Antibacterial and Anti-pathogenic Activity of Soil Organism Extracts from an Archaeological Site

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Abstract and Background

Although the discovery of antibiotics revolutionized medicine as it is today, obstacles such as antibiotic resistance still pose a threat to the world population. Antibiotic resistance is the ability of bacteria to resist the effects of a medication that once was used to treat the same microbe (Adedeji, 2016). Genetic mutations in strains of bacteria and competitive advantage can result in microbial resistance against antibiotics (Adedeji, 2016). Overuse and misuse of antibiotics are also primary reasons for the rapid emergence of resistant bacteria (Ventola, 2015). Resistant organisms are incredibly more difficult to treat, requiring higher doses or alternative medications that can be far more costly and harmful for individuals. For this reason, the study of antibiotic, anti-pathogenic, and anti-cancer activity is becoming increasingly more prevalent.

The purpose of this study is to isolate new antibiotic and anti-pathogenic compounds from soil bacteria excavated from an archaeological site in the ancient Roman city of Pollentia on the island of Mallorca, Spain. Bacteria were collected from five different layers of soil, isolated, and cultured onto yeast or potato dextrose media. Bacterial products were extracted from liquid cultures and activity was tested against *Staphylococcus aureus*, *Escherichia coli*, and *Pythium ultimum*, and separated on silica TLC and flash chromatography with hexane, ethyl acetate, and methanol. Two of the promising cultures had significant activity. The second round of flash chromatography of OIV-4d had 10 of the 16 fractions inhibiting growth by 5-10 mm, while OIX-8m had 7 of the 12 fractions inhibiting growth by 0.5-7 mm against *S. aureus* and *E. coli*. Inhibition of *P. ultimum* were observed in 10 of the 16 fractions of OIV-4d and 3 of the 12 fractions of OIX-8m. Findings from this study indicate that many antibiotics are yet to be discovered.

Figure 1. Isolated actinomycete samples obtained from soil that were collected at an archaeological burial site. Left colony is OIX-8m and right colony is OIV-4d.

Materials and Methods

1. Soil bacteria were extracted from an archaeological dig site in Mallorca, Spain and grown on various agar media.
2. The bacteria of interest were grown in sterile liquid media for 4-6 weeks on a lab shaker. After allowing two weeks of growth on the lab shaker, DNA was extracted with a DNA extraction powersoil kit (Figure 1).
3. After allowing appropriate growth, cultures were separated with separatory funnel extraction and dichloromethane.
4. The solvents in the separated cultures were evaporated using a rotovap and left to dry.
5. Once dried, the samples were dissolved at known concentrations to be used for silica TLC chromatography, using a 7:1 chloroform:methanol solvent. TLC was viewed under 365 nm UV light, under 254 nm UV light, and after, was stained with sulfuric acid and vanilla, then heated (Figure 2).
6. The dissolved extracts were tested against bacterium *S. aureus* and *E. Coli* overnight, and against the water mold *P. ultimum* for up to eight days.
7. For samples that showed promise in fighting against *S. aureus*, *E. coli*, and *P. ultimum*, the samples were further separated into fractions using Reveleris X2 Flash Chromatography to try and isolate the unique fraction that contains inhibitory activity (Figure 3).
8. These fractions were evaporated, underwent TLC analysis, and were tested against *S. aureus*, *E. coli*, and *P. ultimum* using the same methods above.

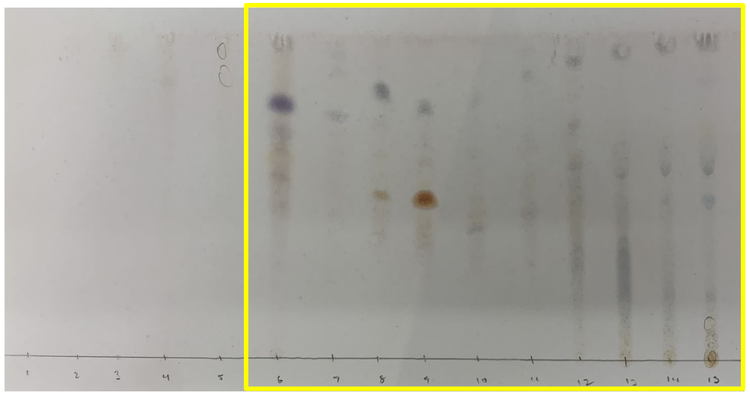

Figure 2. Thin layer chromatography of second flash chromatography results of sample OIV-4d with sulfuric acid and vanilla stain. Using 7:1 chloroform:methanol as a solvent, TLC was completed to separate the polar and nonpolar components of the fractions after evaporation. The components that migrated a farther distance on the TLC were determined to be more nonpolar because they interacted stronger with the nonpolar solvent, whereas, the more polar components in each fraction had less migration up the TLC. The yellow boxed column indicate fractions that had significant activity against *S. aureus, E. coli, and P. ultimum.*

Table 1. Second flash chromatography bacterial inhibition assay results against *S. aureus*, *E. coli,* and *P. ultimum*. A positive (+) mark indicates unsuccessful inhibition of pathogen growth. A negative (-) mark indicates successful inhibition of pathogen growth. Some cultures displayed partial inhibition of pathogen growth which is indicated by +/-. Cultures that exhibited significant antibacterial or anti-pathogenic activity are highlighted in gray.

Fraction	Pythium Day 1-7	E. Coli (gram -)	Staph (gram +)
Control (Methanol)	+	n/a	0mm
Ampicillin	n/a	n/a	22 mm
1	+	n/a	0 mm
2	+	n/a	2 mm
3	+	n/a	4 mm
4	+	n/a	3 mm
5	+	n/a	2 mm
6	-	n/a	6 mm
7	-	n/a	10 mm
8	-	n/a	9 mm
9	-	n/a	9 mm
10	-	n/a	5 mm
11	-	n/a	5 mm
12	-	n/a	6 mm
13	-	n/a	5 mm
14	-	n/a	6 mm
15	-	n/a	6 mm
16	-	n/a	2 mm

Fraction	Pythium Day 1-7	E. Coli (gram -)	Staph (gram +)
Control (Methanol)	+	0	0
Ampicillin	n/a	16 mm	14 mm
1	+/-	0 mm	0 mm
2	+	0 mm	0 mm
3	+	0 mm	0 mm
4	+	3 mm	0 mm
5	+	7 mm	4 mm
6	+	4 mm	2 mm
7	+	2.5-3 mm	2 mm
8	-	0 mm	2 mm
9	+	0 mm	0.5 mm
10	-	1 mm	2 mm
11	-	0.5 mm	0.5 mm
12	+	0 mm	0 mm

Results

- Our results show that extracts from isolated soil bacteria show significant antibacterial or anti-pathogenic properties.
- The soil bacteria that showed antibacterial activity against *S. aureus* and *E. coli* included 10 out of the 16 fractions of OIV-4d and 3 of the 12 fractions of OIX-8m (Table 1).
- Fraction 7, 8 and 9 of OIV-4d showed the greatest inhibitory activity of *S. aureus* at a distance of 9-10 mm.
- Fraction 5 of OIX-8m showed the greatest inhibitory activity of *S. aureus* at a distance of 4 mm and *E. coli* at a distance of 7 mm (Table 1).
- Fractions 6-16 of OIV-4d and 8, 10, and 11 of OIX-8m showed anti-pathogenic activity against *P. ultimum* growth for more than eight days.
- Comparing the first and second round of flash chromatography results indicate that some fractions may work synergistically to inhibit microbes, but once isolated, are inactive.
- Further flash chromatography analysis of Fraction 6-16 of OIV-4d's showed that the new fraction from 5-14 possessed antibiotic and anti-pathogenic properties as it inhibited *P. ultimum* and *S. aureus.*

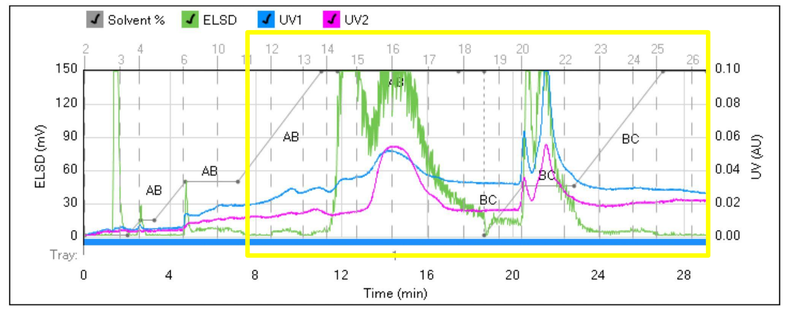

Figure 3. Sample OIV-4d was fractionated by Reveleris X2 flash chromatography using silica gel. The solvents used for separation were hexane, ethyl acetate, and methanol, and were administered in this order.

Conclusion

- Each bacteria culture showed different strengths in inhibiting growth of *S. aureus*, *E. coli*, and *P. ultimum*, due to each extract having varying chemical composition.
- Flash chromatography results indicated that the location of out antibiotic is located in the middle to polar end of the spectrum, allowing us to narrow down our searches.
- When fractioned, OIX-8m and OIV-4d showed less antibacterial and anti-pathogenic activity than initial testing possibly due to the concentration of the fraction being too low or because activity may be synergistic between the fraction components. In order to combat this experimental obstacle, more bacteria culture needs to be grown for future testing to ensure greater concentration.
- We were able do a third round of flash chromatography on sample OIV-4d to create as much separation of compounds on a standard TLC paper, with plans of doing a TLC with an aluminum backing to allow for a laser NMR to identify the unknown compounds.
- Further spectroscopic analysis is needed to identify the exact chemical molecule that is responsible for antibacterial or anti-pathogenic activity.
- Future research will run PCR tests on the isolated bacteria DNA. To identify the specific bacteria, the isolated DNA will need to be compared to national databases and cross referenced. However, results from a peer group (Alex Temple) indicate that our soil samples are most likely from the *Streptomyces* group of soil bacteria.

References

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