Candida albicans*; SC: *Saccharomyces cerevisiae*; MT: *Mycobacterium tuberculosis*; MS: *Mycobacterium smegmatis*

**Table A2.1.** Antimicrobial activity of extracts in the NPRG collection before normalization<sup>a</sup>.

Extract	SA	EF	PA	EC	CA	SC	MT	MS
MYEX8-355	0.0	0.0	0.0	0.0	0.0	0.0	3.6	7.6
MYEX8-356	0.0	0.0	0.0	0.0	0.0	0.0	10.4	0.0
MYEX8-357	11.8	3.8	0.0	1.8	5.0	2.4	6.9	4.3
MYEX8-358	0.0	0.0	0.0	0.0	2.1	2.2	58.5	5.7
MYEX8-359	0.0	0.0	0.8	0.0	3.1	2.9	7.4	0.0
MYEX8-360	0.0	0.0	0.0	0.5	1.1	4.7	3.2	0.0
MYEX8-361	9.3	0.0	0.0	0.0	0.0	8.3	7.6	0.0
MYEX8-368	8.8	0.0	0.0	1.9	3.2	1.0	17.7	0.0
MYEX8-374	4.2	4.6	0.0	1.6	8.0	9.2	16.2	0.0
MYEX8-375	0.0	0.0	0.0	0.0	1.8	0.0	7.6	0.0
MYEX8-376	6.2	0.0	0.0	8.7	1.0	7.8	32.9	0.0
MYEX8-377	0.0	0.0	0.0	0.0	1.3	0.0	0.0	0.0
MYEX8-380	0.0	0.2	0.0	3.3	1.6	0.0	1.4	0.0
MYEX8-381	0.0	3.5	0.0	12.6	1.8	0.0	4.0	0.0
MYEX8-382	64.3	0.0	99.1	45.1	3.7	23.0	97.8	82.0
MYEX8-383	25.3	0.0	0.0	1.7	0.0	0.0	25.0	0.0
MYEX8-384	0.0	3.1	0.0	0.0	100.0	0.0	22.5	0.0
MYEX8-385	0.0	6.6	0.6	5.8	0.0	0.0	10.3	0.0
MYEX8-392	0.0	0.0	0.0	0.0	1.5	0.0	0.0	0.0
MYEX8-393	14.8	1.0	0.0	0.0	5.3	4.8	34.9	0.0

<sup>a</sup> Mean percentage inhibition (n=3); All negative values removed and represented as zeros. SA: *Staphylococcus aureus*; EF: *Enterococcus faecium*; PA: *Pseudomonas aeruginosa*; EC: *Escherichia coli*; CA: *Candida albicans*; SC: *Saccharomyces cerevisiae*; MT: *Mycobacterium tuberculosis*; MS: *Mycobacterium smegmatis*

**Table A2.1.** Antimicrobial activity of extracts in the NPRG collection before normalization<sup>a</sup>.

Extract	SA	EF	PA	EC	CA	SC	MT	MS
MYEX8-394	14.7	0.0	0.0	7.3	2.5	9.2	54.2	71.8
MYEX8-395	4.2	7.3	0.0	0.0	8.2	0.0	7.7	57.1
MYEX8-397	6.3	8.7	0.2	0.0	2.2	0.0	8.9	0.0
MYEX8-398	34.2	1.4	3.9	6.2	18.2	30.8	84.0	22.8
MYEX9-401	83.2	64.5	22.2	43.6	44.5	10.7	13.8	0.0
MYEX9-402	11.9	1.9	4.5	5.0	9.6	3.8	12.6	21.8
MYEX9-403	39.4	15.8	0.0	2.8	8.9	29.5	62.7	12.6
MYEX9-404	16.1	22.1	3.5	9.6	2.7	16.7	14.7	3.2
MYEX9-405	8.6	20.4	12.0	8.4	0.0	19.4	25.3	10.6
MYEX9-406	8.2	0.0	1.8	12.5	4.4	17.0	0.0	1.0
MYEX9-407	0.6	0.0	3.3	2.9	5.2	23.7	0.0	12.4
MYEX9-408	0.0	8.6	13.7	10.3	4.2	0.0	0.2	13.8
MYEX9-409	0.0	5.2	0.0	1.2	29.2	89.7	14.2	11.8
MYEX9-410	0.0	87.7	29.9	0.0	14.3	21.4	25.3	0.0
MYEX9-411	99.1	0.0	6.6	0.0	6.8	56.0	56.0	0.0
MYEX9-412	0.0	99.2	32.7	8.4	8.8	2.2	56.8	0.0
MYEX9-413	1.9	0.7	0.0	5.0	5.7	17.6	0.0	7.0
MYEX9-414	38.9	0.0	18.6	1.0	2.3	11.6	11.6	11.9
MYEX9-415	0.0	0.0	0.0	0.0	2.0	17.8	0.0	73.7
MYEX9-416	0.0	0.0	5.9	0.0	5.6	21.5	0.0	3.8

<sup>a</sup> Mean percentage inhibition (n=3); All negative values removed and represented as zeros. SA: *Staphylococcus aureus*; EF: *Enterococcus faecium*; PA: *Pseudomonas aeruginosa*; EC: *Escherichia coli*; CA: *Candida albicans*; SC: *Saccharomyces cerevisiae*; MT: *Mycobacterium tuberculosis*; MS: *Mycobacterium smegmatis*

**Table A2.2.** Antimicrobial activity of extracts in the NPRG collection from first sample group half dilution testing before normalization<sup>a</sup>.

Extract	Endophyte	SA	EF	PA	EC	CA	SC	MT	MS
MYEX2-090	KP1-123A	99.8	100.0	11.4	42.3	0.0	98.6	0.0	6.4
MYEX3-110	KP1-131A	100.0	12.0	1.3	15.5	0.1	9.4	93.3	0.0
MYEX4-177	KP1-123B	100.0	92.6	12.1	32.7	0.0	98.8	89.7	0.0

<sup>a</sup> Mean percentage inhibition (n=3); All negative values removed and represented as zeros. SA: *Staphylococcus aureus*; EF: *Enterococcus faecium*; PA: *Pseudomonas aeruginosa*; EC: *Escherichia coli*; CA: *Candida albicans*; SC: *Saccharomyces cerevisiae*; MT: *Mycobacterium tuberculosis*; MS: *Mycobacterium smegmatis*

**Table A2.3.** Antimicrobial activity of extracts in the NPRG collection from first sample group half dilution testing after normalization<sup>a</sup>.

Extract	Endophyte	SA	EF	PA	EC	CA	SC	MT	MS
MYEX2-090	KP1-123A	1.0	1.0	0.1	0.4	0.0	1.0	0.0	0.1
MYEX3-110	KP1-131A	1.0	0.1	0.0	0.2	0.0	0.1	0.9	0.0
MYEX4-177	KP1-123B	1.0	0.9	0.1	0.3	0.0	1.0	0.9	0.0

<sup>a</sup> Mean percentage inhibition (n=3); All negative values removed and represented as zeros. SA: *Staphylococcus aureus*; EF: *Enterococcus faecium*; PA: *Pseudomonas aeruginosa*; EC: *Escherichia coli*; CA: *Candida albicans*; SC: *Saccharomyces cerevisiae*; MT: *Mycobacterium tuberculosis*; MS: *Mycobacterium smegmatis*

**Table A2.4.** Antimicrobial activity of extracts in the NPRG collection from second sample group half dilution testing before normalization<sup>a</sup>.

Extract	Endophyte	SA	EF	PA	EC	CA	SC	MT	MS
MYEX2-069	TC2-015	52.9	0.0	6.1	6.1	0.0	0.0	100.0	0.0
MYEX2-073	TC2-029	89.0	0.0	5.9	28.7	1.5	100.0	99.3	0.0
MYEX7-320	KP1-175H	0.0	0.0	5.5	15.7	2.3	0.0	54.7	100.0
MYEX7-334	JAJ1-081	53.3	0.0	0.0	100.0	24.0	33.3	94.2	7.3
MYEX8-384	JAJ1-005	0.0	3.1	0.0	0.0	100.1	0.0	22.5	0.0

<sup>a</sup> Mean percentage inhibition (n=3); All negative values removed and represented as zeros. SA: *Staphylococcus aureus*; EF: *Enterococcus faecium*; PA: *Pseudomonas aeruginosa*; EC: *Escherichia coli*; CA: *Candida albicans*; SC: *Saccharomyces cerevisiae*; MT: *Mycobacterium tuberculosis*; MS: *Mycobacterium smegmatis*

**Table A2.5.** Antimicrobial activity of extracts in the NPRG collection from second sample group half dilution testing after normalization<sup>a</sup>.

Extract	Endophyte	SA	EF	PA	EC	CA	SC	MT	MS
MYEX2-069	TC2-015	0.5	0	0.1	0.1	0	0	1	0
MYEX2-073	TC2-029	0.9	0.0	0.1	0.3	0.0	1.0	1.0	0.0
MYEX7-320	KP1-175H	0.0	0.0	0.1	0.2	0.0	0.0	0.5	1.0
MYEX7-334	JAJ1-081	0.5	0.0	0.0	1.0	0.2	0.3	0.9	0.1
MYEX8-384	JAJ1-005	0.0	0.0	0.0	0.0	1.0	0.0	0.2	0.0

<sup>a</sup> Mean percentage inhibition (n=3); All negative values removed and represented as zeros. SA: *Staphylococcus aureus*; EF: *Enterococcus faecium*; PA: *Pseudomonas aeruginosa*; EC: *Escherichia coli*; CA: *Candida albicans*; SC: *Saccharomyces cerevisiae*; MT: *Mycobacterium tuberculosis*; MS: *Mycobacterium smegmatis*

**Table A2.6.** Antimicrobial activity of commercially available antimicrobials in the NPRG collection from third sample group half dilution testing before normalization<sup>a</sup>.

Antibiotic	SA	EF	PA	EC	CA	SC	MT	MS
Actinomycin D	100.0	99.8	17	0.0	0.0	1.7	95.3	0.0
Amoxicillin	100.0	27.3	12.7	1.1	1.3	0.0	8.4	2.5
Ciprofloxacin	99.8	8.8	100.0	100.0	4.0	0.0	93.9	54.3
Gentamicin	96.4	0.0	1.2	100.0	3.3	0.0	57.6	0.0
Tetracycline	99.9	99.8	27.9	100.0	4.7	5.3	80.0	84.9
Vancomycin	100.0	0.0	21.6	0.0	0.0	1.0	79.4	50.6

<sup>a</sup> Mean percentage inhibition (n=3); All negative values removed and represented as zeros. SA: *Staphylococcus aureus*; EF: *Enterococcus faecium*; PA: *Pseudomonas aeruginosa*; EC: *Escherichia coli*; CA: *Candida albicans*; SC: *Saccharomyces cerevisiae*; MT: *Mycobacterium tuberculosis*; MS: *Mycobacterium smegmatis*

**Table A2.7.** Antimicrobial activity of commercially available antimicrobials in the NPRG collection from third sample group half dilution testing after normalization<sup>a</sup>.

Antibiotic	SA	EF	PA	EC	CA	SC	MT	MS
Actinomycin D	1.0	1.0	0.2	0.0	0.0	0.0	1.0	0.0
Amoxicillin	1.0	0.3	0.1	0.0	0.0	0.0	0.1	0.0
Ciprofloxacin	1.0	0.1	1.0	1.0	0.0	0.0	0.9	0.5
Gentamicin	1.0	0.0	0.0	1.0	0.0	0.0	0.6	0.0
Tetracycline	1.0	1.0	0.3	1.0	0.0	0.1	0.8	0.8
Vancomycin	1.0	0.0	0.2	0.0	0.0	0.0	0.8	0.5

<sup>a</sup> Mean percentage inhibition (n=3); All negative values removed and represented as zeros. SA: *Staphylococcus aureus*; EF: *Enterococcus faecium*; PA: *Pseudomonas aeruginosa*; EC: *Escherichia coli*; CA: *Candida albicans*; SC: *Saccharomyces cerevisiae*; MT: *Mycobacterium tuberculosis*; MS: *Mycobacterium smegmatis*

**Table A2.8.** Antimicrobial activity of extracts obtained for matrix effect testing from the NPRG collection before normalization<sup>a</sup>.

Extract	Endophyte	SA	EF	PA	EC	CA	SC	MT	MS
MYEX7-311	KP1-131CC	0	0	0	11.7	6.4	3.3	77.1	0
MYEX7-318	JAJ1-093	0	0	25.1	0.8	0.5	0.7	9.5	2.6

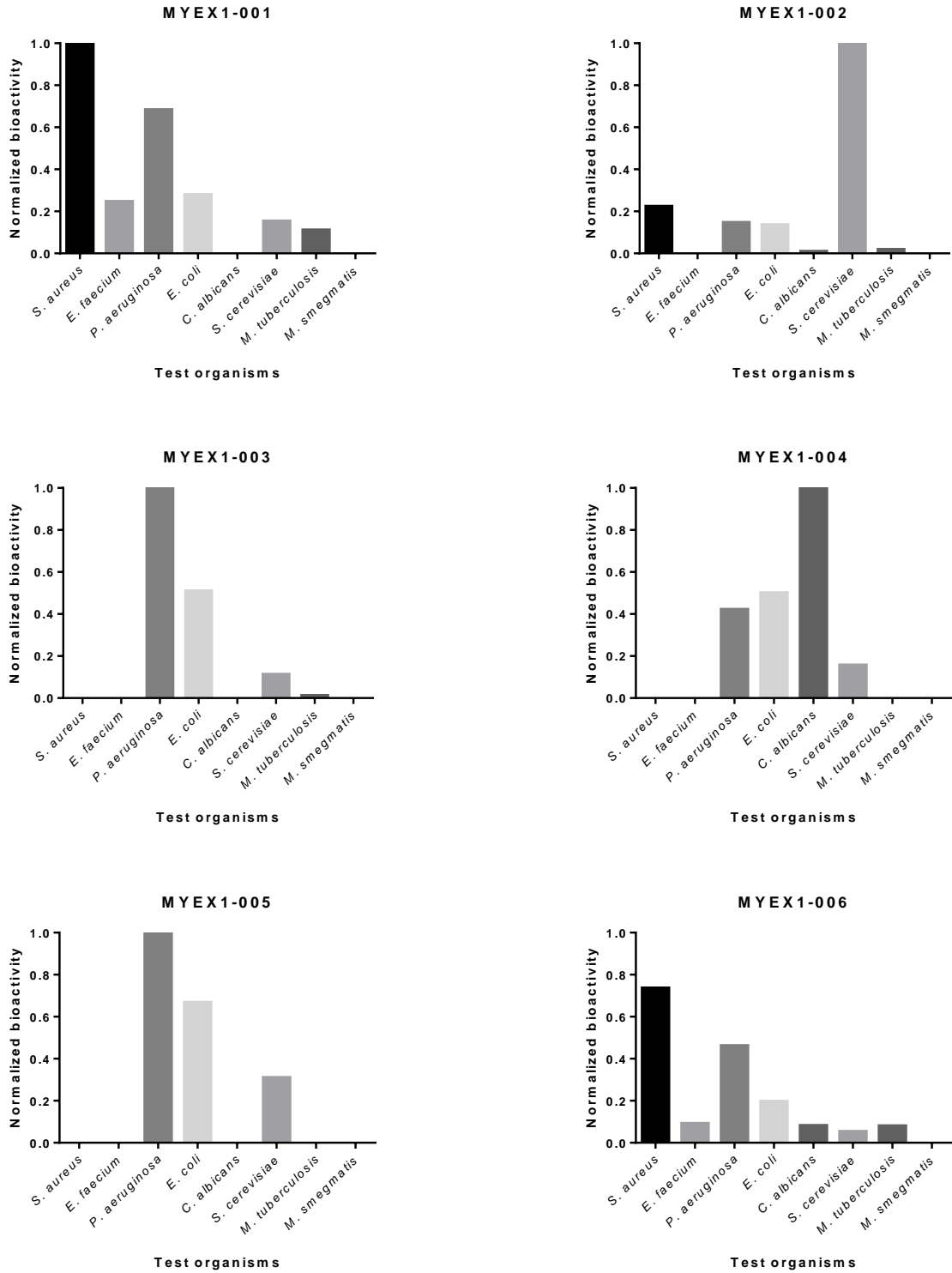
<sup>a</sup> Mean percentage inhibition (n=3); All negative values removed and represented as zeros. SA: *Staphylococcus aureus*; EF: *Enterococcus faecium*; PA: *Pseudomonas aeruginosa*; EC: *Escherichia coli*; CA: *Candida albicans*; SC: *Saccharomyces cerevisiae*; MT: *Mycobacterium tuberculosis*; MS: *Mycobacterium smegmatis*

**Table A2.9.** Antimicrobial activity of extracts obtained for matrix effect testing from the NPRG collection after normalization<sup>a</sup>.

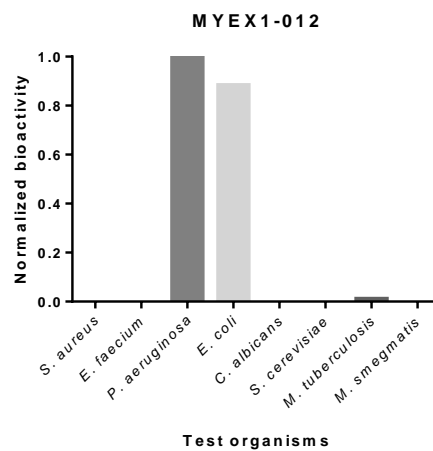
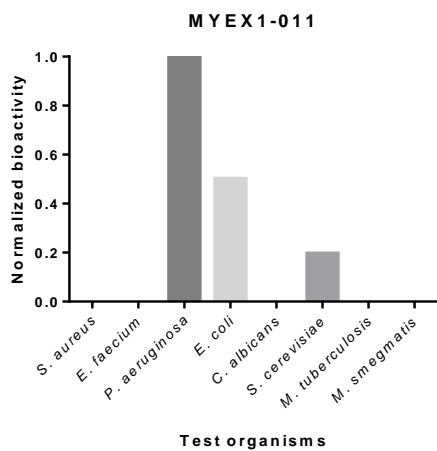
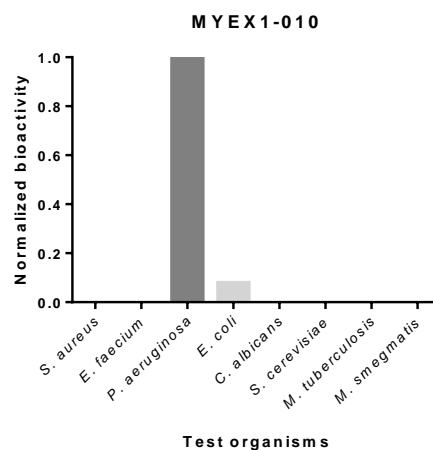
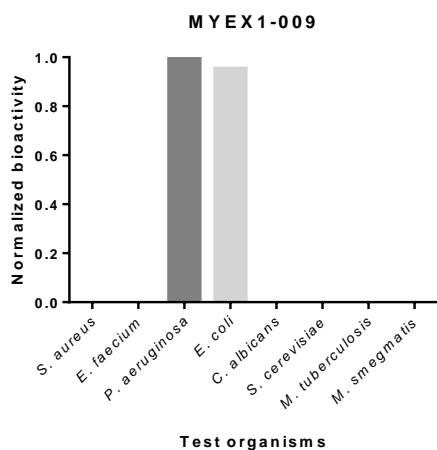
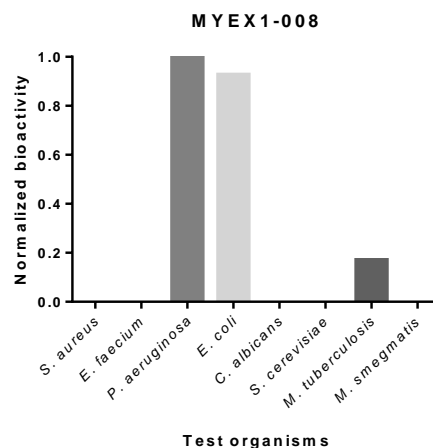
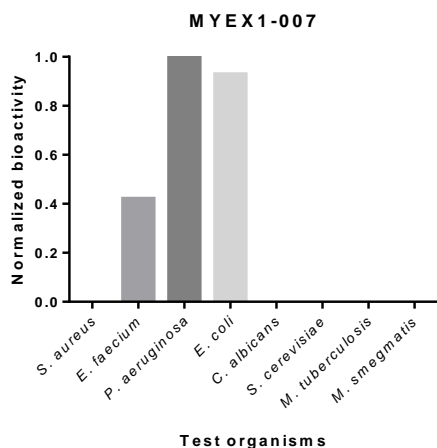
Extract	Endophyte	SA	EF	PA	EC	CA	SC	MT	MS
MYEX7-311	KP1-131CC	0	0	0	0.15	0.08	0.04	1	0
MYEX7-318	JAJ1-093	0	0	1	0.03	0.02	0.03	0.38	0.10

SA: *Staphylococcus aureus*; EF: *Enterococcus faecium*; PA: *Pseudomonas aeruginosa*; EC: *Escherichia coli*; CA: *Candida albicans*; SC: *Saccharomyces cerevisiae*; MT: *Mycobacterium tuberculosis*; MS: *Mycobacterium smegmatis*

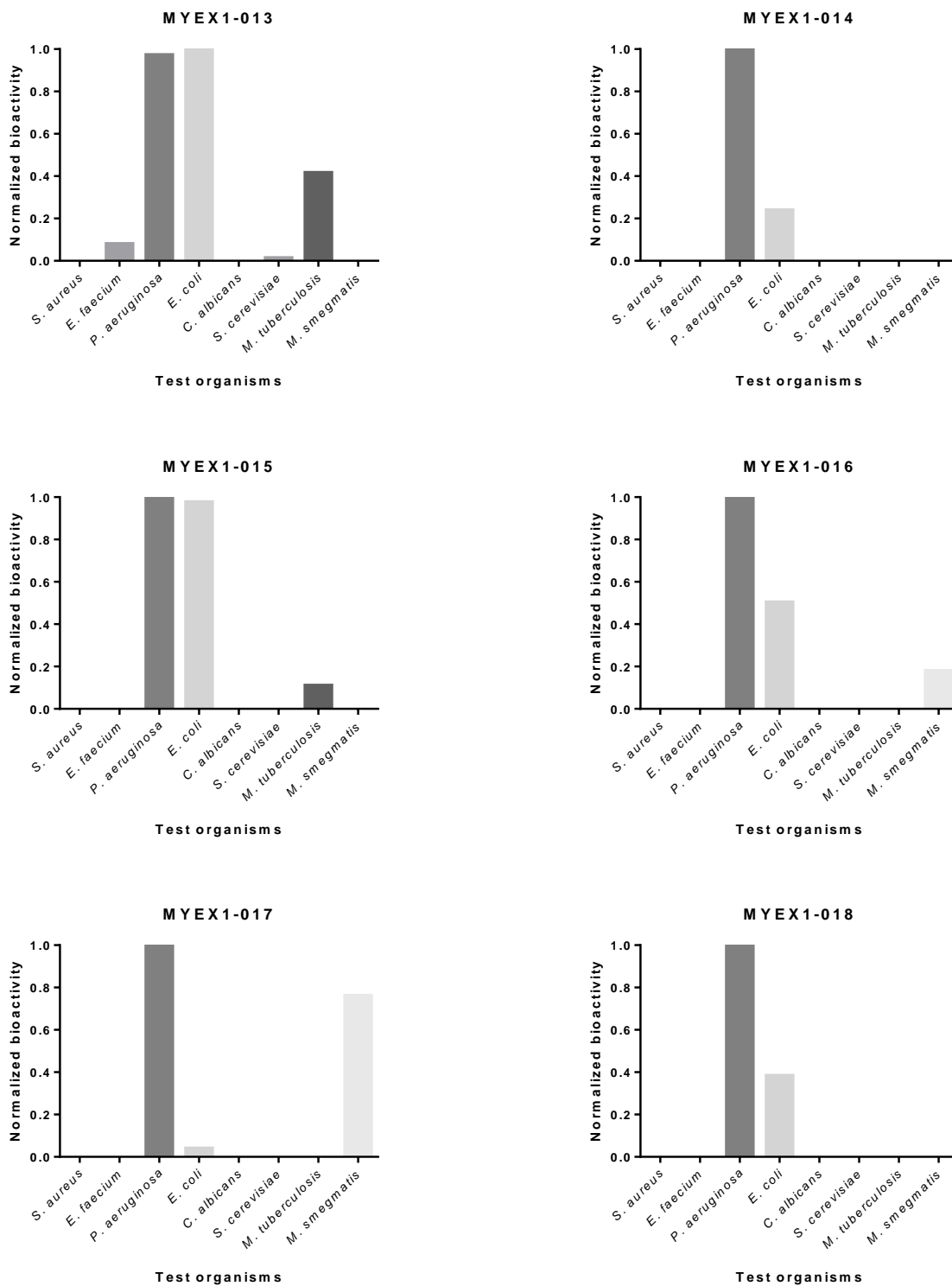
### Appendix 3: Bioactivity profiles



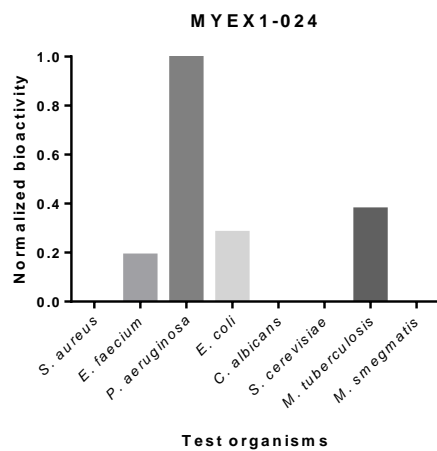
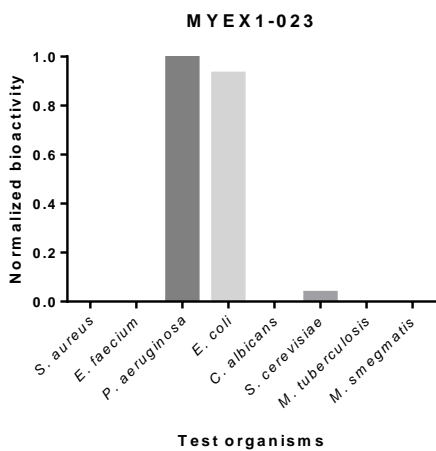
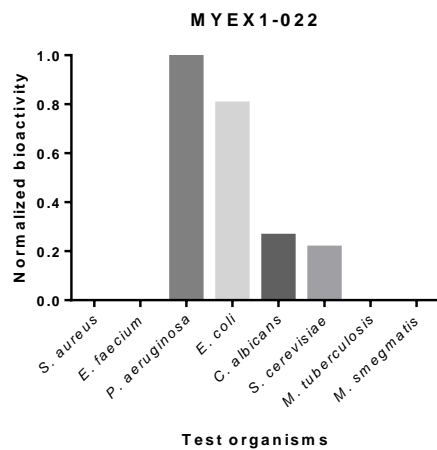
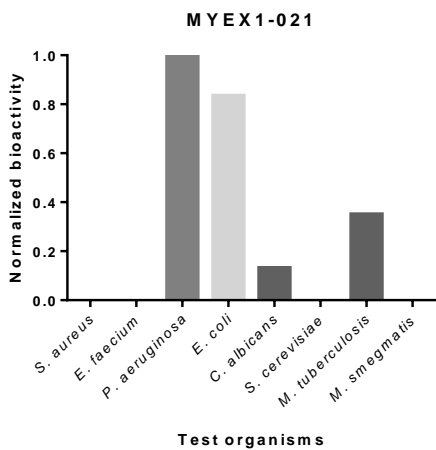
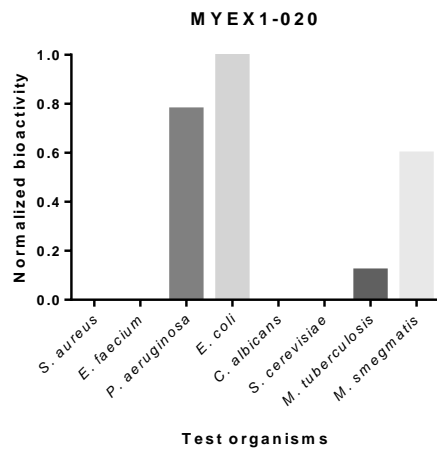
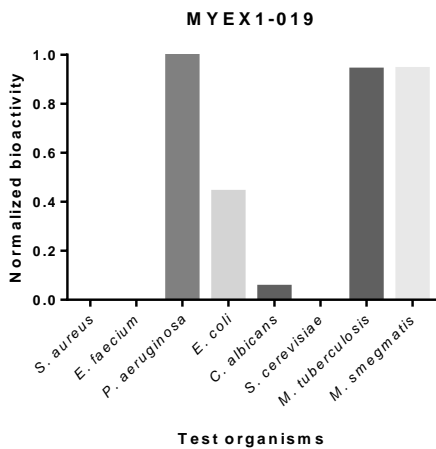
**Figure A3.1.** Bioactivity profiles of extracts in the NPRG collection.



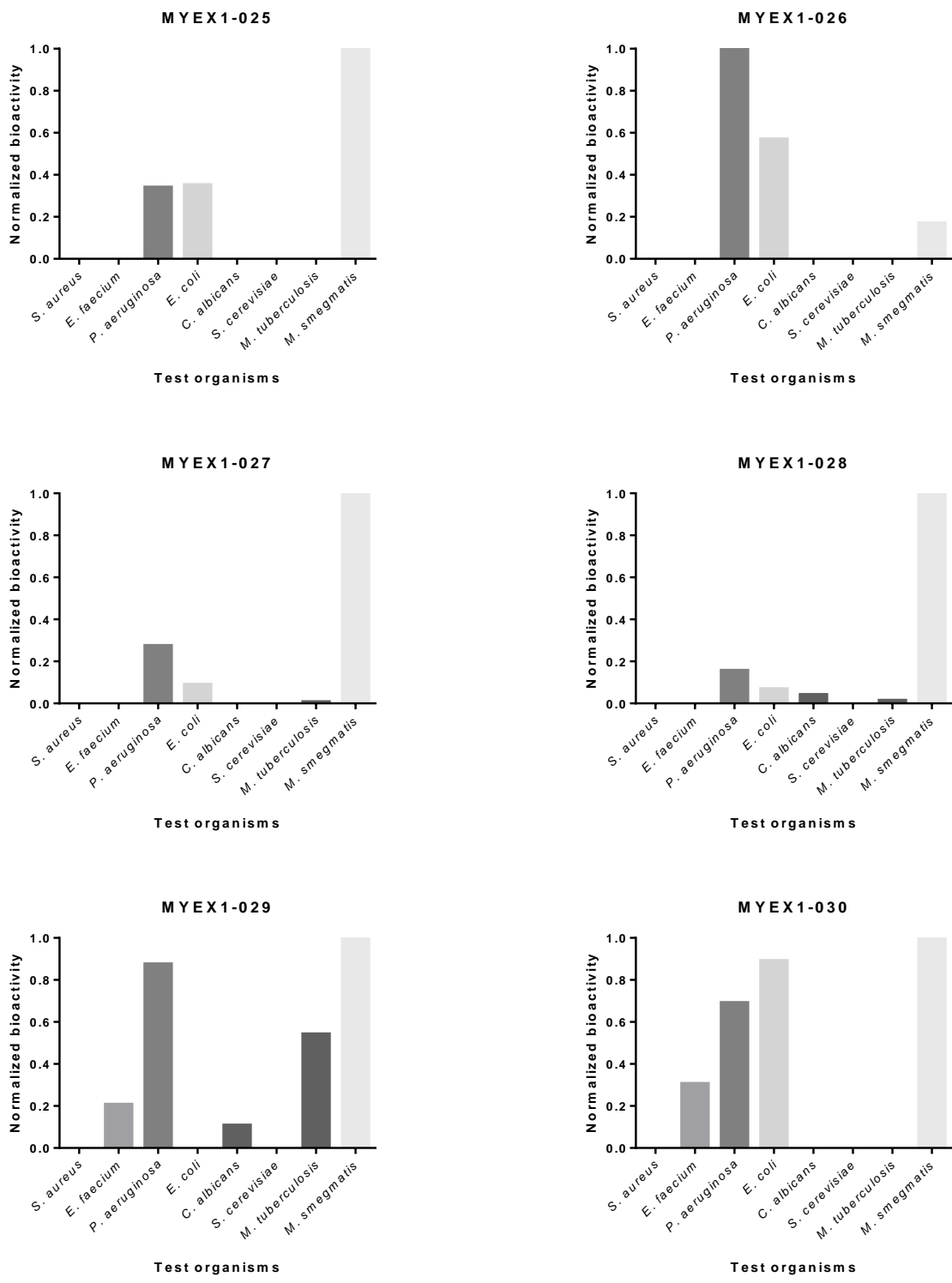
**Figure A3.1.** Bioactivity profiles of extracts in the NPRG collection (continued).



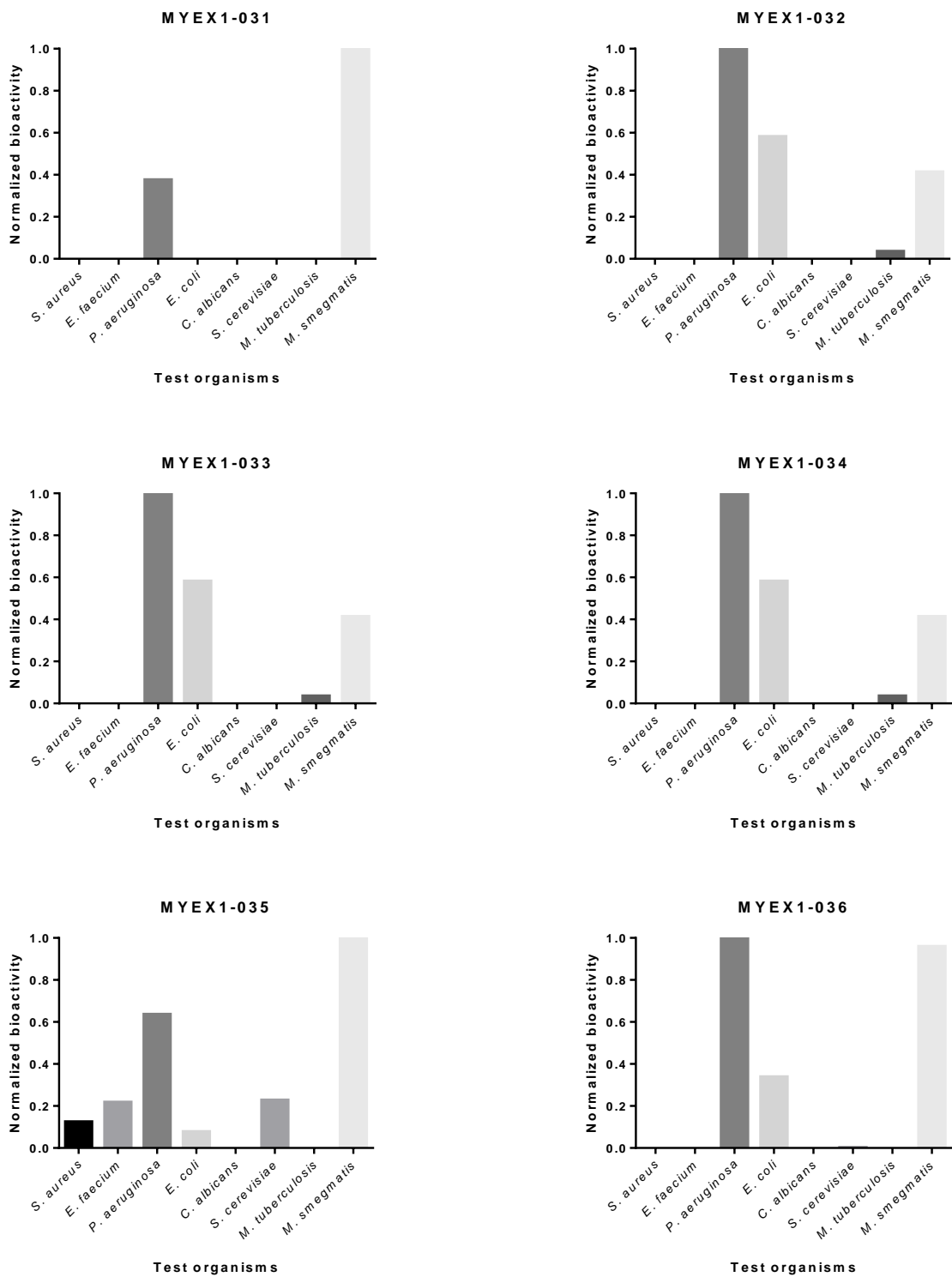
**Figure A3.1.** Bioactivity profiles of extracts in the NPRG collection (continued).



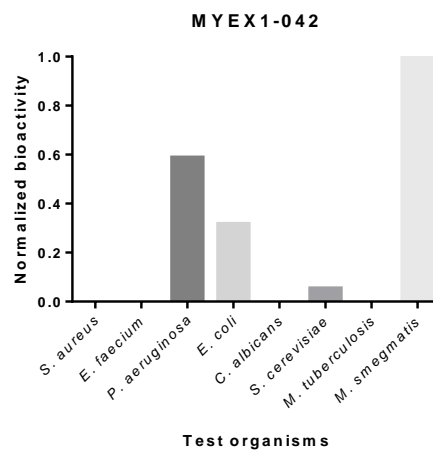
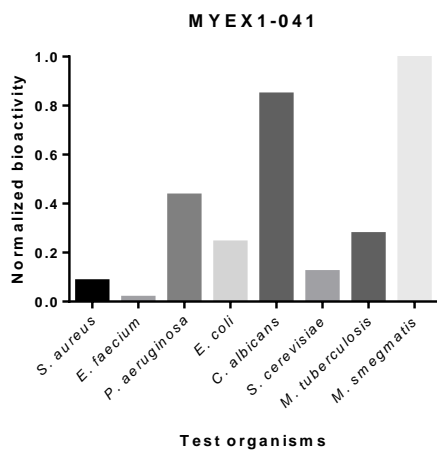
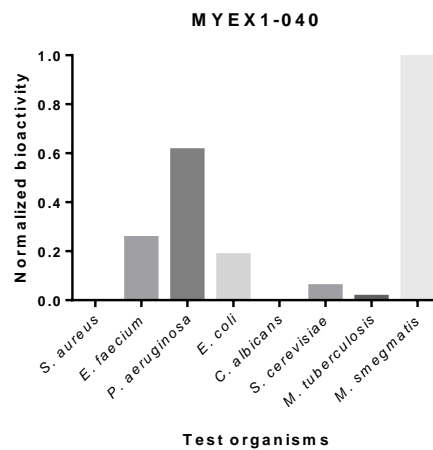
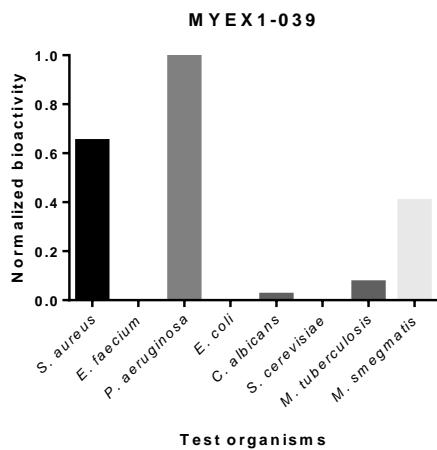
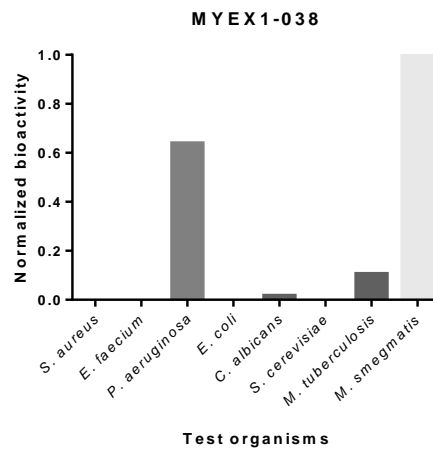
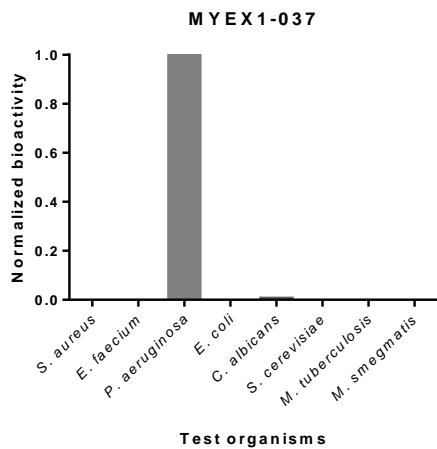
**Figure A3.1.** Bioactivity profiles of extracts in the NPRG collection (continued).



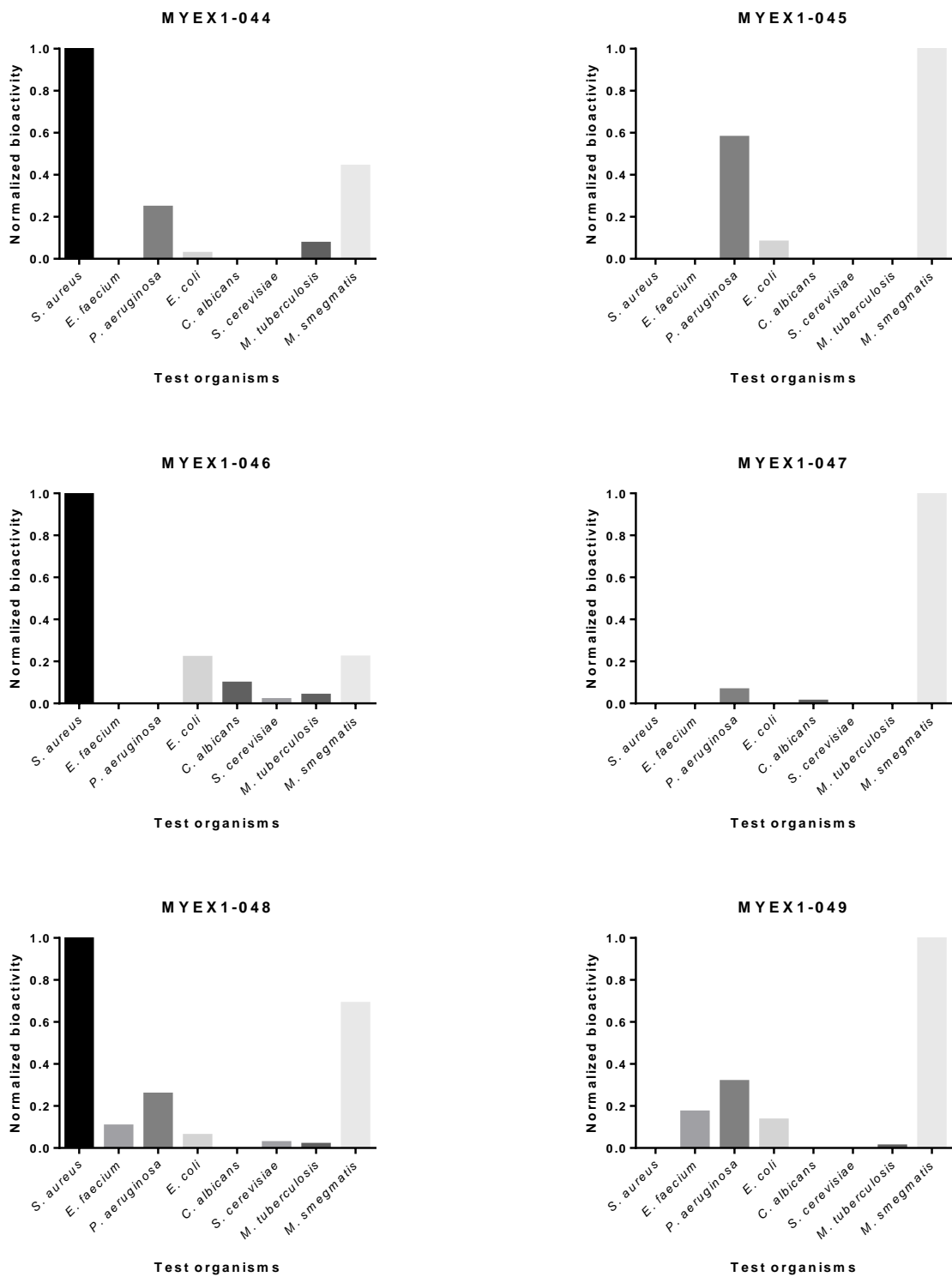
**Figure A3.1.** Bioactivity profiles of extracts in the NPRG collection (continued).



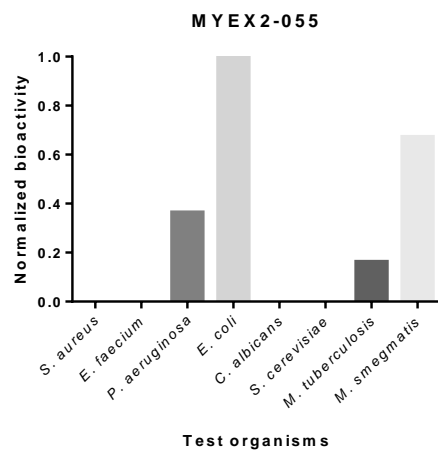
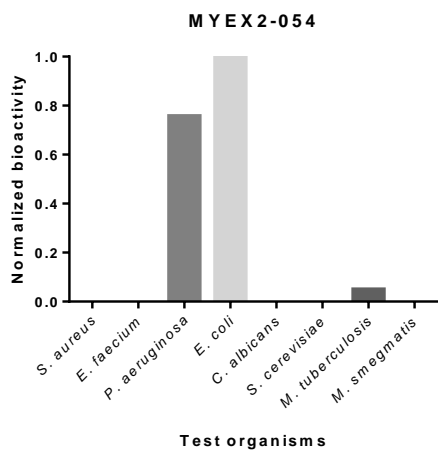
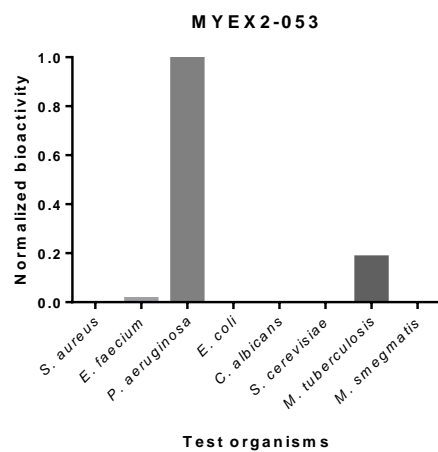
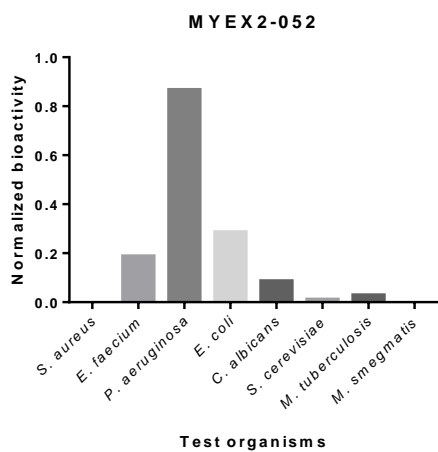
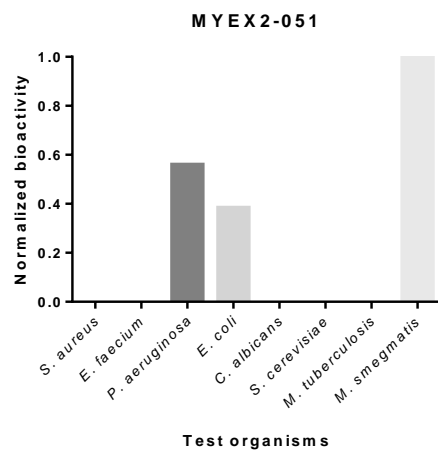
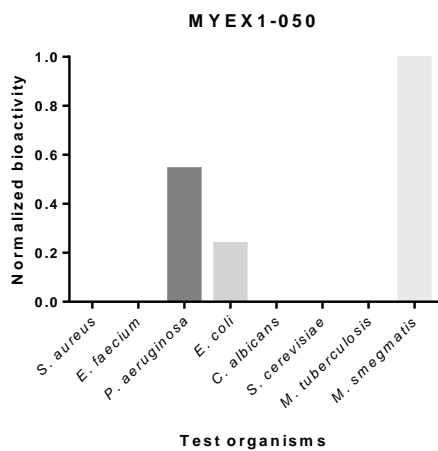
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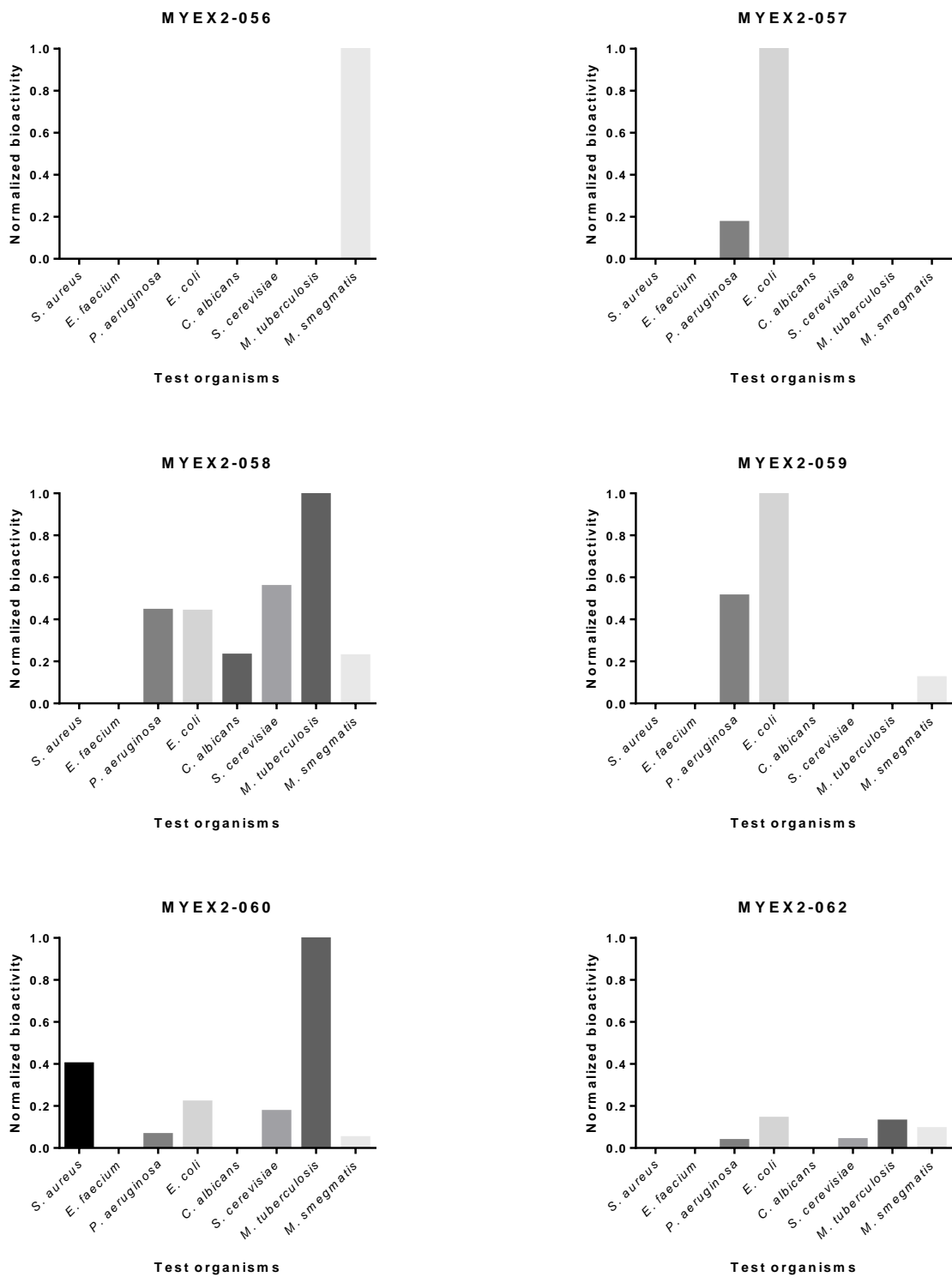
**Figure A3.1.** Bioactivity profiles of extracts in the NPRG collection (continued).



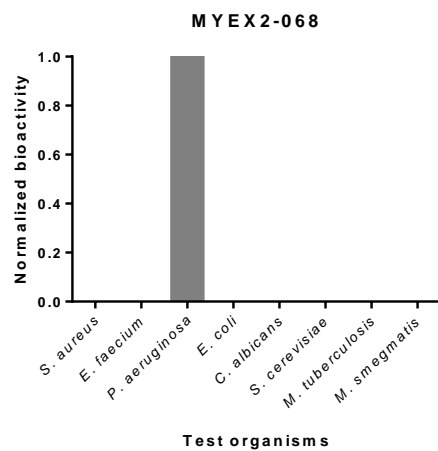
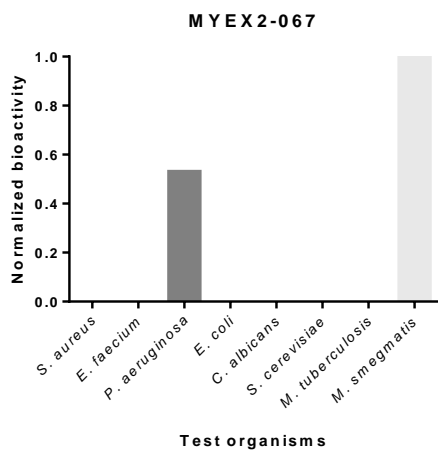
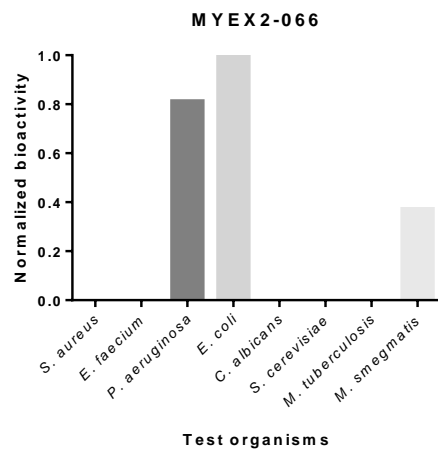
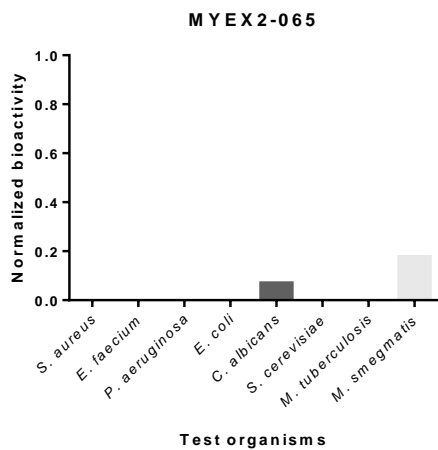
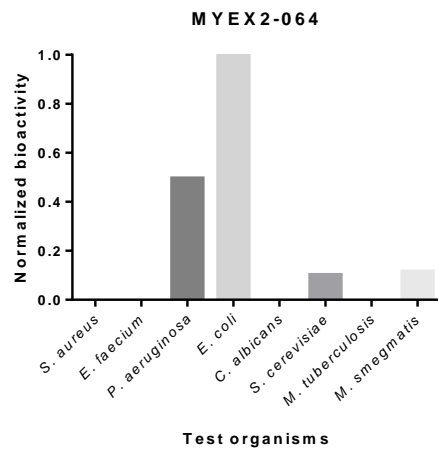
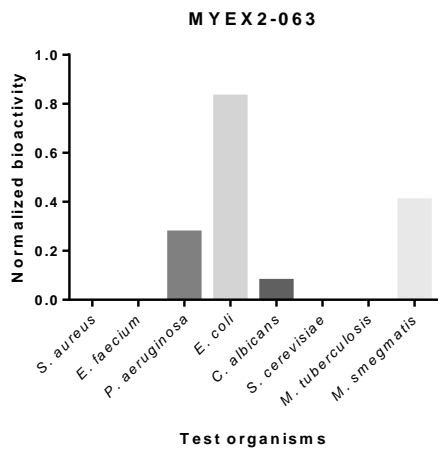
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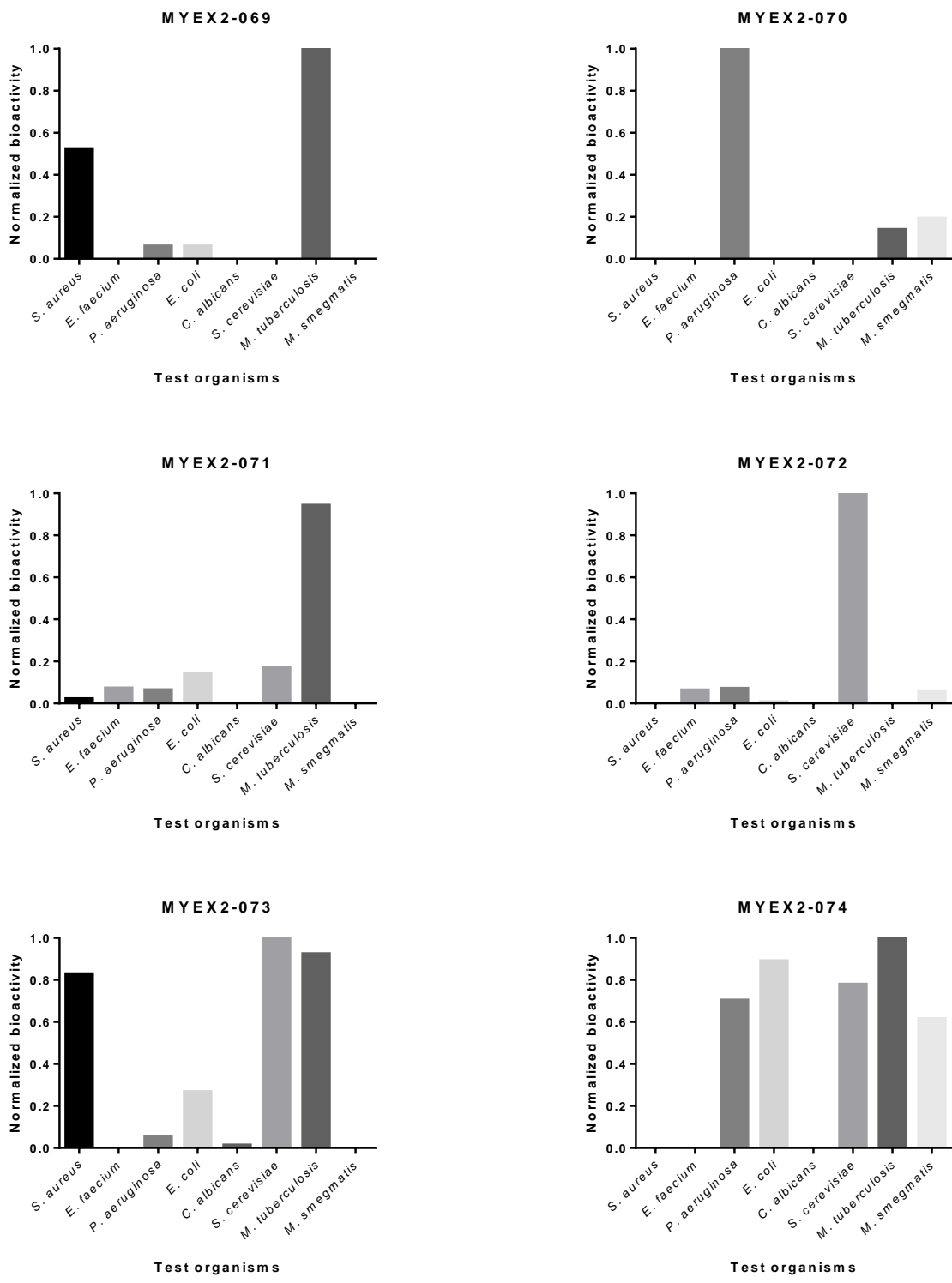
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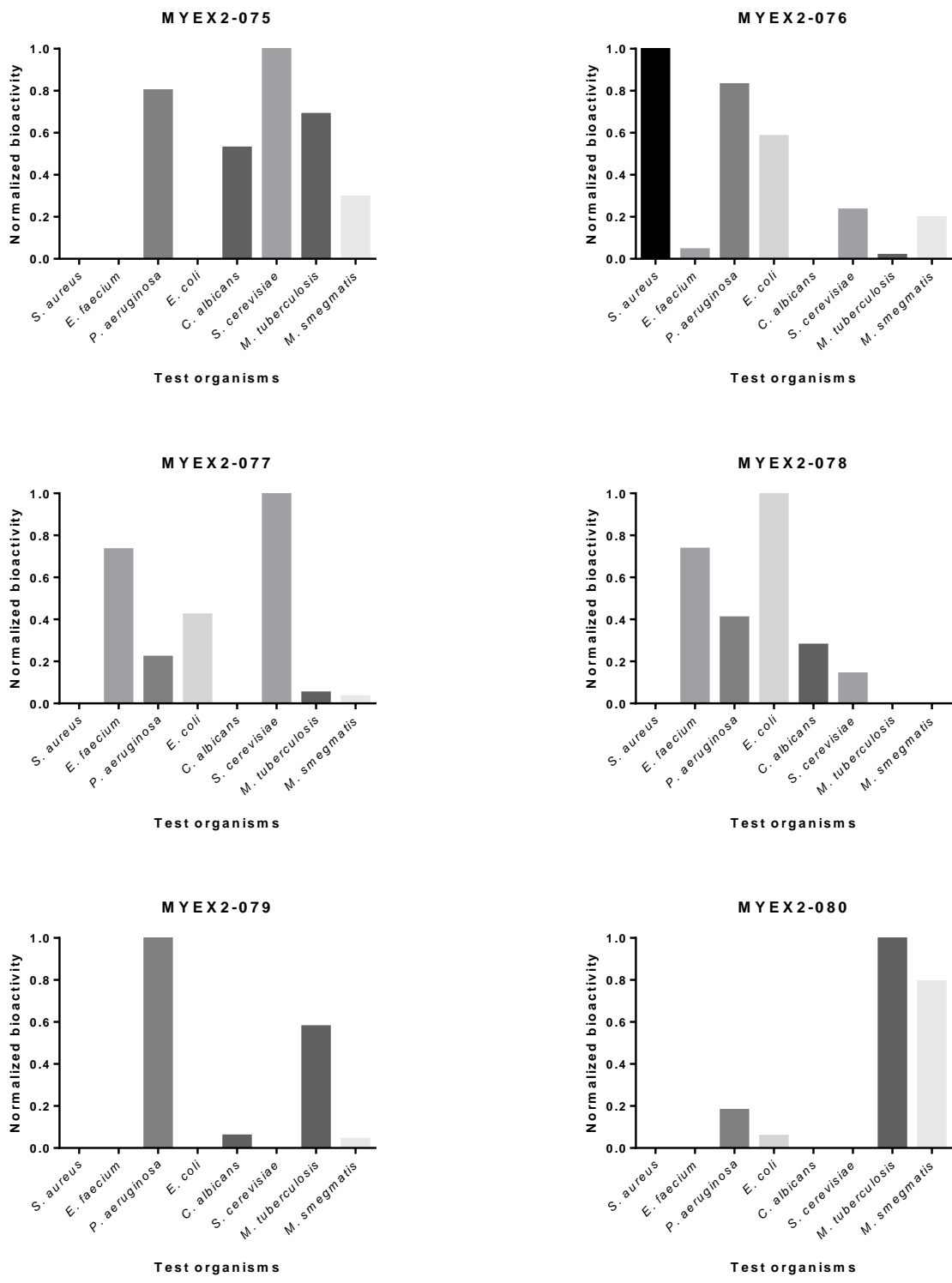
**Figure A3.1.** Bioactivity profiles of extracts in the NPRG collection (continued).



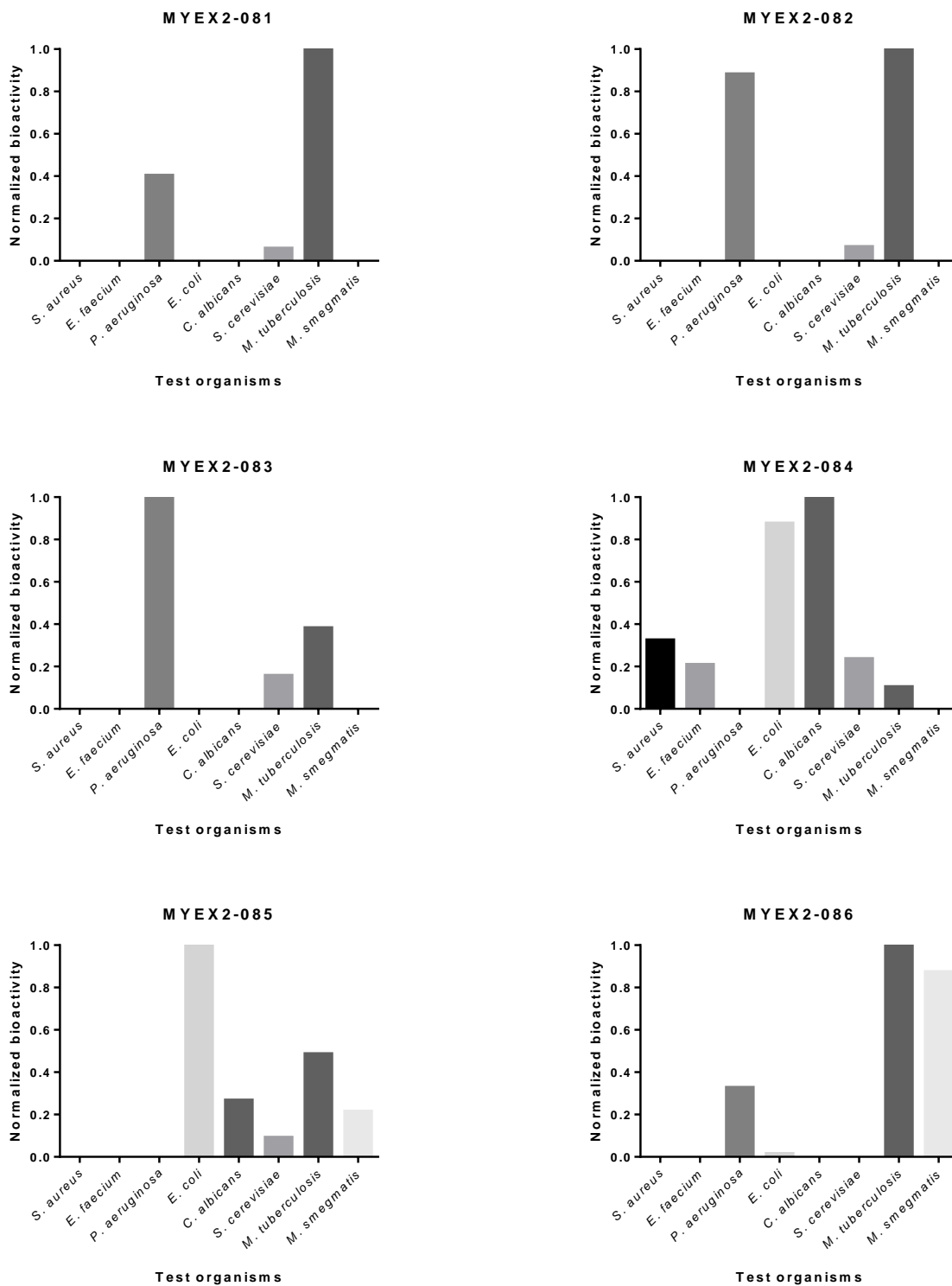
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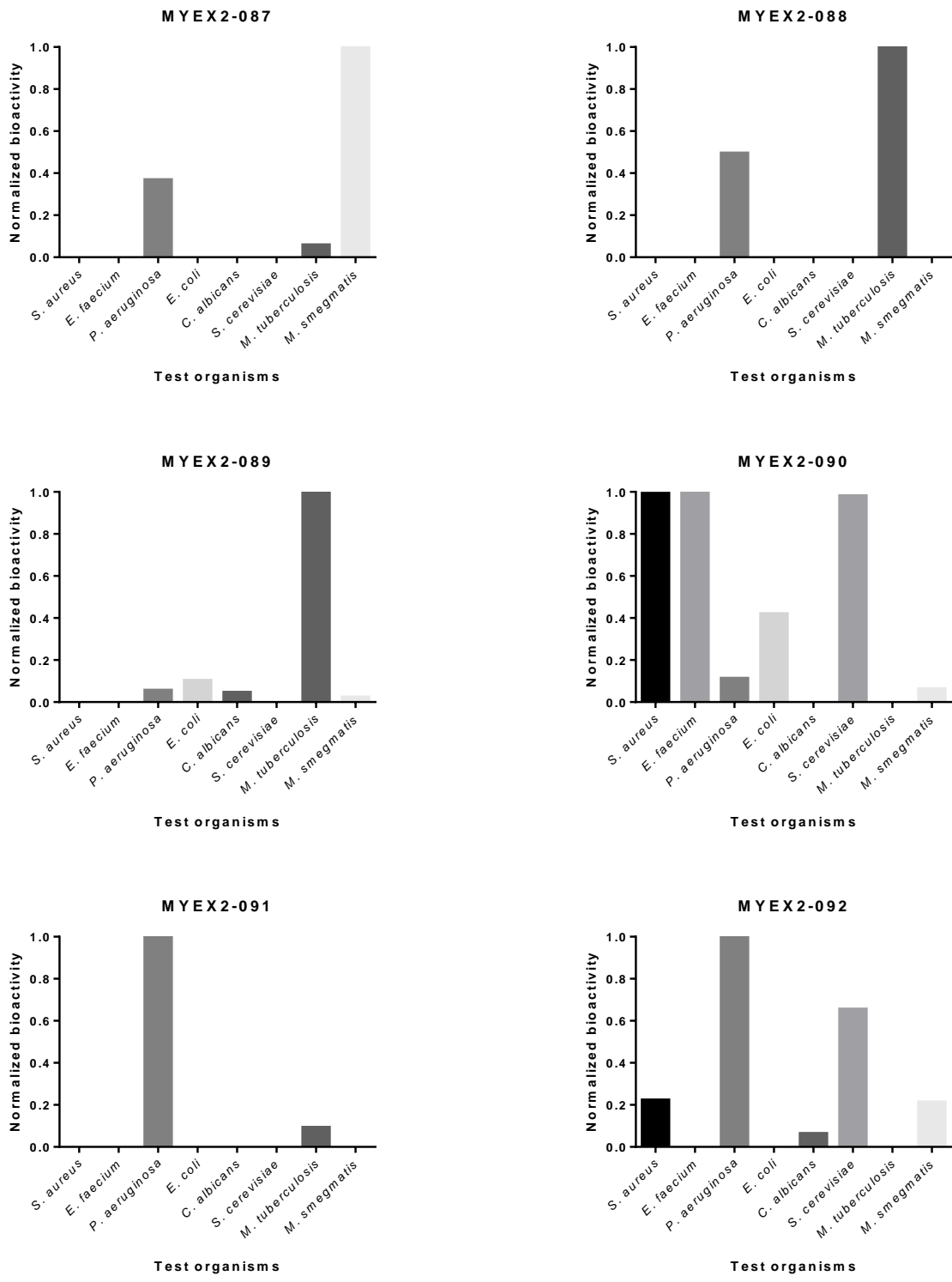
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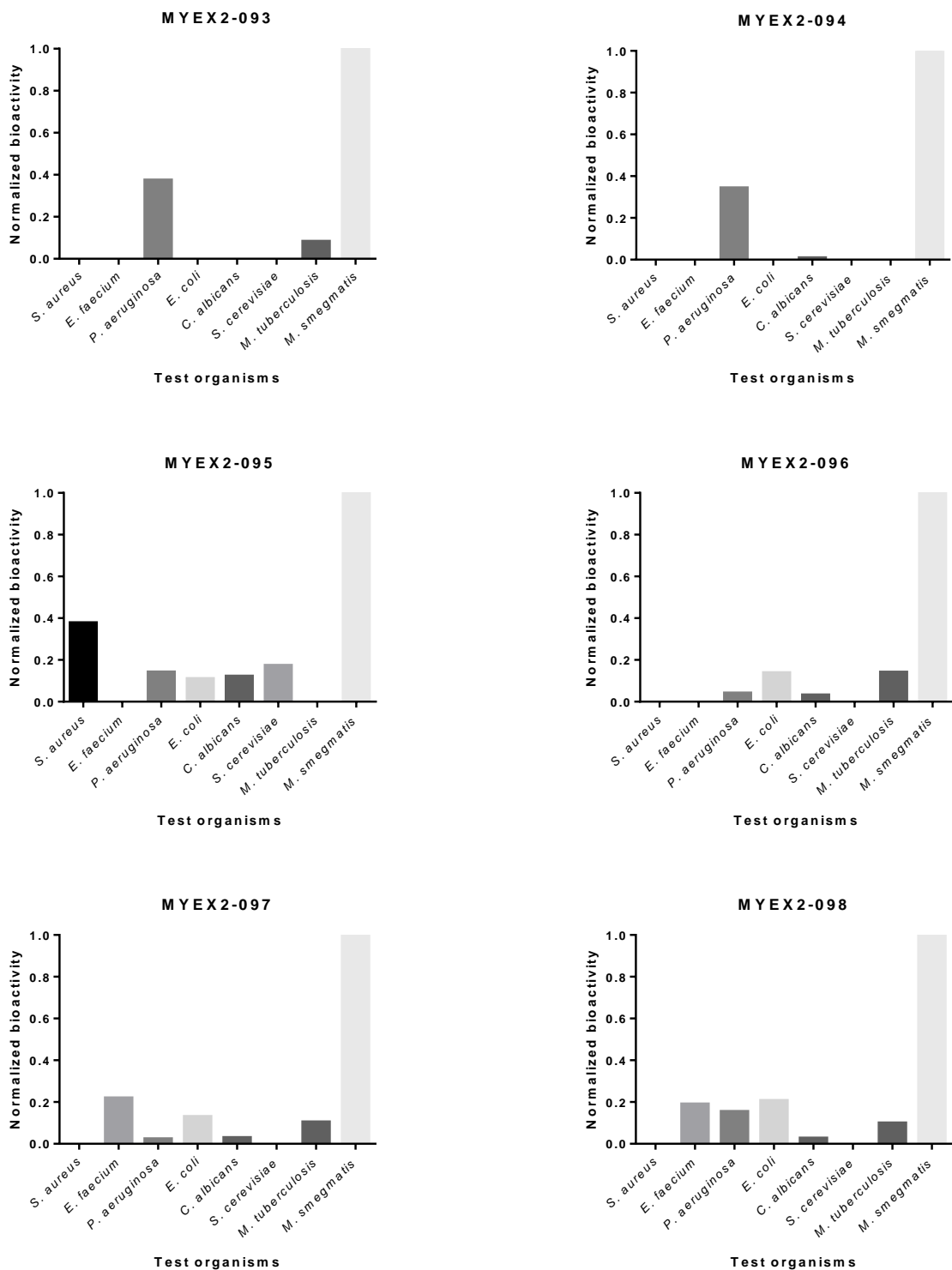
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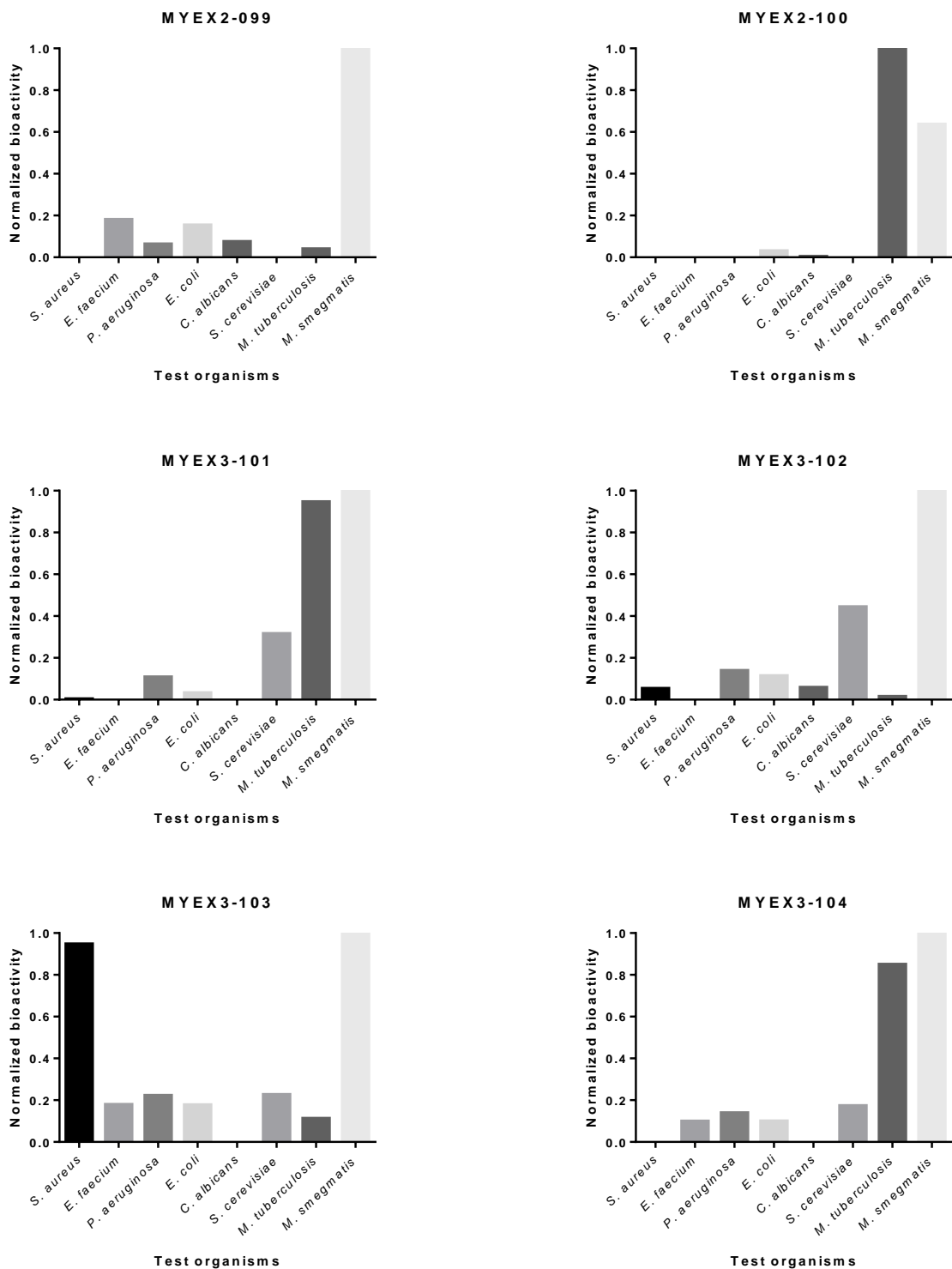
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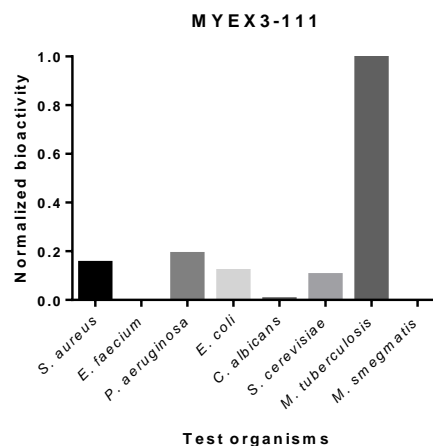
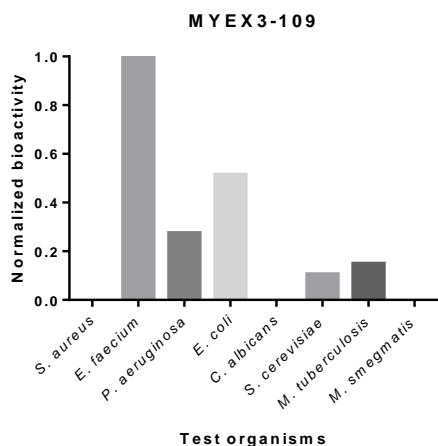
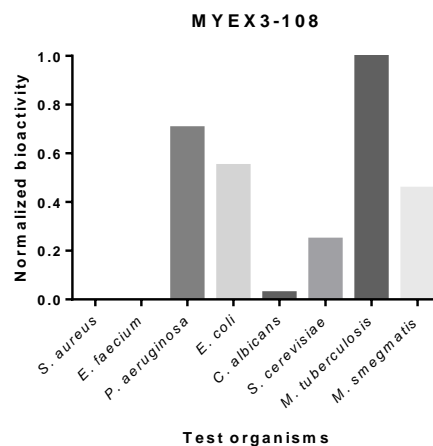
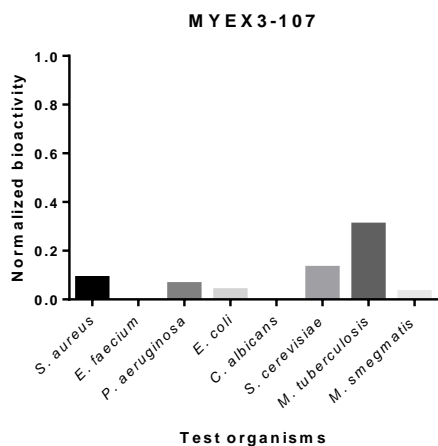
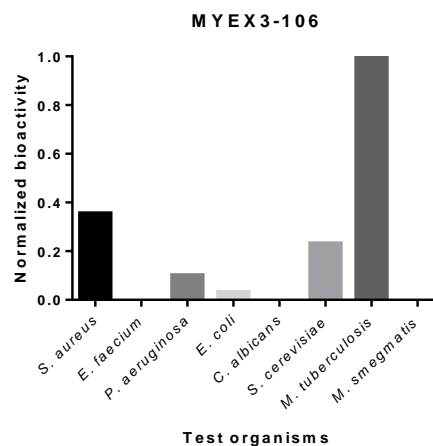
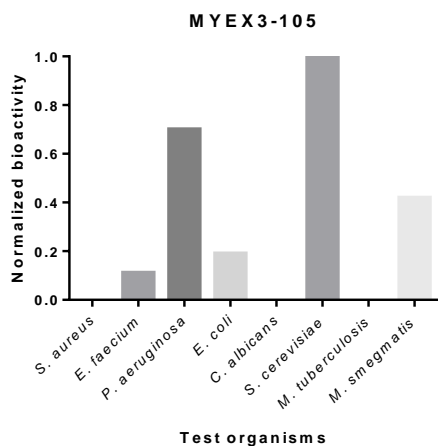
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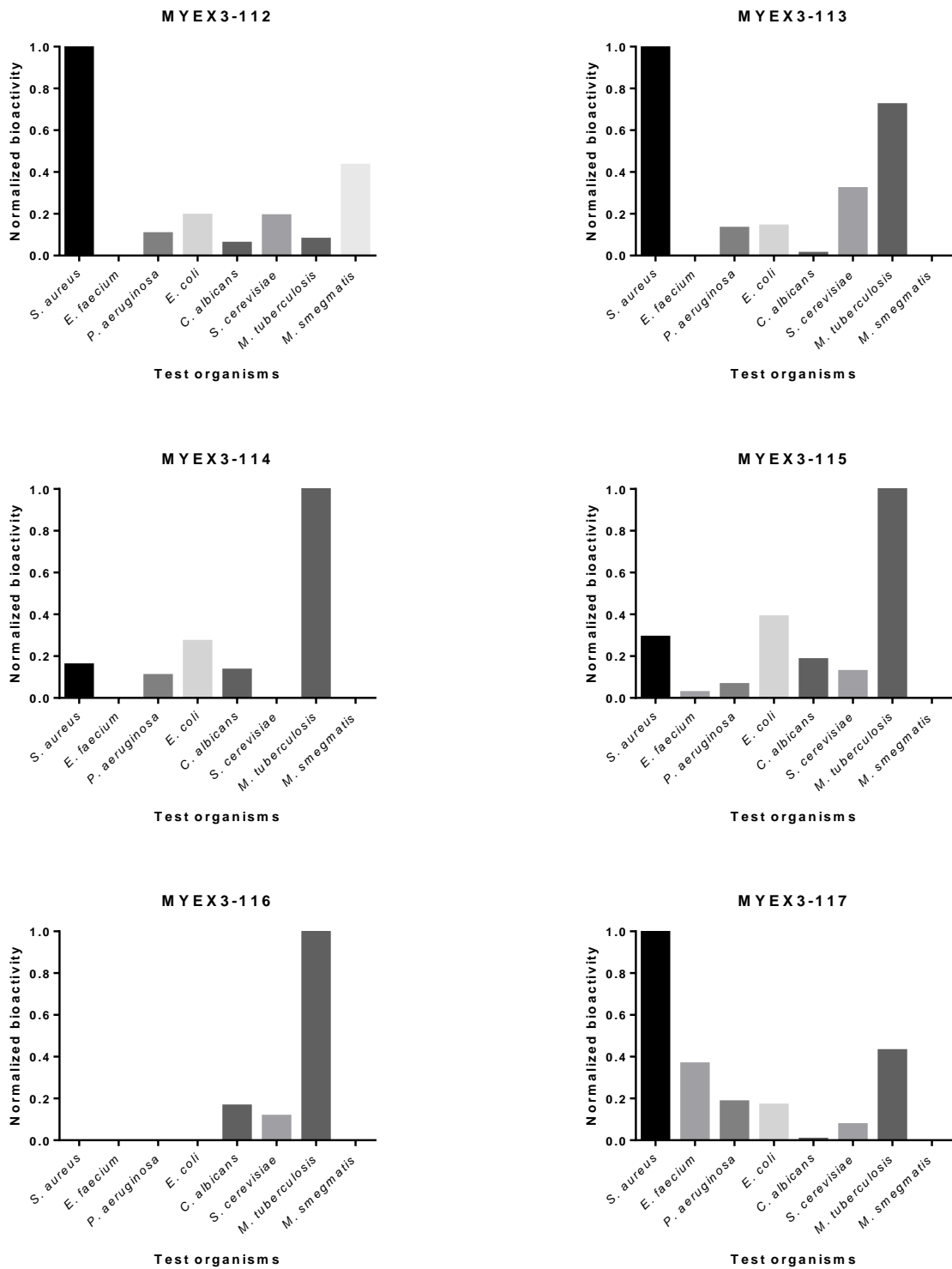
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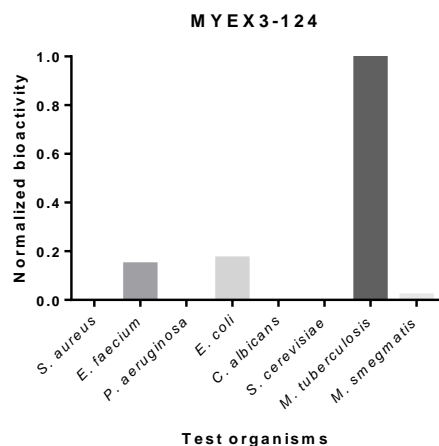
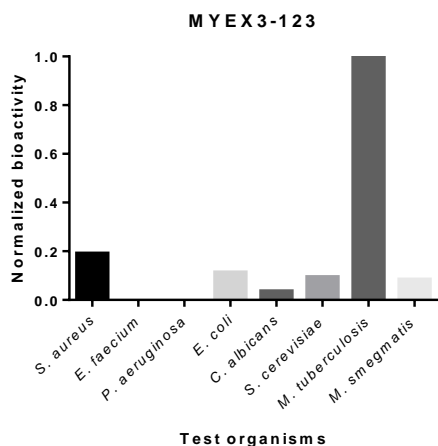
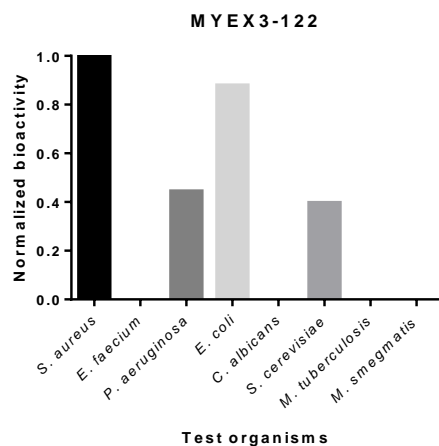
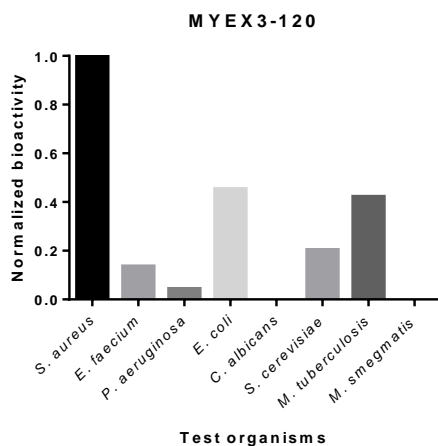
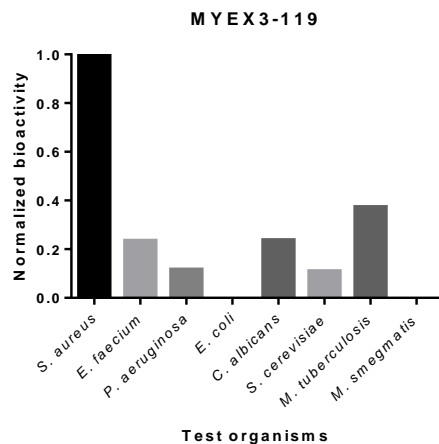
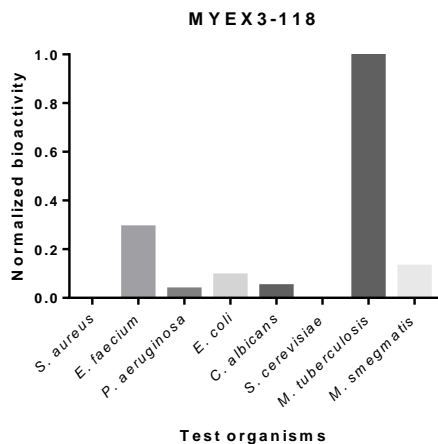
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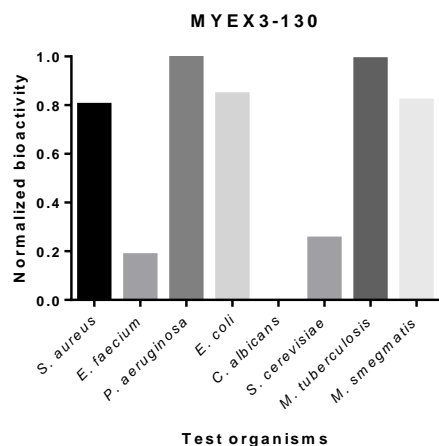
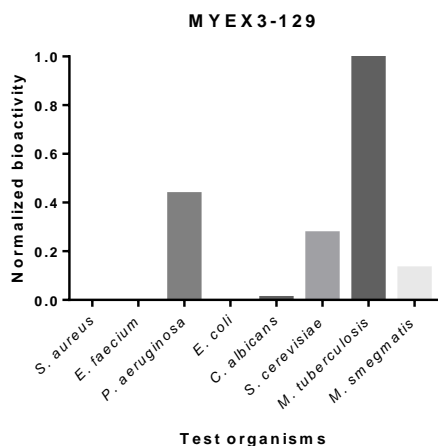
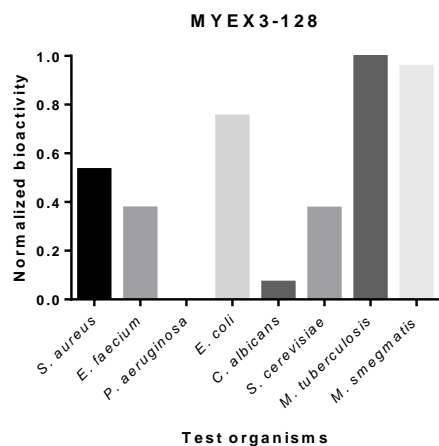
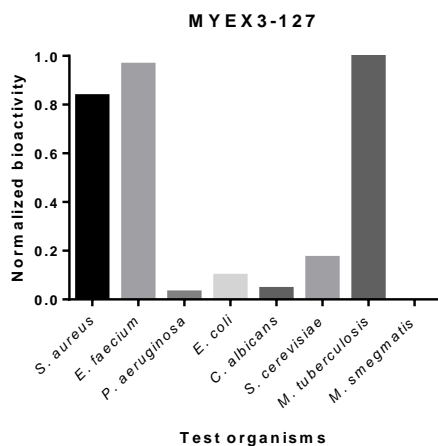
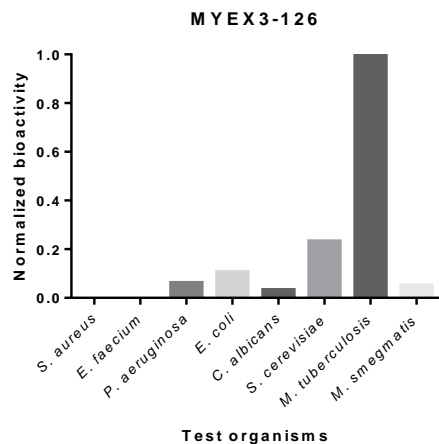
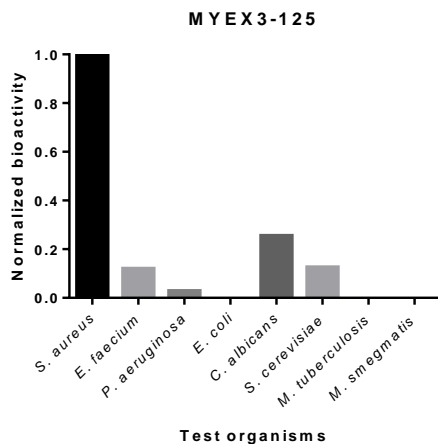
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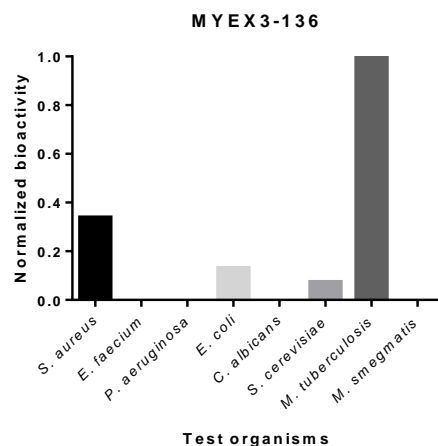
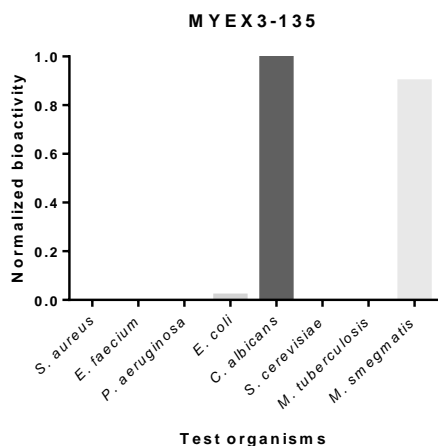
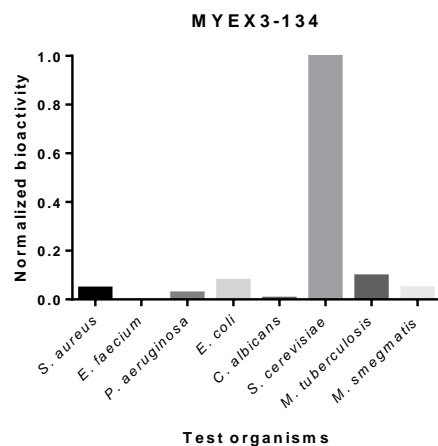
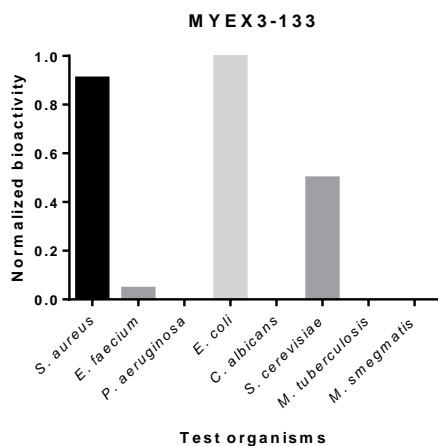
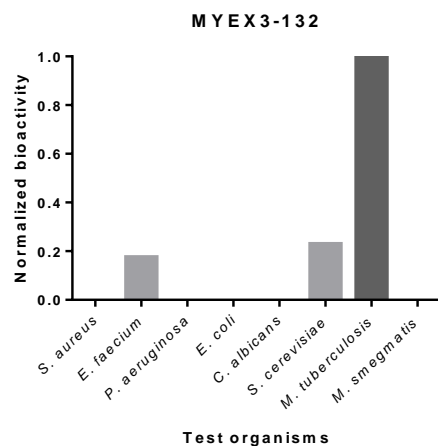
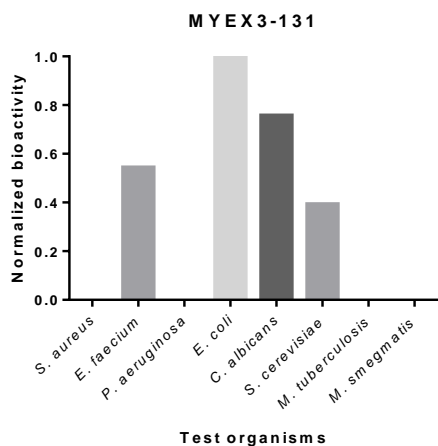
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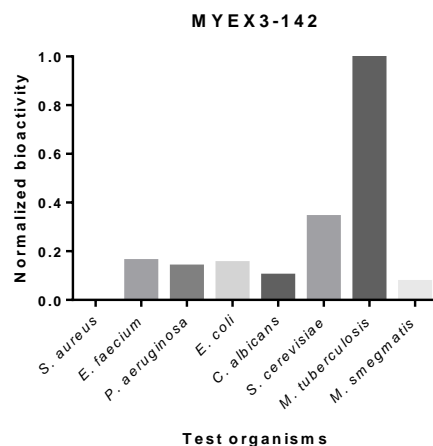
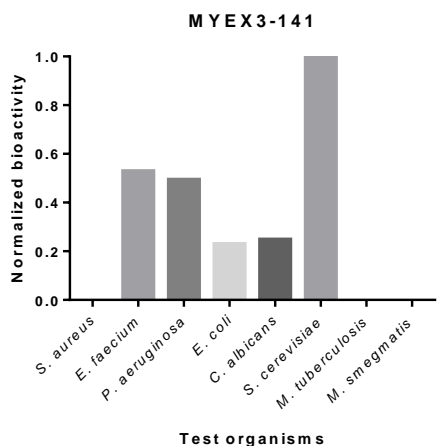
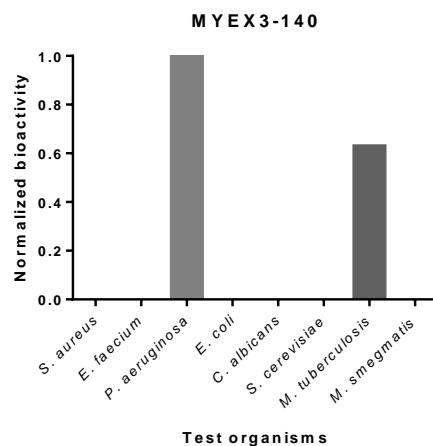
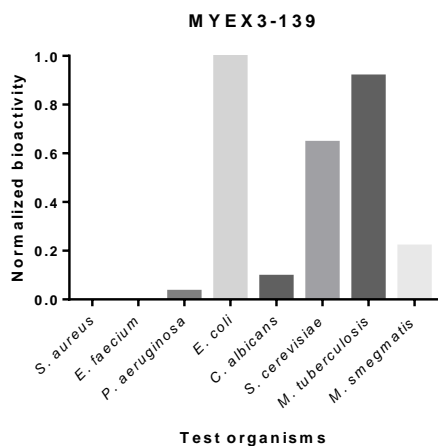
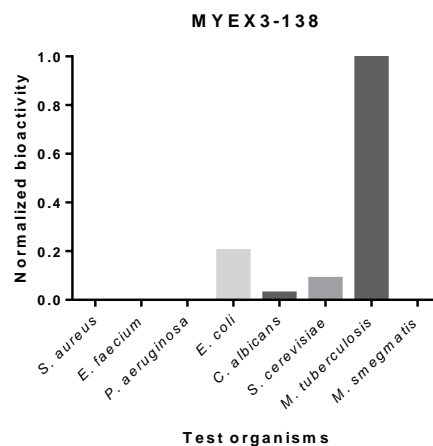
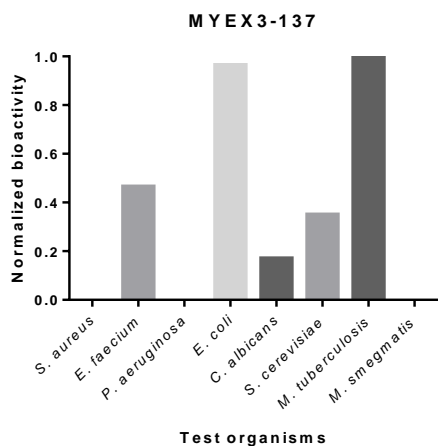
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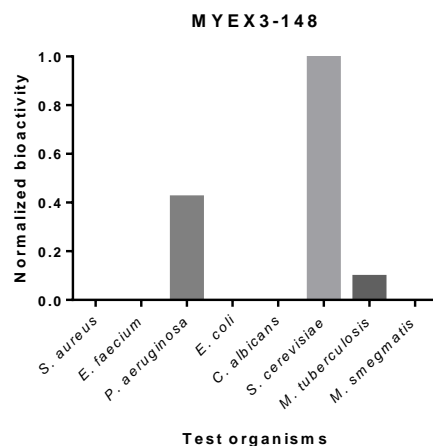
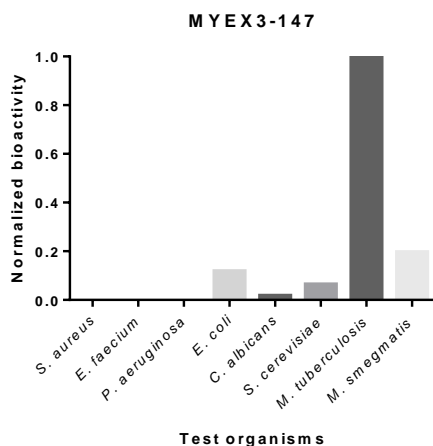
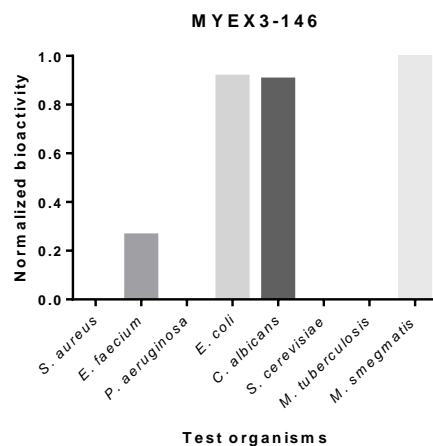
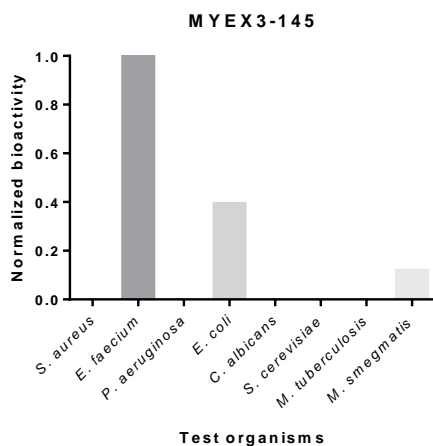
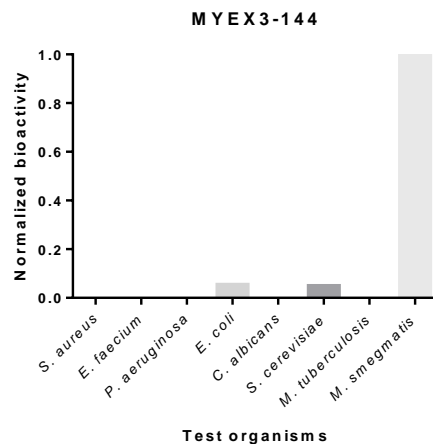
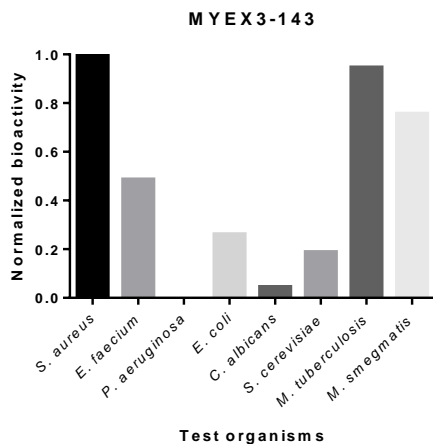
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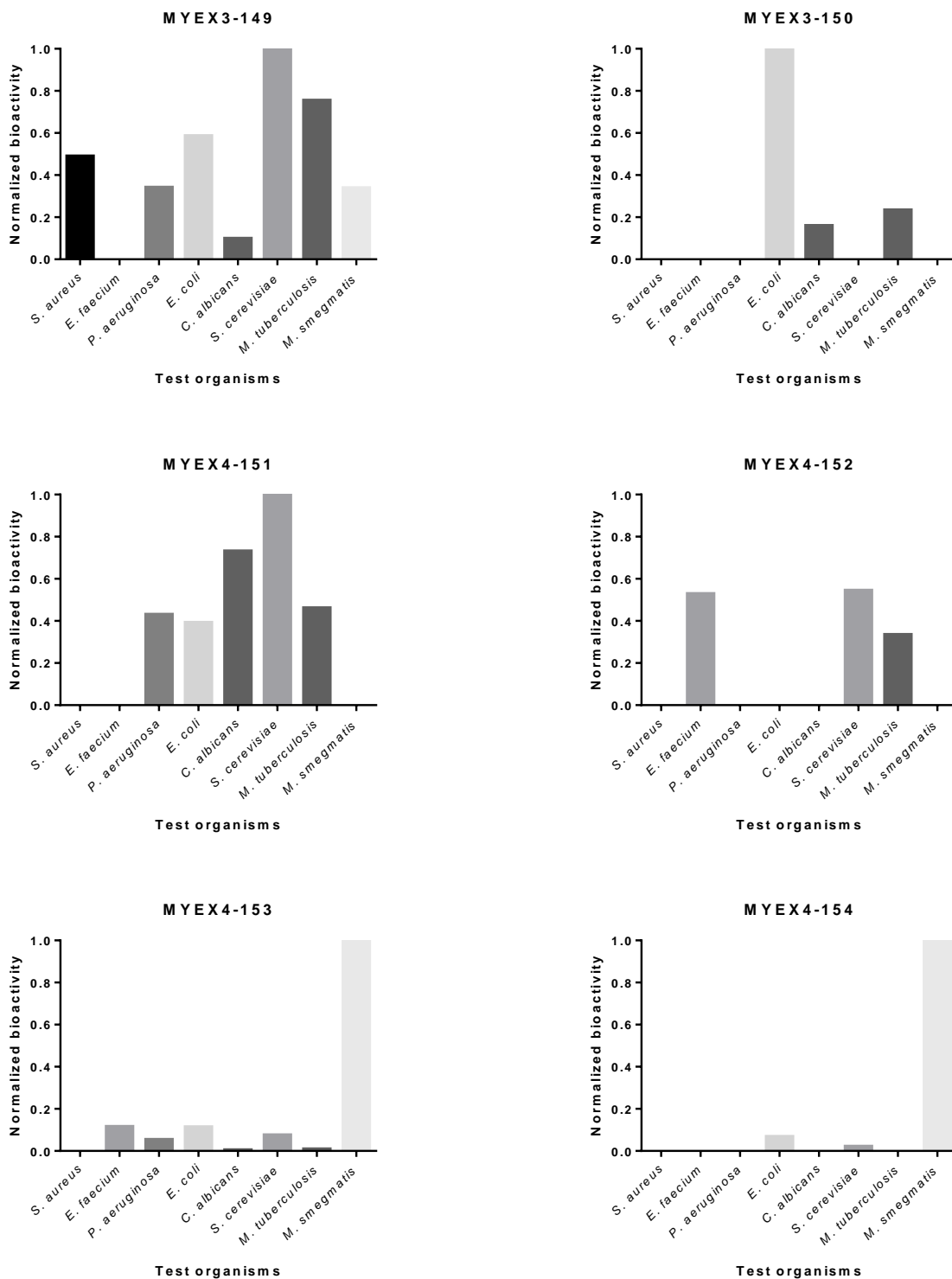
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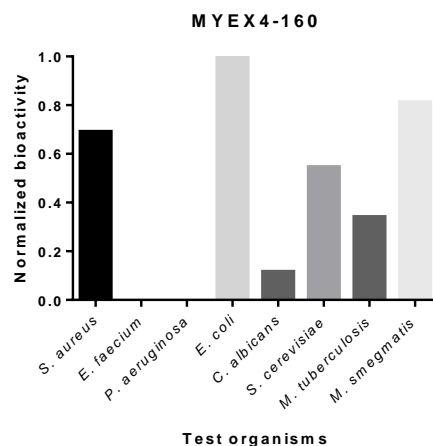
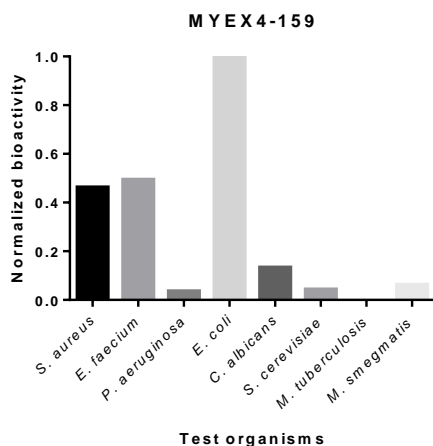
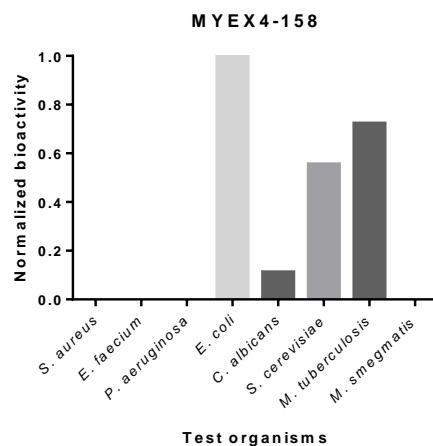
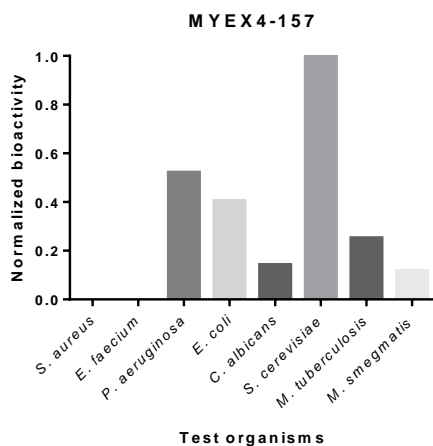
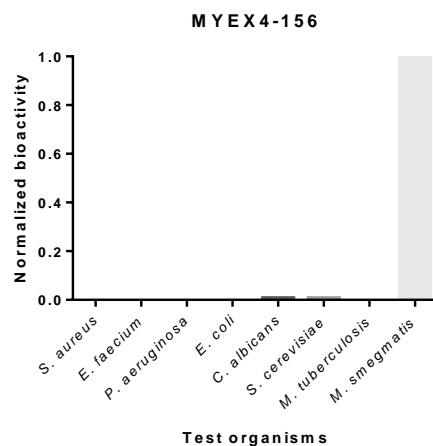
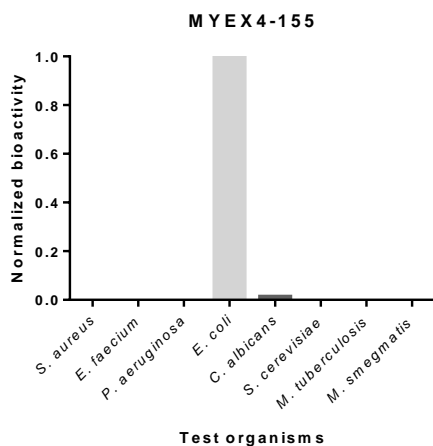
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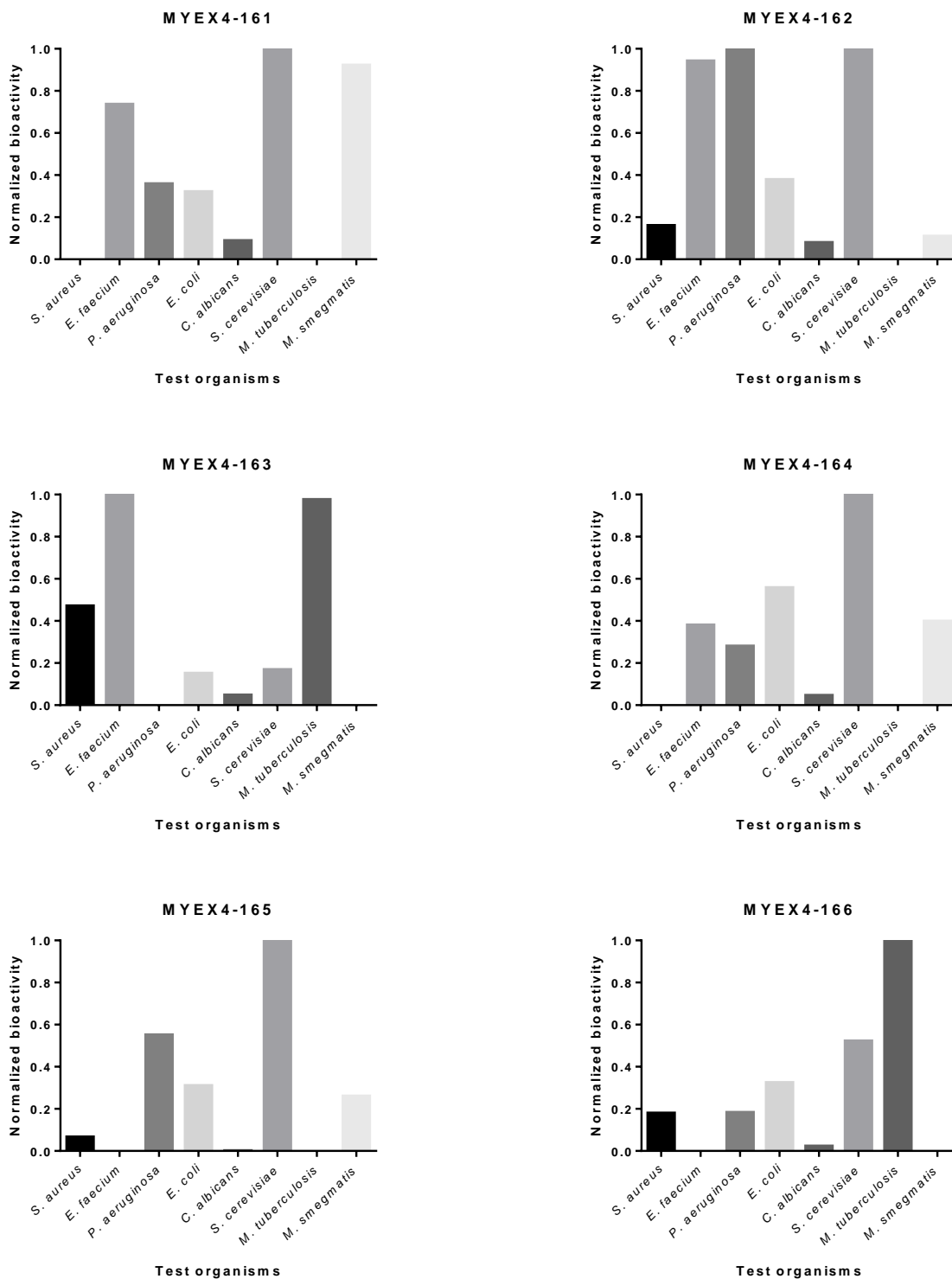
**Figure A3.1.** Bioactivity profiles of extracts in the NPRG collection (continued).



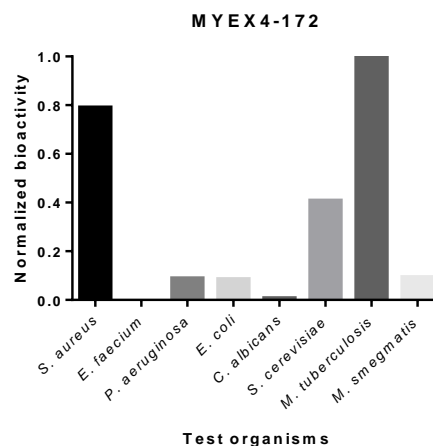
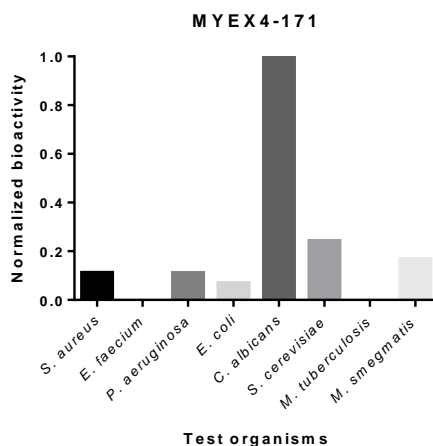
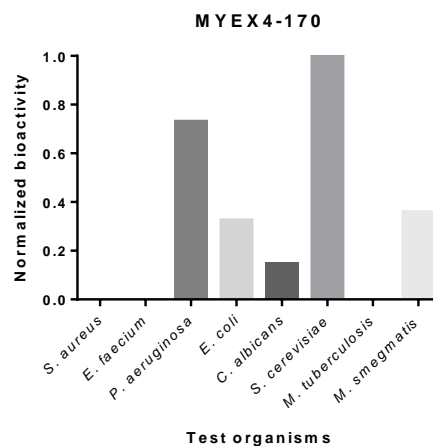
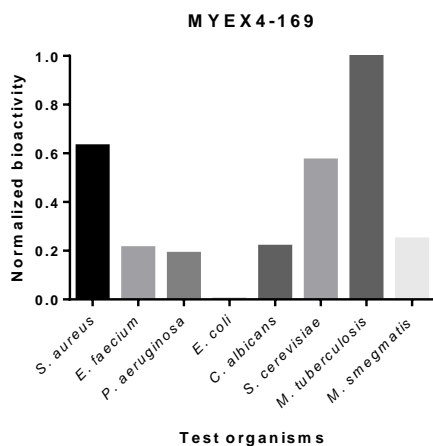
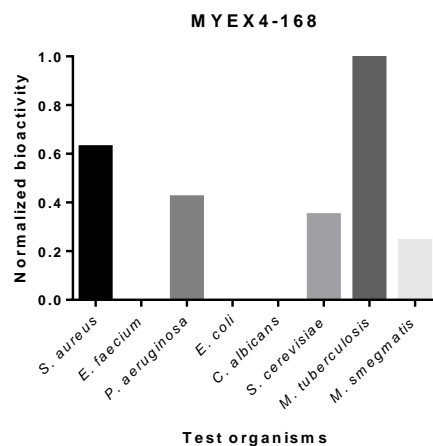
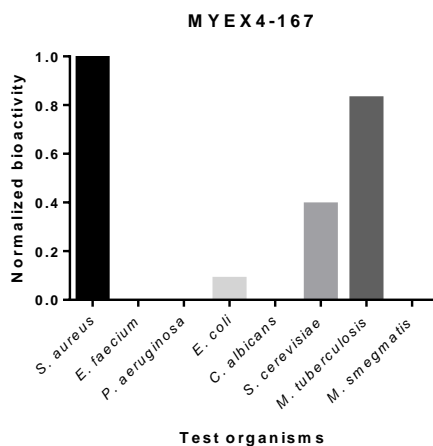
**Figure A3.1.** Bioactivity profiles of extracts in the NPRG collection (continued).



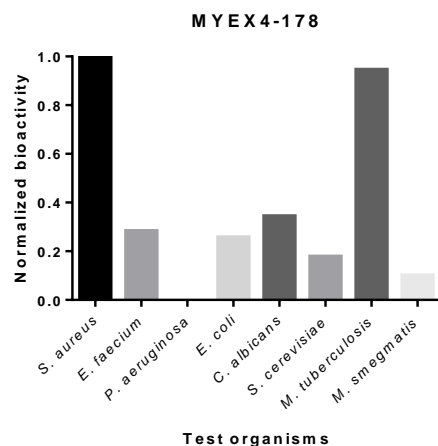
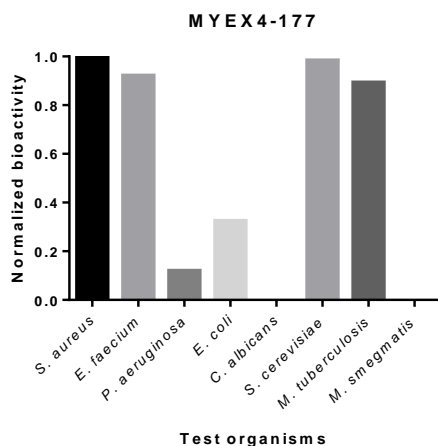
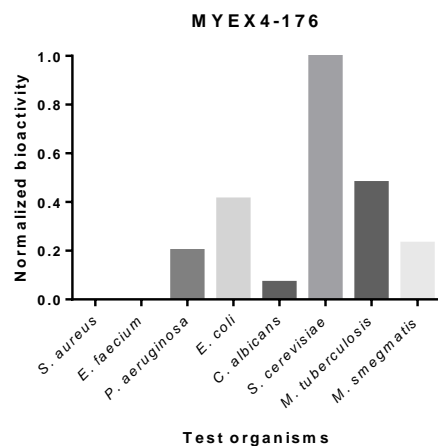
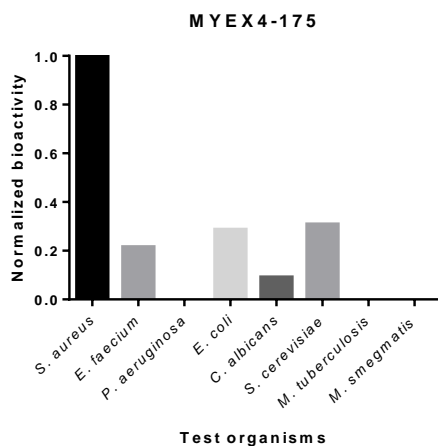
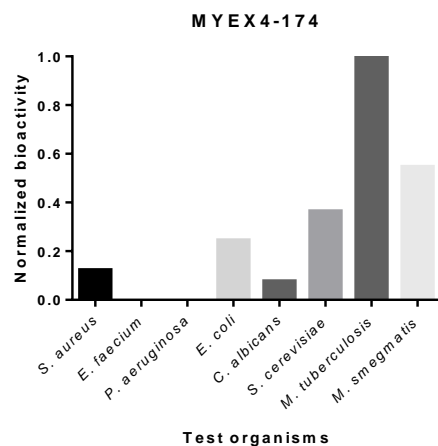
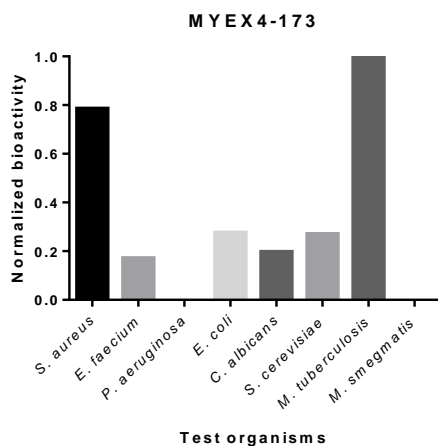
**Figure A3.1.** Bioactivity profiles of extracts in the NPRG collection (continued).



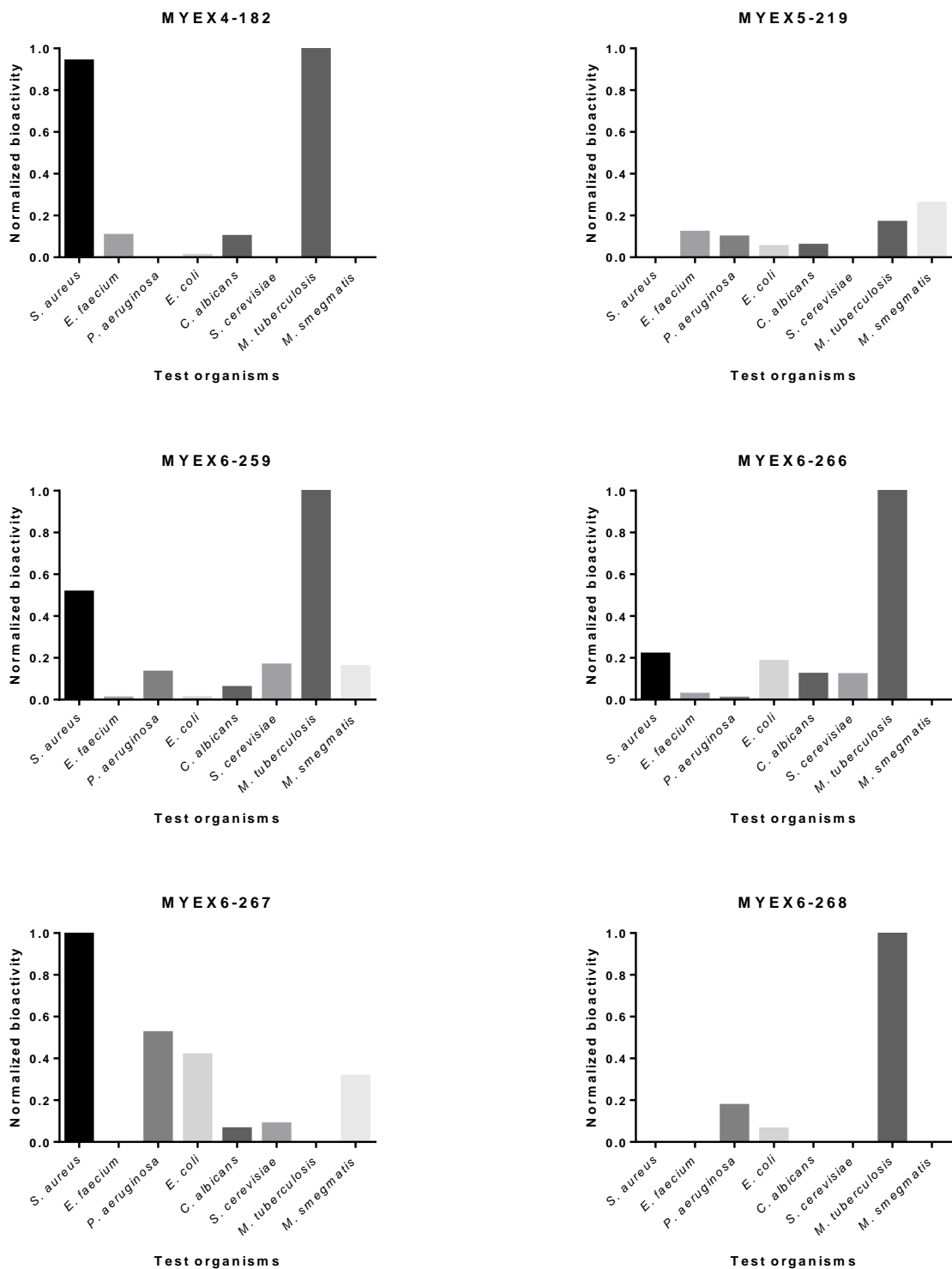
**Figure A3.1.** Bioactivity profiles of extracts in the NPRG collection (continued).



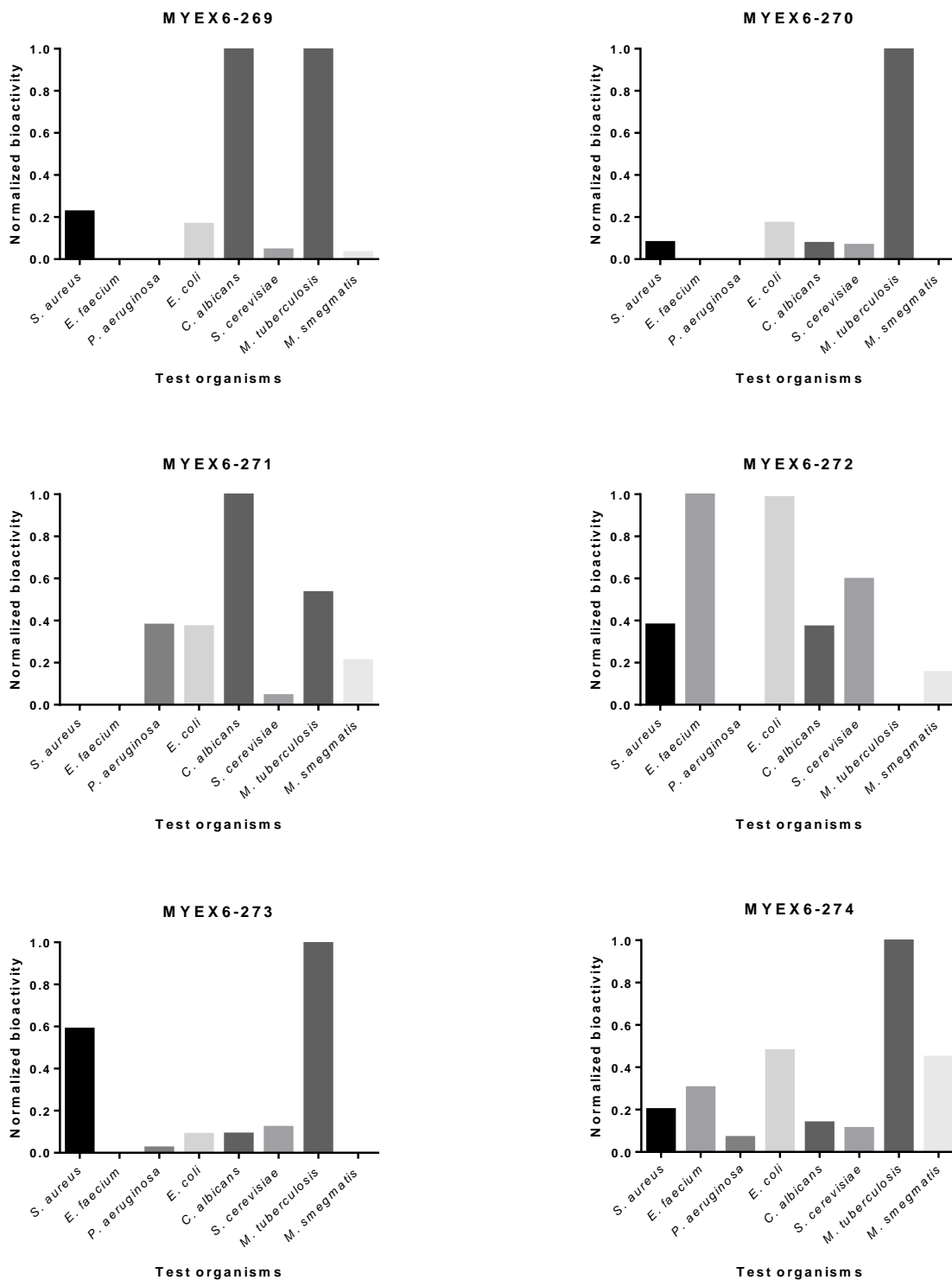
**Figure A3.1.** Bioactivity profiles of extracts in the NPRG collection (continued).



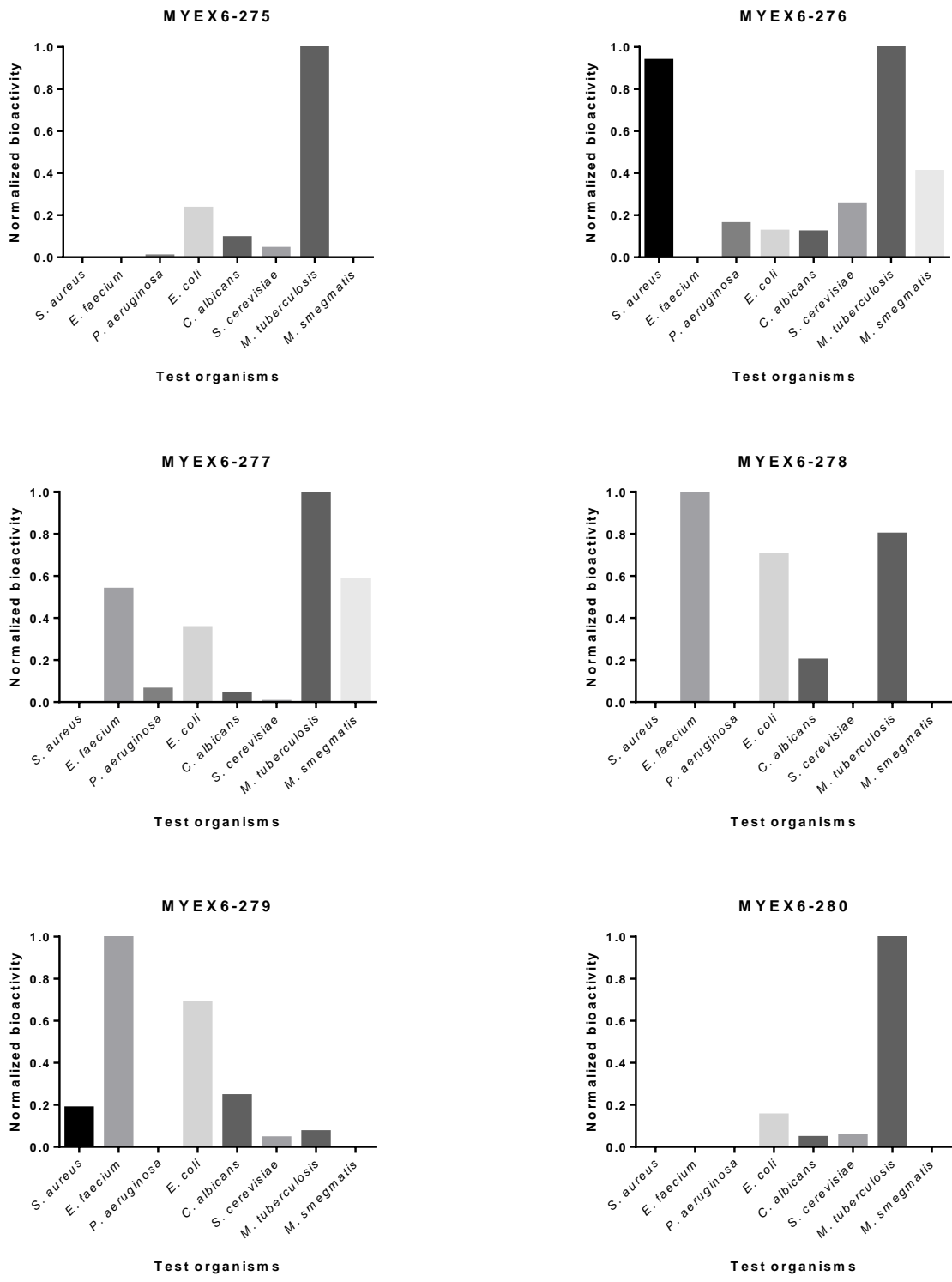
**Figure A3.1.** Bioactivity profiles of extracts in the NPRG collection (continued).



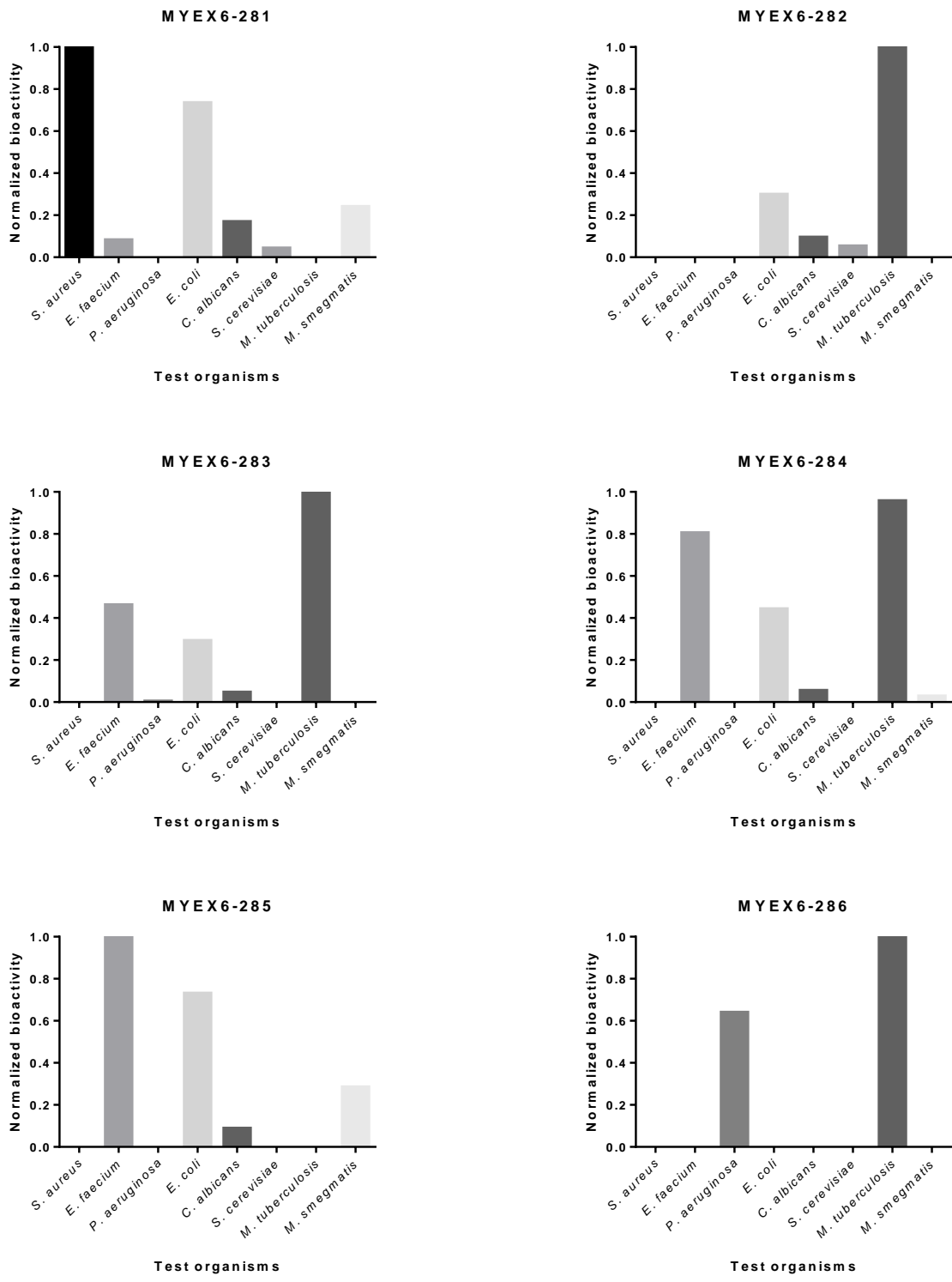
**Figure A3.1.** Bioactivity profiles of extracts in the NPRG collection (continued).



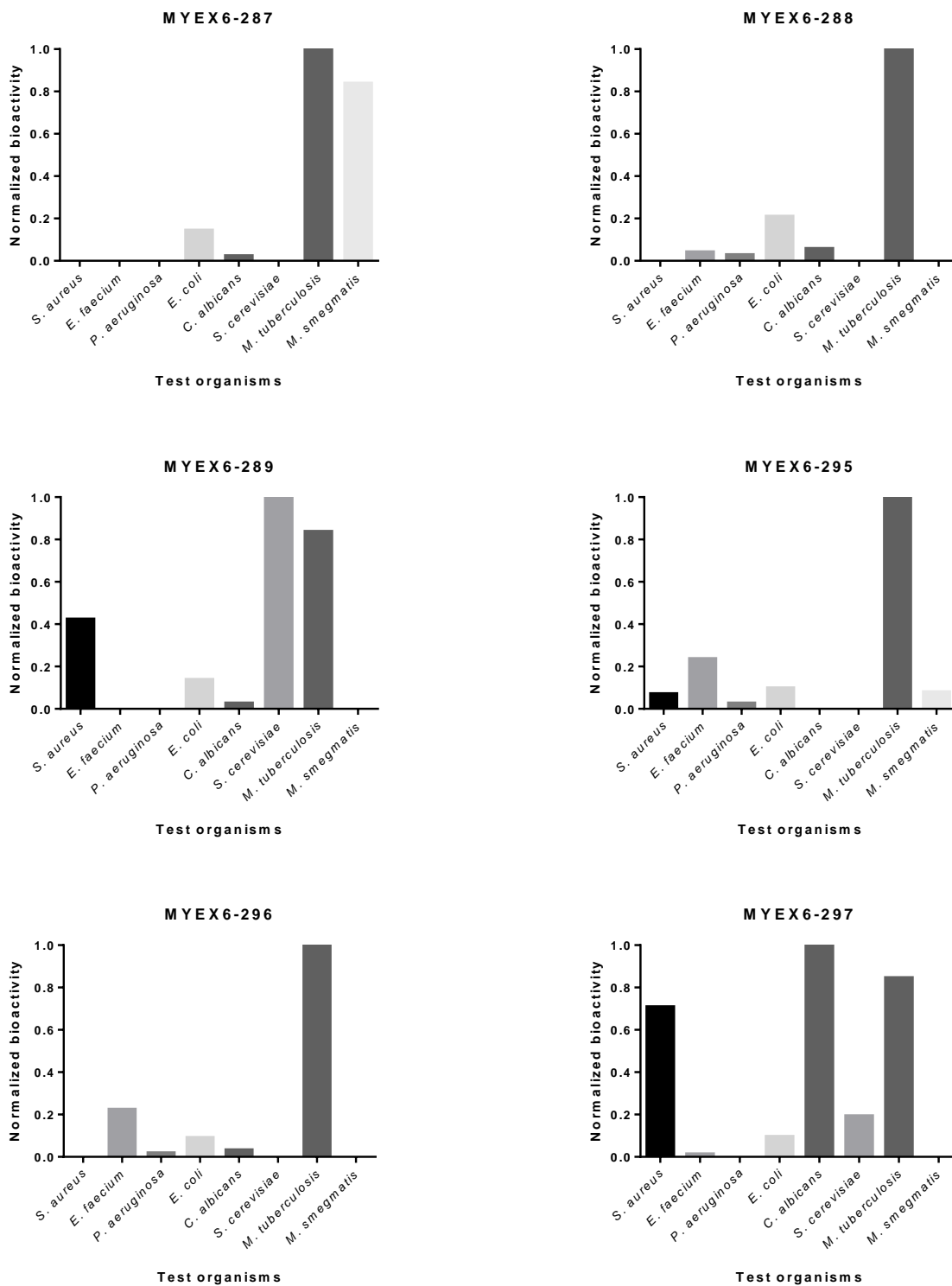
**Figure A3.1.** Bioactivity profiles of extracts in the NPRG collection (continued).



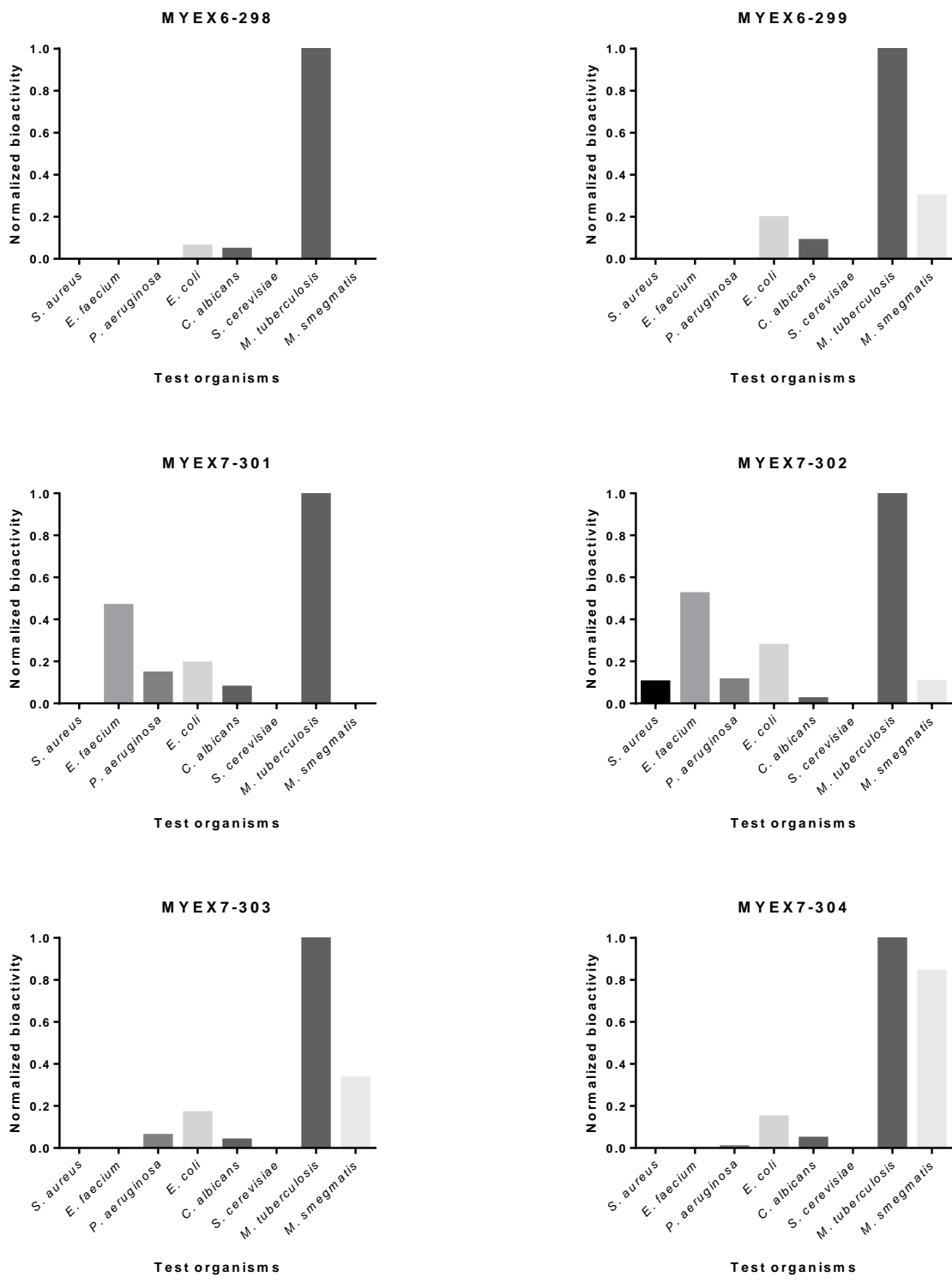
**Figure A3.1.** Bioactivity profiles of extracts in the NPRG collection (continued).



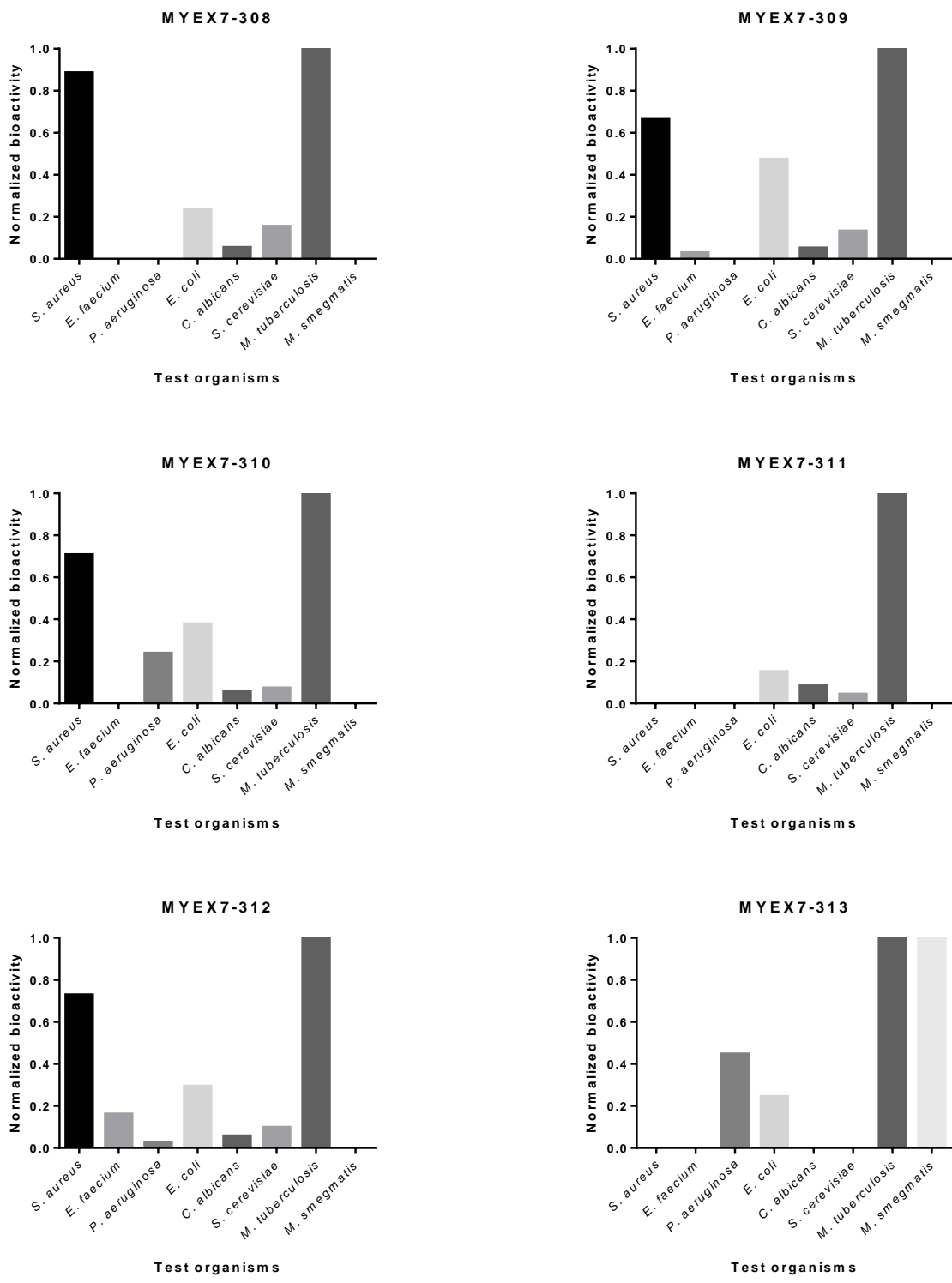
**Figure A3.1.** Bioactivity profiles of extracts in the NPRG collection (continued).



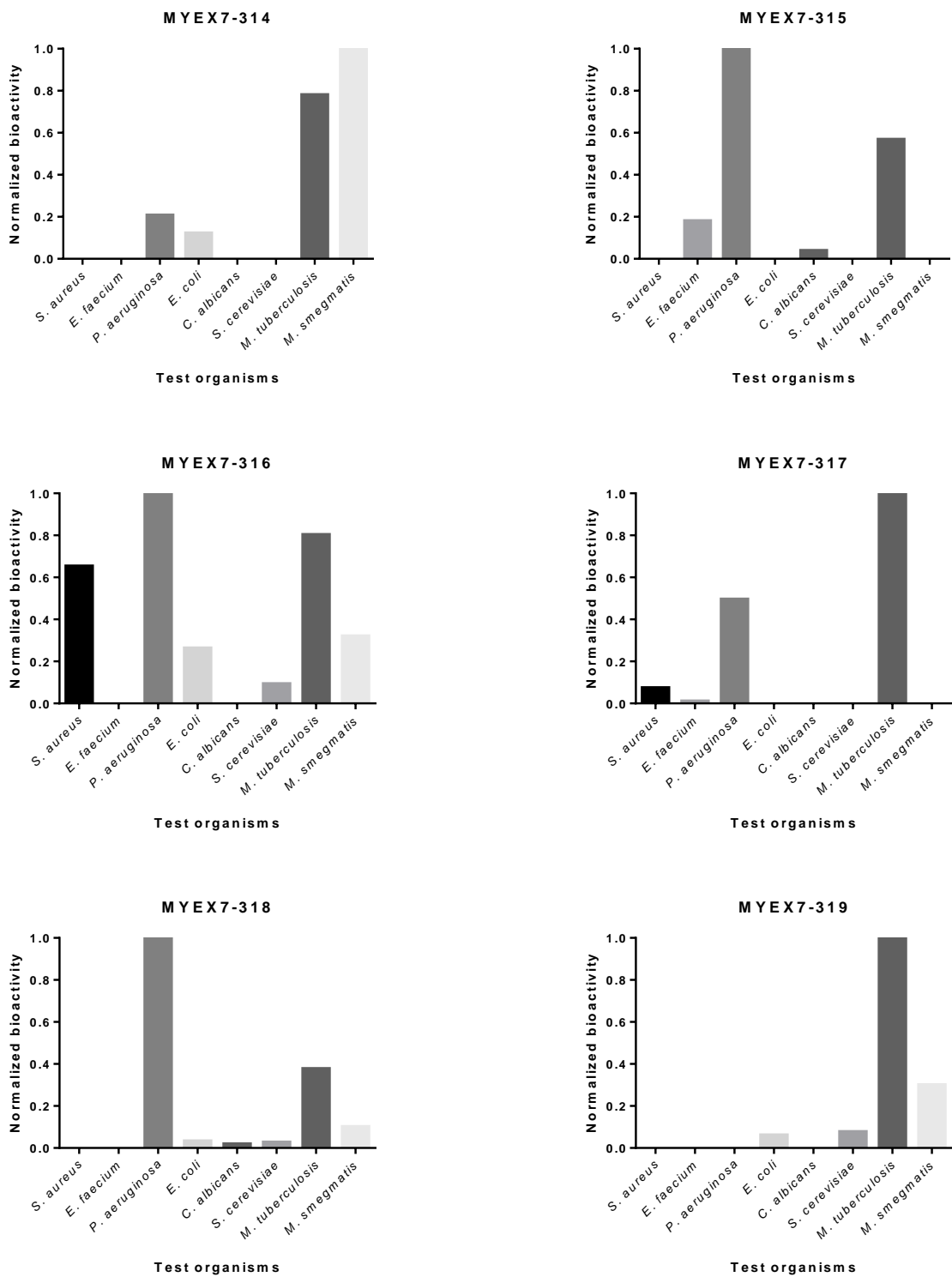
**Figure A3.1.** Bioactivity profiles of extracts in the NPRG collection (continued).



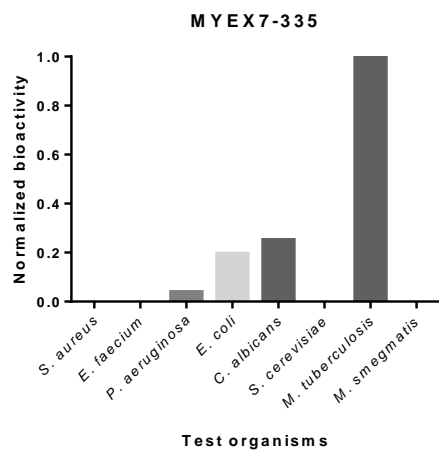
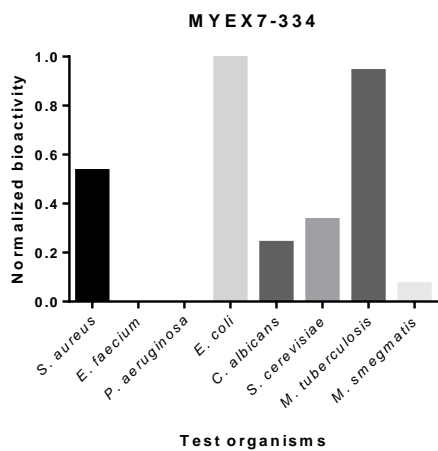
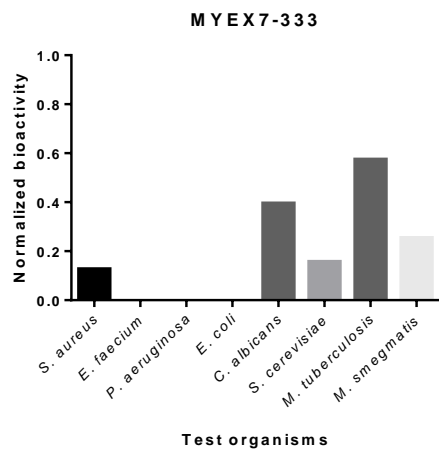
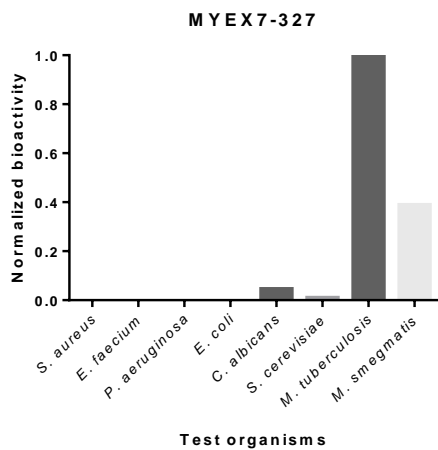
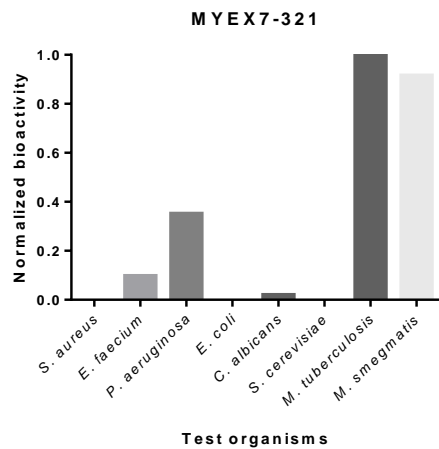
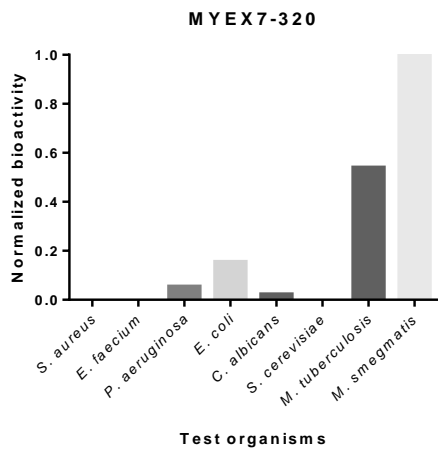
**Figure A3.1.** Bioactivity profiles of extracts in the NPRG collection (continued).



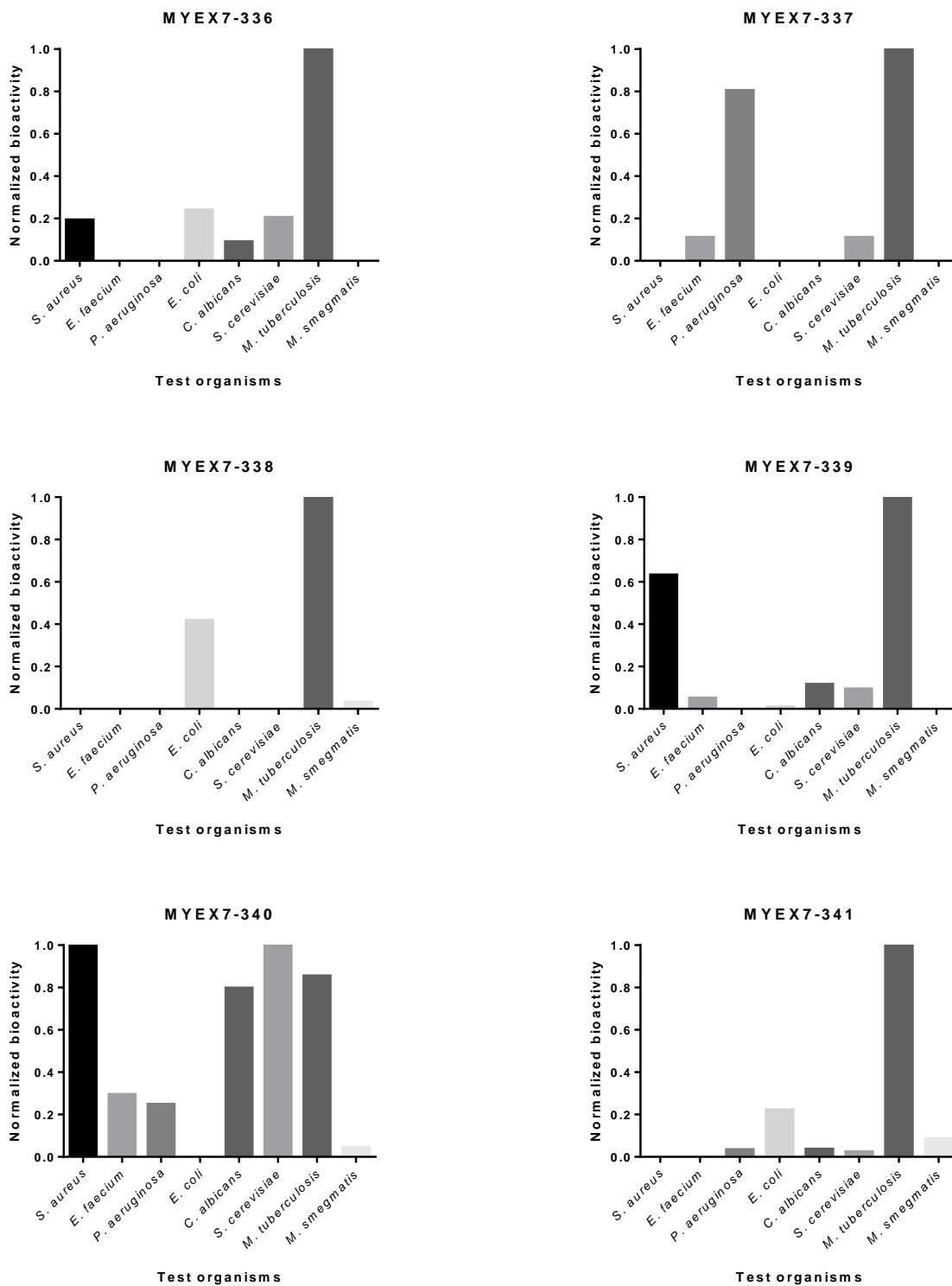
**Figure A3.1.** Bioactivity profiles of extracts in the NPRG collection (continued).



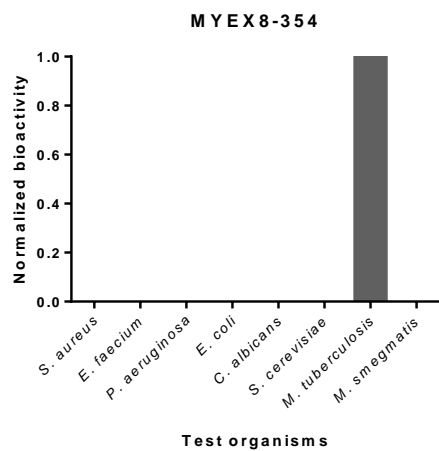
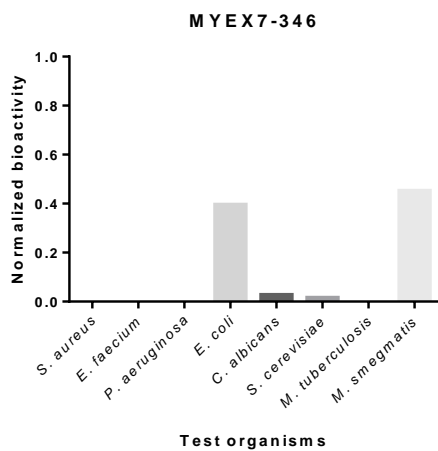
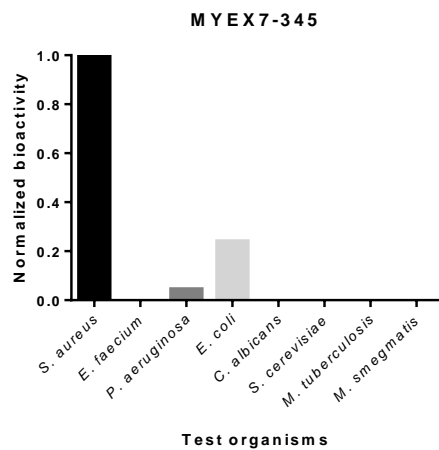
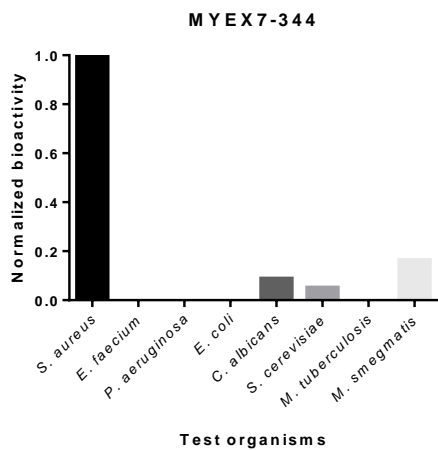
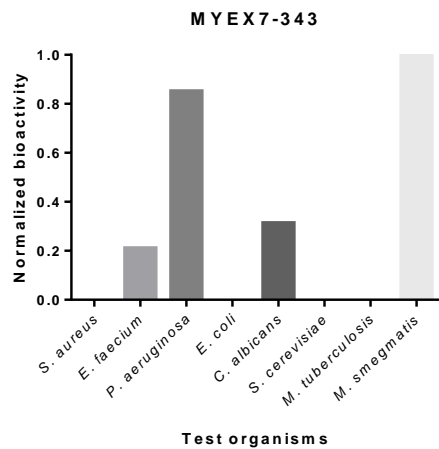
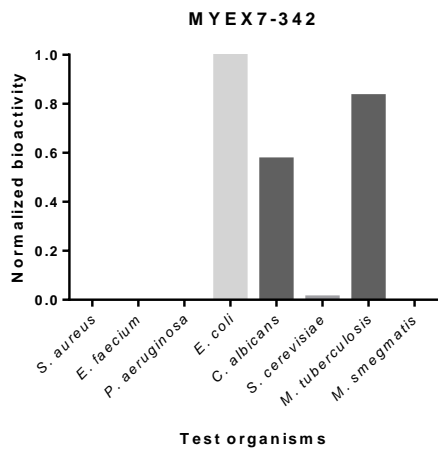
**Figure A3.1.** Bioactivity profiles of extracts in the NPRG collection (continued).



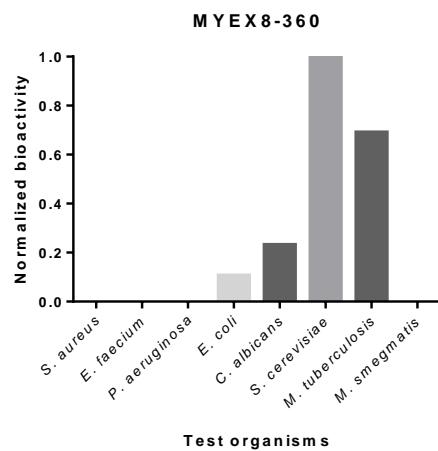
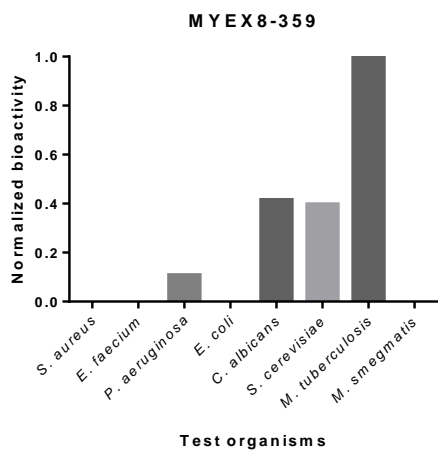
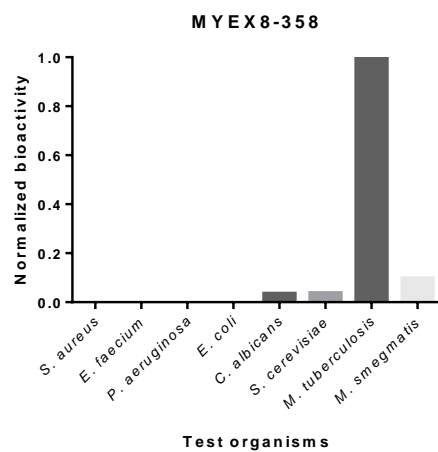
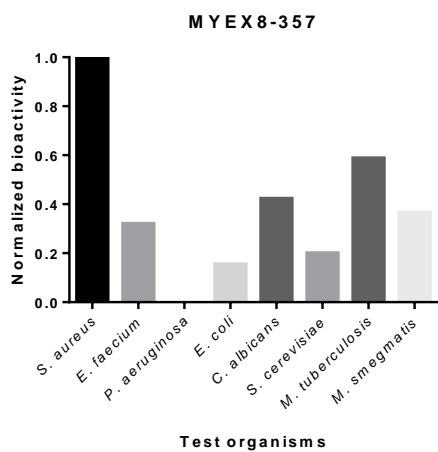
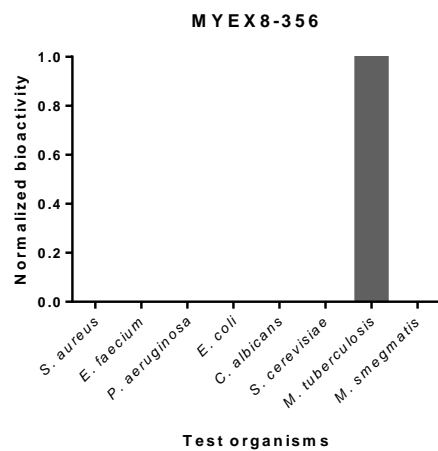
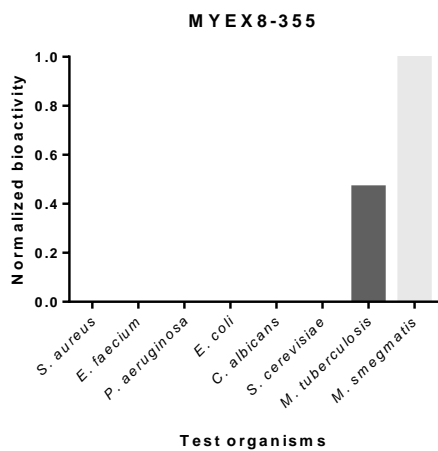
**Figure A3.1.** Bioactivity profiles of extracts in the NPRG collection (continued).



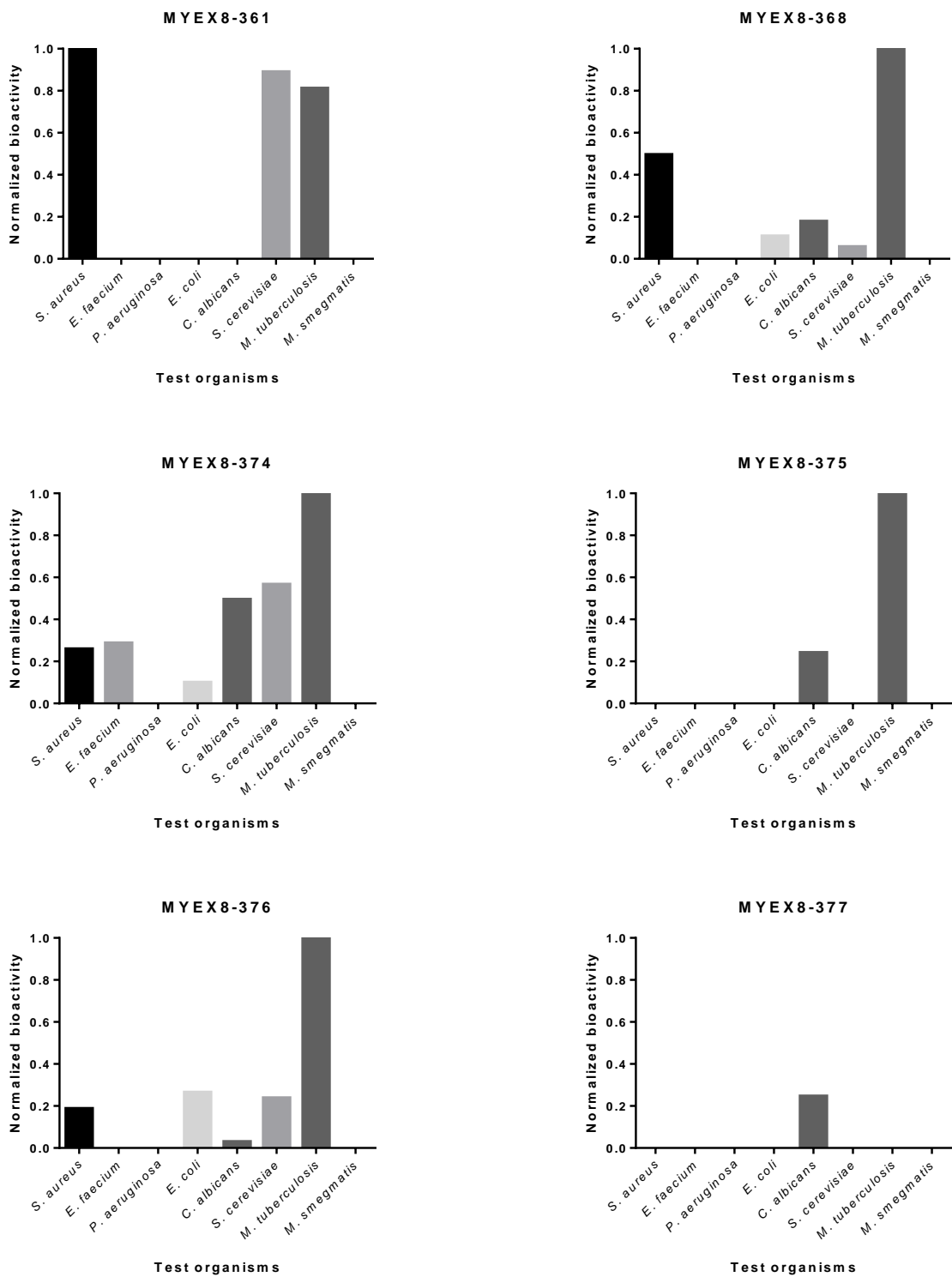
**Figure A3.1.** Bioactivity profiles of extracts in the NPRG collection (continued).



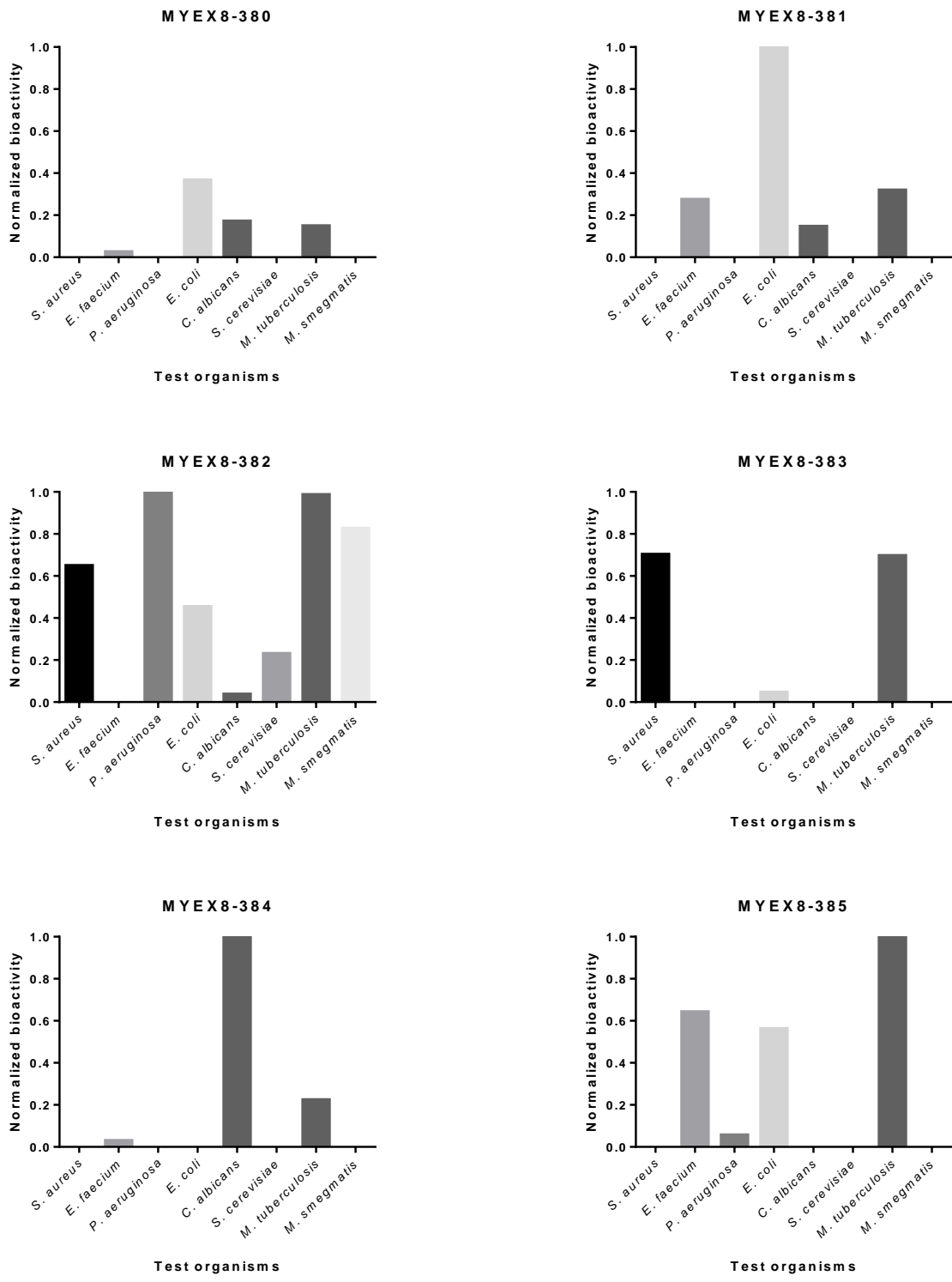
**Figure A3.1.** Bioactivity profiles of extracts in the NPRG collection (continued).



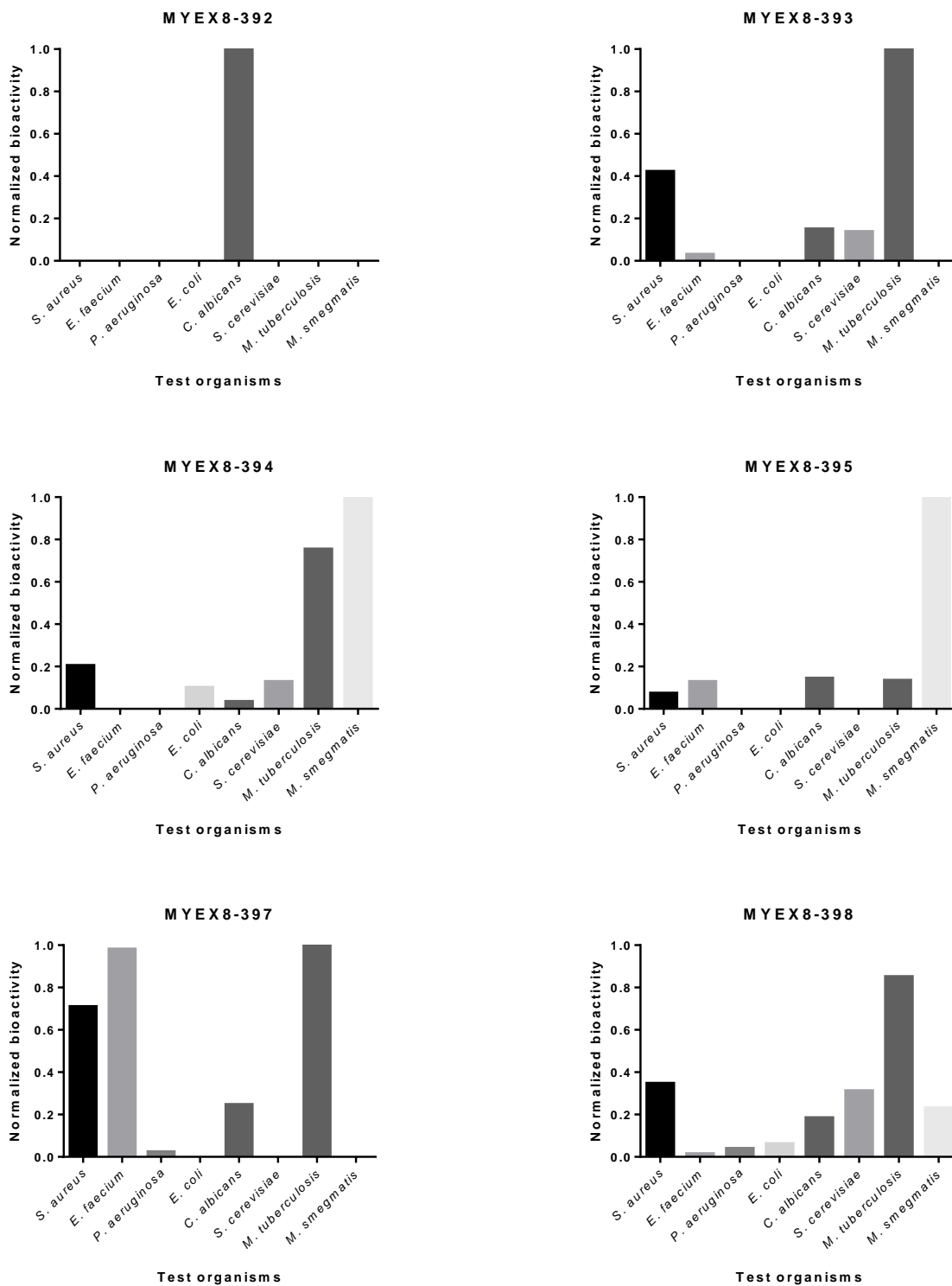
**Figure A3.1.** Bioactivity profiles of extracts in the NPRG collection (continued).



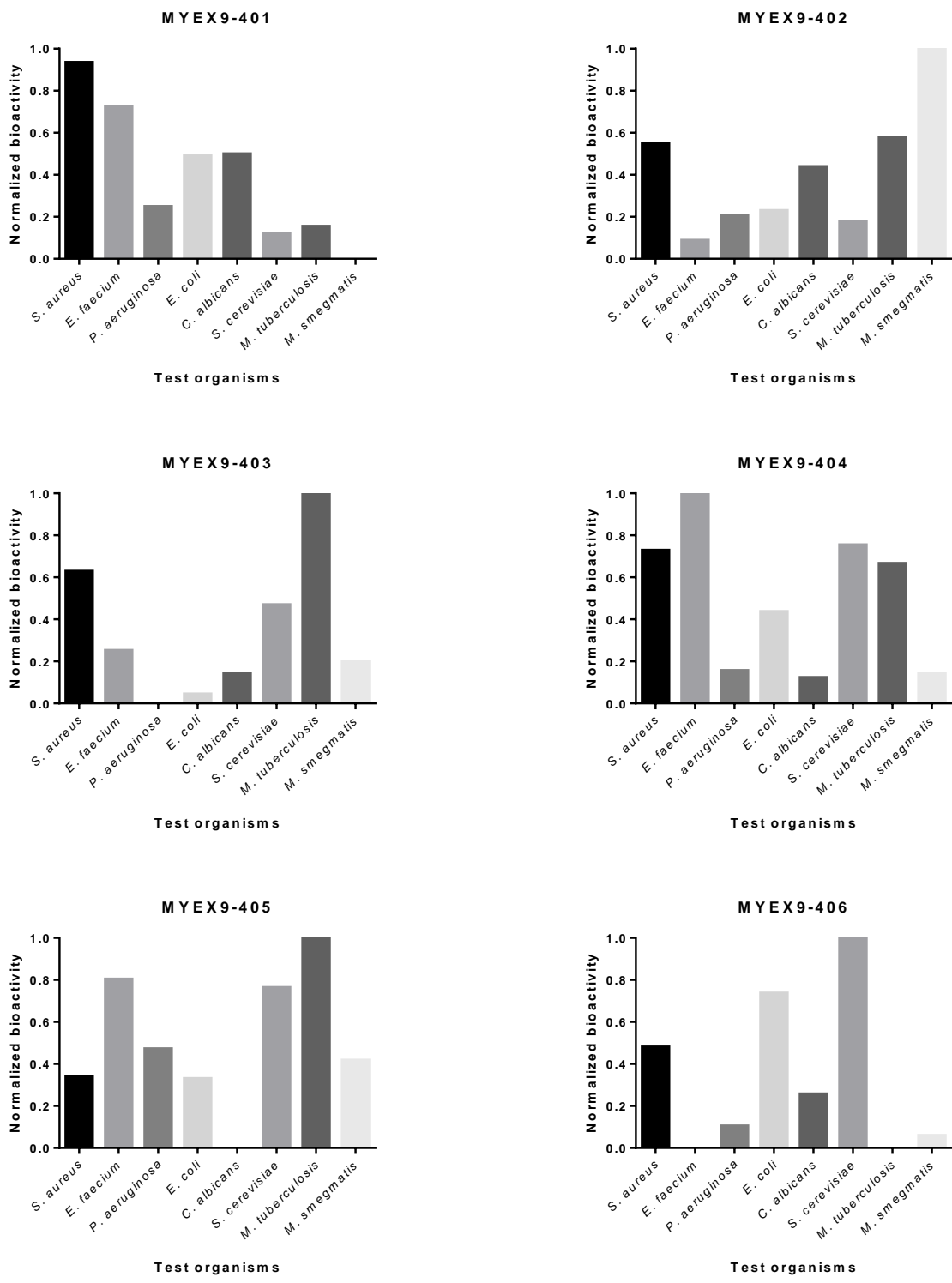
**Figure A3.1.** Bioactivity profiles of extracts in the NPRG collection (continued).



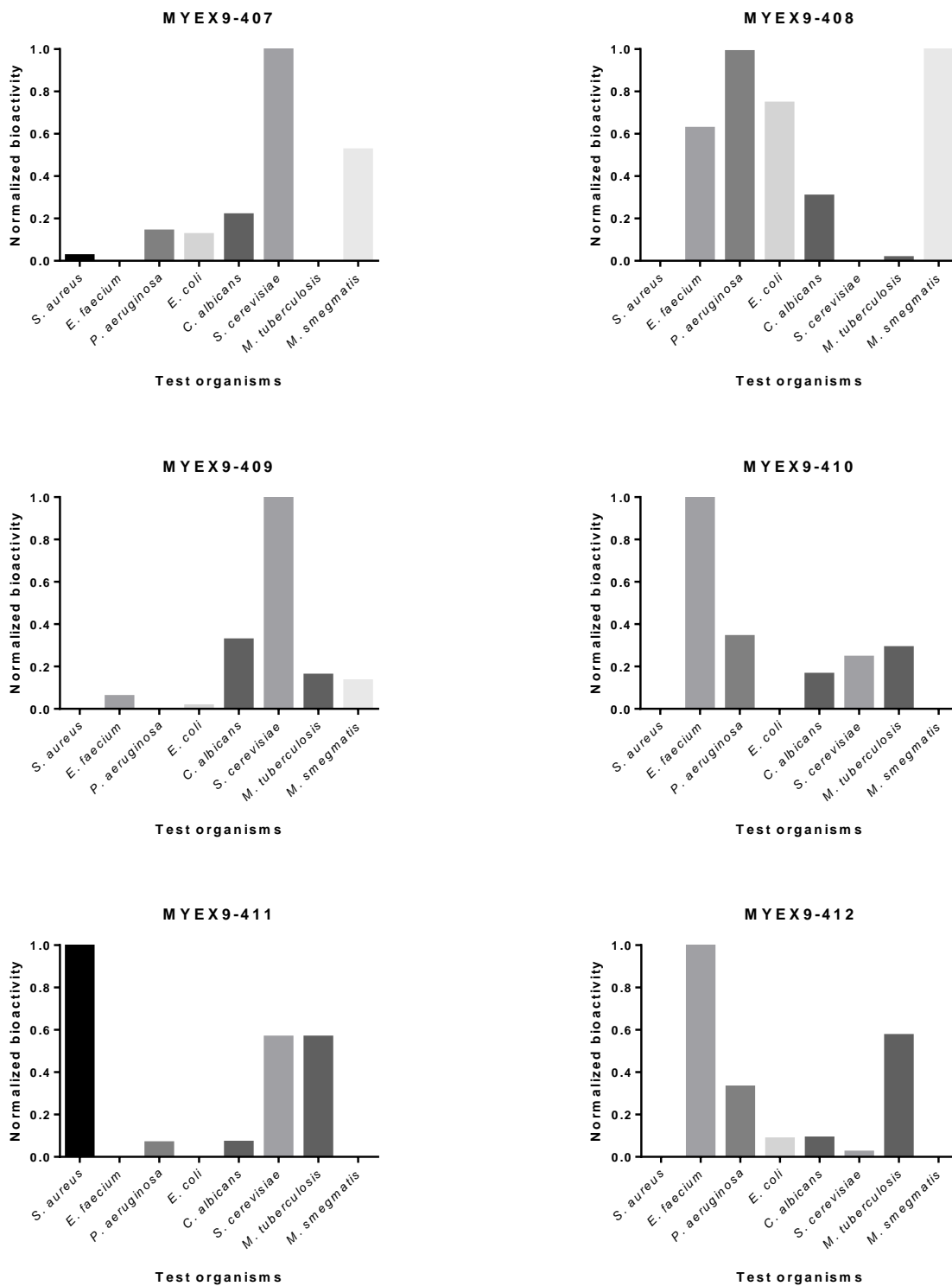
**Figure A3.1.** Bioactivity profiles of extracts in the NPRG collection (continued).



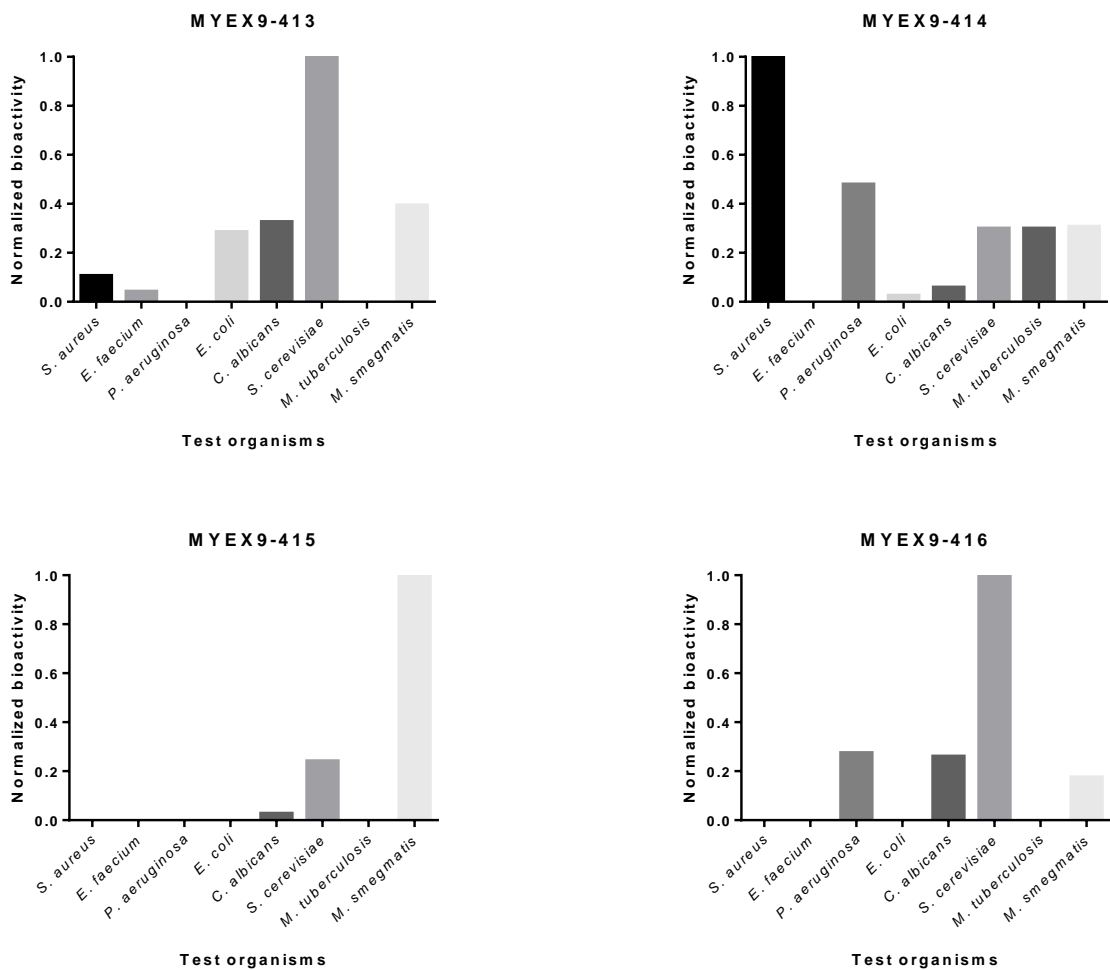
**Figure A3.1.** Bioactivity profiles of extracts in the NPRG collection (continued).



**Figure A3.1.** Bioactivity profiles of extracts in the NPRG collection (continued).

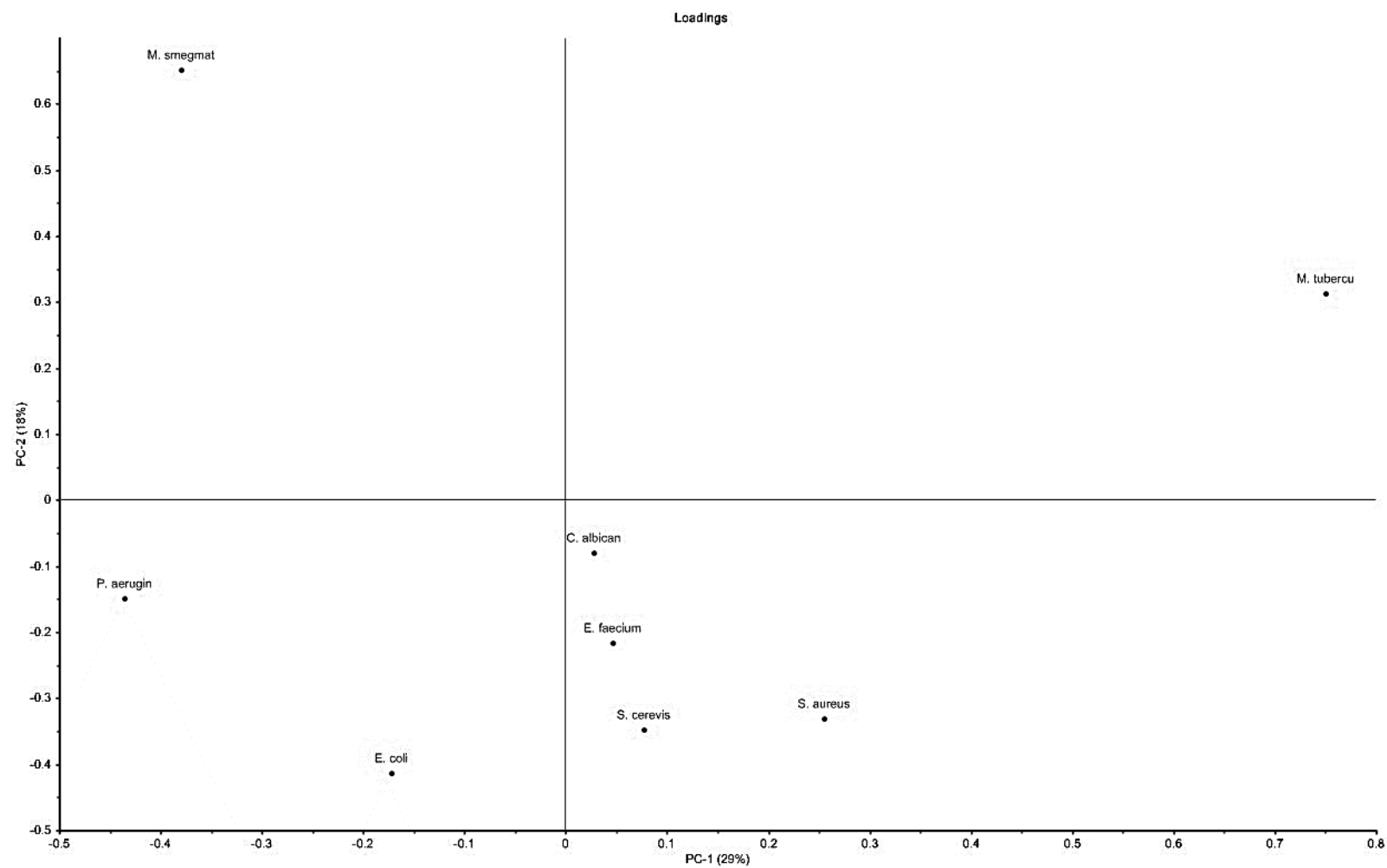


**Figure A3.1.** Bioactivity profiles of extracts in the NPRG collection (continued).



**Figure A3.1.** Bioactivity profiles of extracts in the NPRG collection (continued).

#### Appendix 4: Principal component analyses plots



**Figure A4.1.** Loading plots for principal component 1 plotted against principal component 2 from the principal component analysis of bioactivity profiles obtained from all extracts from endophytes in NPRG collection.



## Appendix 5: List of outliers from principal component analysis

**Table A5.1.** List of extract outliers from principal component analysis as well as their respective isolate for all two hundred and eighty endophytic fungal isolates in the Natural Products Research Group library collection.

Extract	Endophyte
MYEX1-001	TC2-078
MYEX1-002	TC2-073
MYEX1-004	TC2-062
MYEX1-019	TC2-033
MYEX1-030	TC2-039
MYEX1-039	TC2-013
MYEX1-041	TC2-084
MYEX1-048	TC2-020
MYEX2-062	TC2-071
MYEX2-072	TC2-064
MYEX2-074	TC2-077
MYEX2-075	TC2-019
MYEX2-077	TC2-055
MYEX2-078	KP2-005A
MYEX2-082	KP2-013G
MYEX2-084	KP1-063F
MYEX2-085	KP1-063L
MYEX2-090	KP1-123A
MYEX2-092	KP1-135E2
MYEX3-103	KP1-131Q
MYEX3-105	KP1-131Y
MYEX3-109	KP1-175K
MYEX3-122	KP1-131I
MYEX3-125	KP2-001F
MYEX3-127	KP2-029D
MYEX3-128	KP1-045C
MYEX3-130	KP2-033B
MYEX3-131	KP1-045J
MYEX3-133	KP1-045A
MYEX3-134	KP2-033A

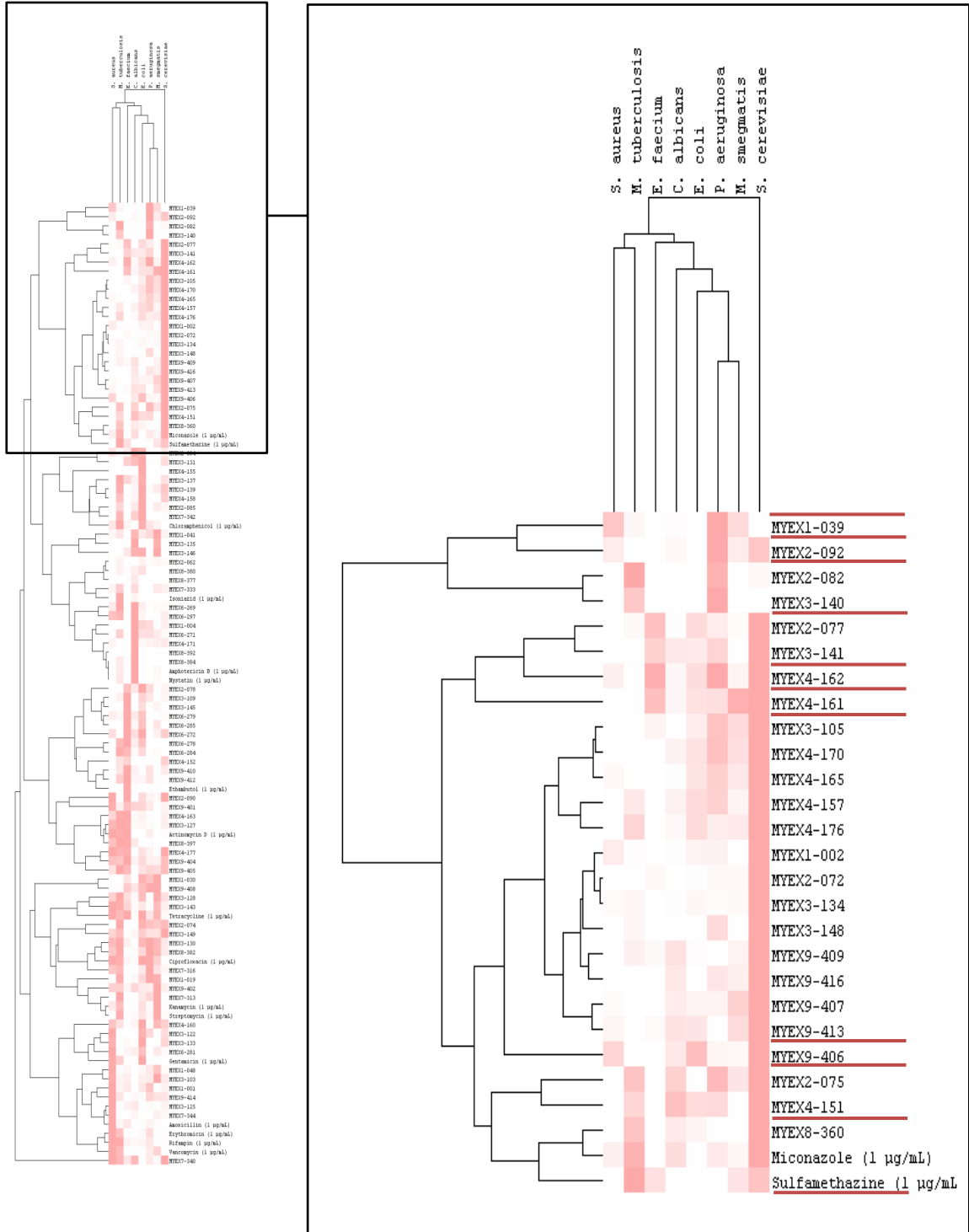
**Table A5.1.** List of extract outliers from principal component analysis as well as their respective isolate for all two hundred and eighty endophytic fungal isolates in the Natural Products Research Group library collection.

Extract	Endophyte
MYEX3-135	KP2-033D
MYEX3-137	KP1-115A
MYEX3-139	KP2-029B
MYEX3-140	KP1-045G
MYEX3-141	KP2-033F
MYEX3-143	KP1-119E
MYEX3-145	KP2-033C
MYEX3-146	KP1-119B
MYEX3-148	KP1-021A
MYEX3-149	KP1-025B
MYEX4-151	KP1-063A
MYEX4-152	KP1-063B
MYEX4-155	KP1-069B
MYEX4-157	KP1-069D
MYEX4-158	KP1-069E
MYEX4-160	KP1-143A
MYEX4-161	KP1-143B
MYEX4-162	KP1-143C
MYEX4-163	KP1-143D
MYEX4-165	KP1-123C
MYEX4-170	KP1-131K
MYEX4-171	KP1-089A
MYEX4-176	KP1-119D
MYEX4-177	KP1-123B
MYEX6-269	KP1-131S
MYEX6-271	KP1-175E
MYEX6-272	KP1-175L
MYEX6-278	KP2-033G
MYEX6-279	KP1-131V
MYEX6-281	KP1-131R

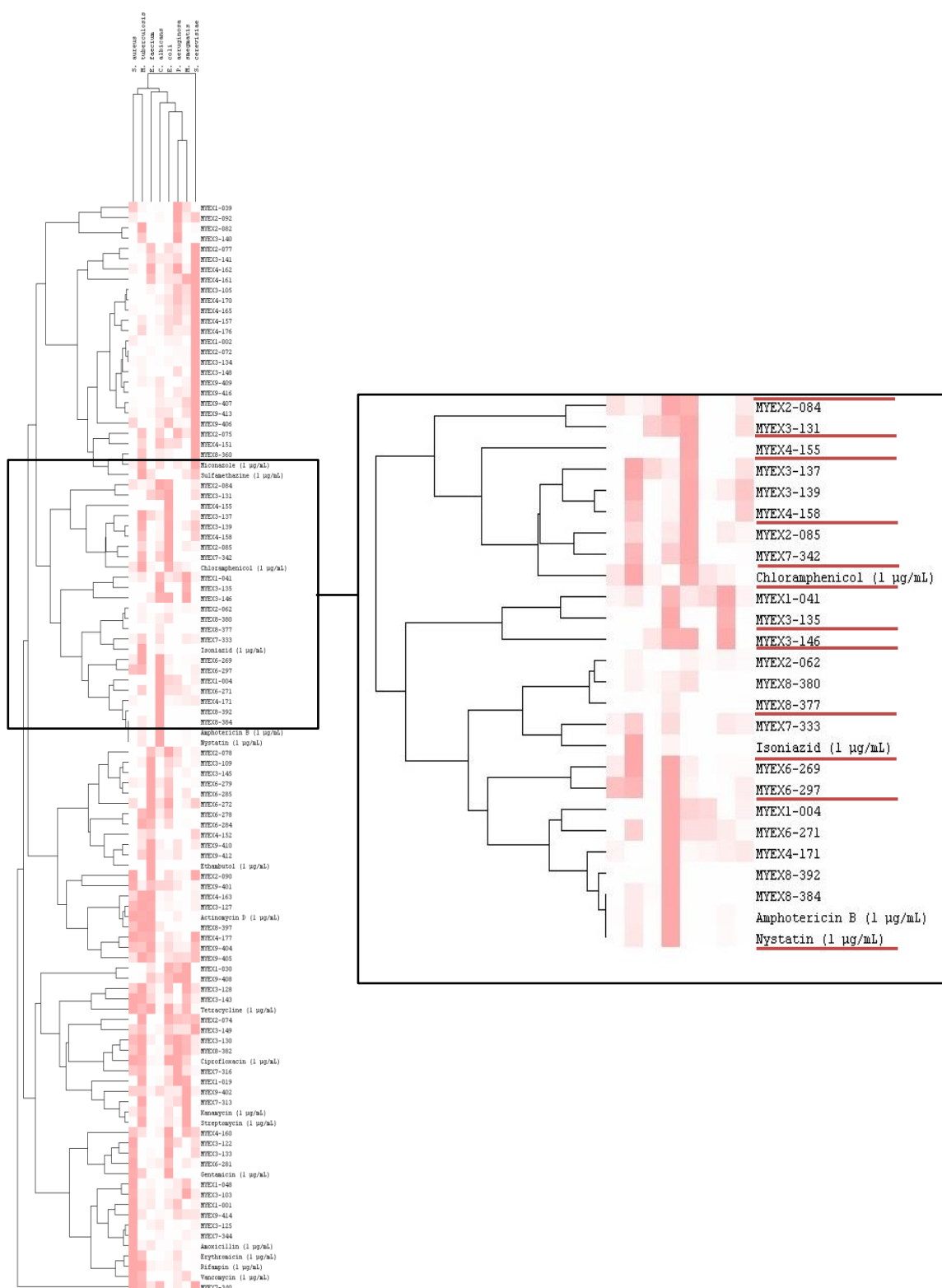
**Table A5.1.** List of extract outliers from principal component analysis as well as their respective isolate for all two hundred and eighty endophytic fungal isolates in the Natural Products Research Group library collection.

Extract	Endophyte
MYEX6-284	KP1-017D
MYEX6-285	TC2-047
MYEX6-297	TC3-014
MYEX7-313	KP1-175F
MYEX7-316	JAJ1-113
MYEX7-333	JAJ1-025
MYEX7-340	JAJ1-073
MYEX7-342	JAJ1-097
MYEX7-344	JAJ1-069
MYEX8-360	JAJ1-145
MYEX8-377	SC1-085H
MYEX8-380	JAJ1-129
MYEX8-382	SC1-079G
MYEX8-384	JAJ1-005
MYEX8-392	SC1-063C
MYEX8-397	SC1-065E
MYEX9-401	SC1-077G
MYEX9-402	CT1-006B
MYEX9-404	CT1-011A
MYEX9-405	CT1-012A
MYEX9-406	CT1-013A
MYEX9-407	CT1-016A
MYEX9-408	KP1-131J
MYEX9-409	SC1-065G
MYEX9-410	JAJ1-117
MYEX9-412	JAJ1-013
MYEX9-413	CT1-008A
MYEX9-414	CT1-012B
MYEX9-416	JAJ1-109

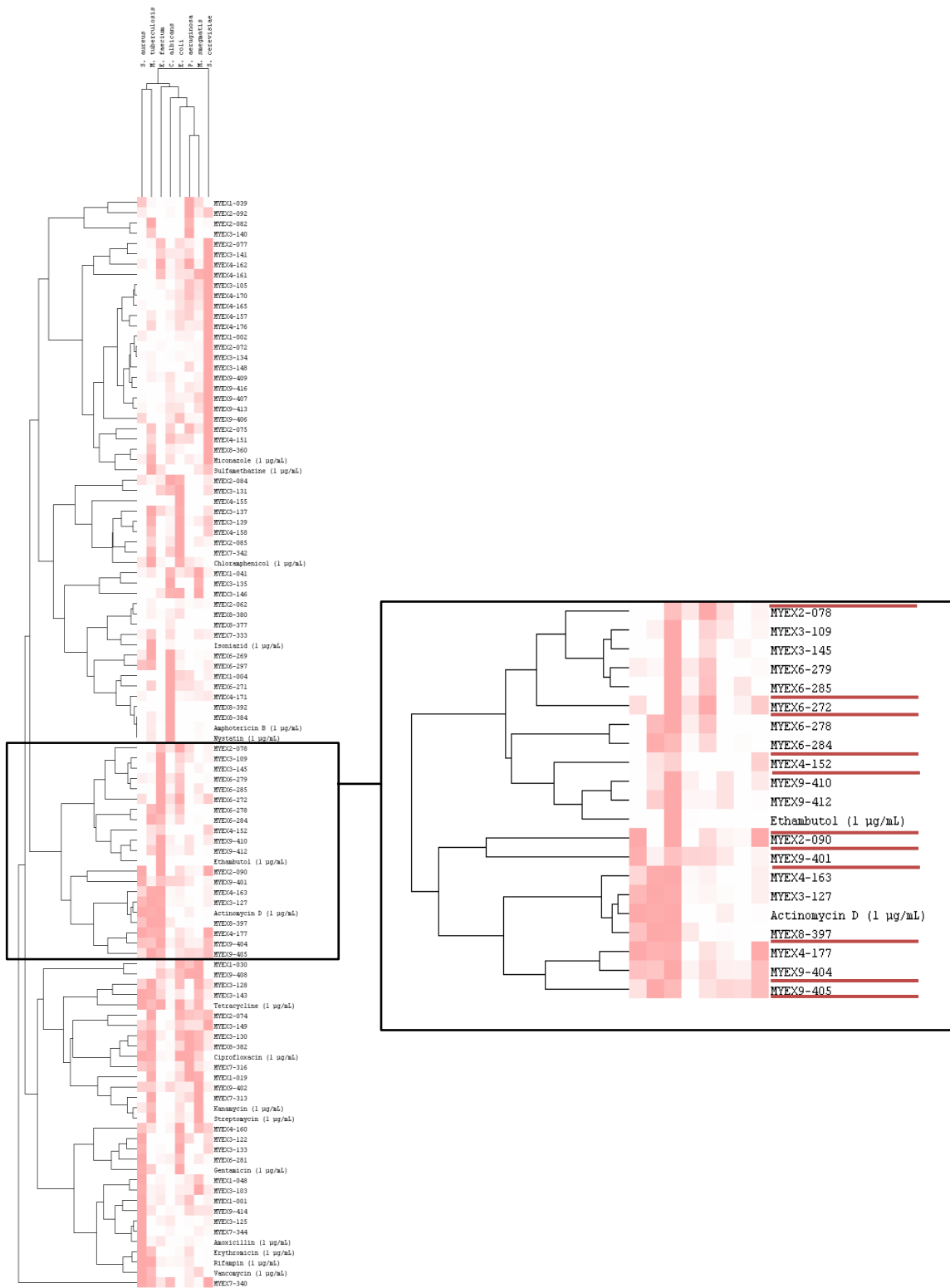
## Appendix 6: Hierarchical cluster analysis



**Figure A6.1.** Hierarchical cluster analysis (Euclidean distance, average linkage) of bioactivity profiles of extracts in NPRG collection. Red lines are separating the labels of clusters that showed correlations of  $\geq 0.80$ .

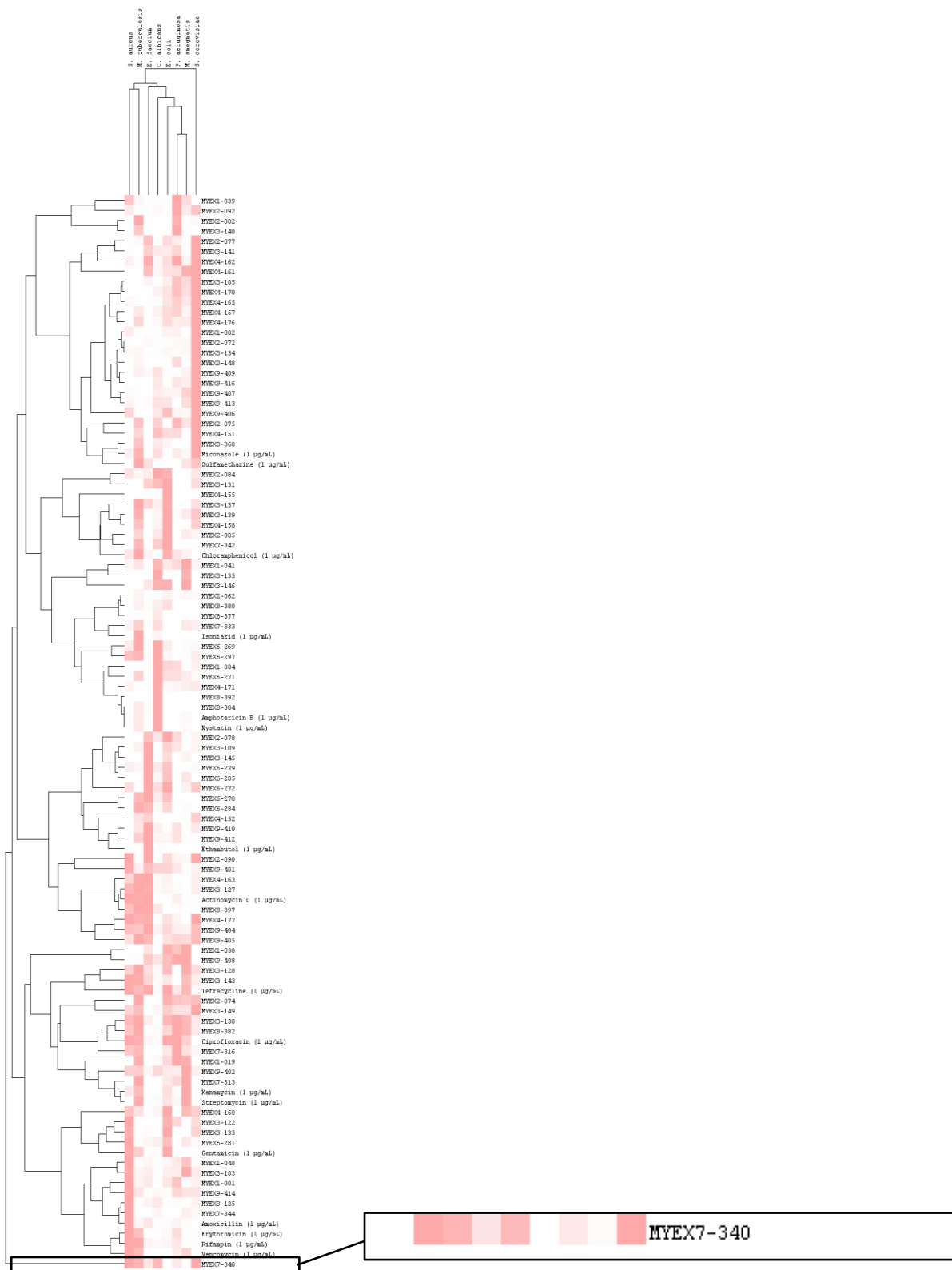


**Figure A6.1.** Hierarchical cluster analysis (Euclidean distance, average linkage) of bioactivity profiles of extracts in NPRG collection. Red lines are separating the labels of clusters that showed correlations of  $\geq 0.80$  (continued).



**Figure A6.1.** Hierarchical cluster analysis (Euclidean distance, average linkage) of bioactivity profiles of extracts in NPRG collection. Red lines are separating the labels of clusters that showed correlations of  $\geq 0.80$  (continued).





**Figure A6.1.** Hierarchical cluster analysis (Euclidean distance, average linkage) of bioactivity profiles of extracts in NPRG collection. Red lines are separating the labels of clusters that showed correlations of  $\geq 0.80$  (continued).

## Curriculum Vitae

**Candidate's full name:** Marija Veljanovska

### Universities attended

University of New Brunswick, Saint John

2013-2017 Bachelor of Science with Honours

2017- Master of Science

### Conference presentations:

BioActives CREATE Workshop, University of New Brunswick, Saint John, New Brunswick (August 2019)

Poster Presentation

Title: "Prioritizing a collection of fungal extracts for natural product discovery using bioactivity profiling"

Authors: Mila Veljanovska, **Marija Veljanovska**, Andrew J. Flewelling, John A. Johnson and Christopher A. Gray.

Maritime Natural Products Conference, University of New Brunswick, Saint John, New Brunswick (August 2019)

Poster Presentation

Title: "Prioritizing a collection of fungal extracts for natural product discovery using bioactivity profiling"

Authors: Mila Veljanovska, **Marija Veljanovska**, Andrew J. Flewelling, John A. Johnson and Christopher A. Gray.

Maritime Natural Products Conference, University of New Brunswick, Saint John, New Brunswick (August 2019)

Oral Presentation

Title: "The development and application of a simple bioactivity profiling strategy to natural product discovery"

Authors: **Marija Veljanovska**, John A. Johnson and Christopher A. Gray.

Graduate Research Conference, University of New Brunswick, Fredericton, N.B. (March 2019)

Poster Presentation

Title: "The development and application of a simple bioactivity profiling strategy to natural product discovery from a library of endophytic fungi"

Authors: **Marija Veljanovska**, John A. Johnson and Christopher A. Gray.

School of Graduate Studies, 3 Minute Thesis competition, University of New Brunswick, Fredericton, N.B. (March 2019)

Oral Presentation

Title: "Natural Products Research: developing an approach in drug discovery to save time and money"

Authors: **Marija Veljanovska**, John A. Johnson and Christopher A. Gray.

Guest lecturer for BIOL2485 – Introduction to Microbiology, University of New Brunswick, Saint John, NB (March 2019)

Oral Presentation

Title: “The development and application of a simple bioactivity profiling strategy to natural product discovery from a library of endophytic fungi”

Authors: **Marija Veljanovska**, John A. Johnson and Christopher A. Gray.

Maritime Natural Products Conference, Dalhousie University, Halifax, Nova Scotia (August 2018)

Oral Presentation

Title: “The development and application of a simple bioactivity profiling strategy to natural product discovery from a library of endophytic fungi”

Authors: **Marija Veljanovska**, John A. Johnson and Christopher A. Gray.

Maritime Natural Products Conference, University of Prince Edward Island, Charlottetown, PEI (August 2017)

Poster Presentation

Title: “The Natural Products Research Group fungal endophyte library”

Authors: Julie Therrien, Allyson Bos, Matthew Clinton, Kathleen Complak, Samantha Cox, Trevor Clark, Katelyn Ellsworth, Andrew Flewelling, Lauren Forgrave, Nicholas Morehouse, Artabaz Nazari, Kelsey Pendleton, **Marija Veljanovska**, John A. Johnson and Christopher A. Gray.