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Biotransformation of Praziquantel by Cunninghamella elegans, Umbelopsis ramanniana, and Yarrowia lipolytica Metabolism

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BIOTRANSFORMATION OF PRAZIQUANTEL BY *CUNNINGHAMELLA ELEGANS*,
UMBELOPSIS RAMANNIANA, AND *YARROWIA LIPOLYTICA* METABOLISM

by

SAMUEL CHIVERS

A THESIS

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Samuel Chivers

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Schistosomiasis is a neglected tropical disease rampant in developing countries. Widespread chemotherapy is reliant on a single drug, praziquantel, which increases the risk of resistance and creates an urgent need for the development of new alternatives for treatment. Fungal biotransformation is a well-documented tool in the pharmaceutical synthesis of new drugs. This study examined the efficacy of four fungal strains, *Cunninghamella elegans* (ATCC 9245 and ATCC 8688a), *Umbelopsis ramanniana*, (ATCC 9628) and *Yarrowia lipolytica* (ATCC 20225), in the biotransformation of praziquantel. Colonies were cultured, dosed with praziquantel, incubated for 5, 10 or 15 days to metabolize, after which the cultures were filtered, metabolites extracted and subjected to HPLC analysis. Once the optimal time for metabolization was ascertained cultures were exposed to variable drug doses, either single or double. *C. elegans*, ATCC 8688a, fully metabolizes praziquantel after 15 days producing major peaks at 4.7 and 4.3 minutes; and responded more efficiently to a double dose. After 15 days *C. elegans*, ATCC 9245, had metabolized the majority of praziquantel producing major peaks at 4.7 and 4.3 minutes, and was equally efficient with an increased dose. *U. ramanniana*, fully metabolizes praziquantel after 10 days producing major peaks at 4.9 and 4.5 minutes, however, failed to produce metabolites with increased dosage. *Y. lipolytica*, showed negligible metabolic activity. The praziquantel peak remained relatively unchanged at 8.1 minutes for each time course and was not exposed to variable doses. Purification of the mixture and further testing is required to identify the metabolites obtained.

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Introduction

Schistosomiasis, also referred to as bilharzia, is largely a disease of poverty; particularly prevalent in tropical areas such as sub-Saharan Africa, (Hotez, et al., 2014). Schistosomiasis is caused by blood flukes, *Schistosoma sp.*, of which there are five primary species. *S. mansoni* primarily located in the Africa, the Arabian Peninsula, and South America. Whilst it is also found in rodents and non-human primates, humans remain its main host. *S. haematobium* is commonly found in Africa and the Arabian Peninsula. *S. japonicum* is a zoonotic parasite capable of infecting humans and 40 other mammalian reservoirs, located throughout China and South East Asia, (Ross , et al., 2002). *S. intercalatum* and *S. mekongi* also cause schistosomiasis but are confined to smaller areas and deemed only of local significance.

It is the second most commonly contracted parasitic disease after malaria; affecting approximately 207 million people, (Meister, et al., 2014). 120 million of these are symptomatic and 20 million have contracted a severe form of schistosomiasis. It is estimated that 85% of those infected reside on the African continent in undeveloped regions. South America is also heavily infested; Brazil cites schistosomiasis as the one of the top three public health problems, (Chitsulo, et al., 2000). As such schistosomiasis bears a significant burden, both economically and in terms of public health. The disability adjusted life years lost for schistosomiasis is ~70 million, annually; this is akin to HIV and exceeds both malaria and tuberculosis, (King & Dangerfield-Cha, 2008).

In recent years there has been a relative hiatus in the continued research of schistosomiasis treatment, characterisation, and eradication. Given the low level of investment for the continued research into treatment and prevention of schistosomiasis, this disease is often referred to as the neglected “silent pandemic”, (King, et al., 2005).

A pyrazino-isoquinoline derivative, praziquantel, PZQ, has proven most effective against schistosomiasis, (Woelfle, et al., 2011). The combination of being readily available and cost effective has quickly made it the preferred drug for the wide spread chemotherapy of schistosomiasis, (Alsaqabi & Lofty, 2014). Once ingested praziquantel shows results in less than an hour. It works by damaging the tegument of the worms which causes paralysis. After a single treatment 70-100% of patients cease to excrete eggs; those that still do exhibit a decrease of viable eggs and associated antigen concentration by 95%, (Gryseels & Polderman, 1991). However, there are significant drawbacks with the praziquantel treatment. Firstly, it is ineffective against eggs and juvenile *Schistosoma sp.*. Additionally, tissue dwelling eggs can be excreted several weeks after treatment. Therefore, a second dose of the treatment is required 4-6 weeks after the first, which many patients fail to acquire, (Renganathan & Cioli, 1998) . Additionally, praziquantel is administered as a racemate mixture, however, the anthelmintic activity is only associated with the R-(-)-enantiomer, (Schepmann & Blaschke, 2001). Whereas the S-(-)-enantiomer is biologically inactive and the causation of many side effects linked to the treatment. It is estimated that 80% of the drug is excreted in urine, (Alsaqabi & Lofty, 2014).

Such a heavy reliance on a single treatment and drug with wide spread use exponentially increases the inherent risk of resistance and tolerance by *Schistosoma sp.*, (Woelfle, et al., 2011). Drug resistance and tolerance in disease causing organism is on the rise, (Valentim, et al., 2013). Whilst, as yet, there is no documented cases for wide spread PZQ resistance in *Schistomiasis sp.*. There is evidence of resistance mechanisms that researchers have identified in laboratory isolates which show significantly reduced susceptibility to PZQ, (Couto, et al., 2011). Moreover, it is possible to induce PZQ resistance to *S. mansoni* and *S. japonicum in vivo*, (Wang, et al., 2012). Although the exact mechanisms of resistance is unclear, and has yet to fully present itself,

increased tolerance to treatment has presented itself in the field. *S. mansoni* of many foci have sowed reduced susceptibility. *S. haematobium* has also been documented causing infections in which repeated standard treatment fails to eradicate. Treatment failure of other infections can be as high as 30%, (Wang, et al., 2012). In the absence of an effective vaccine for human helminth infections, repetitive rounds of monotherapies are used, however these increase the risk of both resistance and tolerance, (Taylor, et al., 2017) . Therefore, there is an urgent need to identify and synthesise new PZQ derivatives in order to circumvent this and other drawbacks to the current treatment, (Vale, et al., 2017).

Fungal biotransformation is a widely established model to investigate drug metabolism and produce metabolites *in vitro*, (Dube & Kumar, 2017). The fungal biotransformation of organic compounds is well-documented as a useful tool in the pharmaceutical synthesis of new drugs, (Parshikov, et al., 2012). It is frequently used in lieu of chemical synthesis and other transformation techniques, (Rui, et al., 2005). The experimental procedures for fungal biotransformation offers many advantages; it is relatively simple and has the capacity to be scaled up far beyond other methodologies whilst remaining cost effective, (Moody, et al., 2002).

This study offers a viable procedure for obtaining PZQ derivatives. Where the introduction of modifiable moiety, via fungal biotransformation, can vastly increase the opportunity for derivation of the original structure; possibly be used as a scaffold for the production of novel semi-synthesized compounds. These compounds maybe able to meet the urgent needs to synthesize new derivatives and drug analogs in order to mitigate the risks of drug resistance and tolerance.

Methodology

Fungal Strains

Four fungal strains were selected for this investigation. *Cunninghamella elegans*, ATCC 9245, the most frequently used strain in fungal biotransformation, and *Cunninghamella elegans*, ATCC 8688a, for comparison. *Cunninghamella sp.* are zygomycete fungi studied extensively for their biotransformation capability and their metabolic similarities to humans, (Pothuluri, et al., 1998). *Cunninghamella sp.* expose compounds to diverse metabolic pathways, including region- and stereo selective mechanisms, (Moody, et al., 2002), leading to the creation of novel metabolites, (Zhang, et al., 1996). Previous studies cite *C. elegans* as the most efficient *Cunninghamella sp.* (Moody, et al., 2002).

Umbelopsis ramanniana, ATCC 9628, previously referred to as *Mucor ramannianus*, are saprobic fungi, (Parshikov, et al., 1999), which have been used in the biotransformation of several compounds, (Kang, et al., 2008). *U. ramanniana* has shown promising results of metabolising other quinolones, (Sutherland, et al., 2005). It is equally efficient in the production of derivatives as *C. elegans*, and also used for its similarities to mammalian metabolism, (Parshikov, et al., 2000).

Yarrowia lipolytica, ATCC 20225, is a hemiascomycetous dimorphic fungus that is showing increasing promise in the biotransformation of various industrially important compounds, particularly in processes that involve hydroxylation, (Bankar, et al., 2009). Additionally, *Y. lipolytica* shows similar efficacy to *U. ramanniana* and is particularly efficient at bio-catalysing hydrophobic compounds, of which PZQ is one, (Fickers, et al., 2005).

Culturing and biotransformation

Using a modified protocol derived from Parshikov, et al., 2004, triplicate cultures of fungal mycelia were cultured for 48 (h) in 125 ml of HiMedia, Yeast-Mold, (YM) broth medium. Cultures were incubated at 28°C with constant agitation, 180 rpm. Fungal cultures were exposed

to two experimental conditions. Initially, the optimal time courses were investigated in order to establish a baseline for the comparison of subsequent experimental runs with differing drug doses. After the initial 48 (h) of culturing 40mg/ml of PZQ, suspended in acetone, was added to the broth culture. Where upon cultures were left for a period of 5, 10, or 15 days before metabolite extraction. Secondly, dose comparison was carried out. After the initial 48 (h) of culturing either 40mg/ml, single dose, or 80mg/ml, double dose, of PZQ were added to the cultures; which were then left to metabolize for a number of days as dictated by the results of time courses.

Extraction and purification of metabolites

After metabolization the mycelia was filtered using a buchner funnel and filter paper, with a 20 to 25 μ m particle retention, washed with water and discarded. Metabolites were separated from the retained culture broth via extraction using three equal volumes of 100ml ethyl acetate, Fisher Scientific, EtOAc, and evaporated in vacuum, using a BUCHI rotavapor R II connected to a BUCHI V-700 Vacuum Pump. High-performance liquid chromatography was used to ascertain if, and how much, PZQ has been metabolized. The evaporated samples were dissolved in Acetonitrile and run through a Dionex UltiMate 3000 HPLC System for a total of 20 minutes. Broken down as a gradient starting at 20% acetonitrile in 2% acetic acid to 100% acetonitrile in 16 minutes, followed by 100% acetonitrile for 2 minutes, then reducing to 20% acetonitrile in the final 2 minutes. Injection of 10 μ L of samples were run through a PRP-1 guard column and a Waters X-terra RP₈ 3.5 μ m column. This reverse phase column holds onto the less polar molecules for a longer amount of time within the column. Therefore, in the chromatograms produced any peak occurring before that of PZQ is assumed to be indicative of a metabolite.

Quantification of HPLC

Four standard solutions of PZQ dissolved in acetone were prepared at 1mg/mL, 0.75 mg/mL, 0.5 mg/mL, and 0.1 mg/mL. These standards were run alongside the filtrate samples from the dose courses. During post analysis of chromatogram peaks area under the curve was calculated and compared to the standards and the amount of un-metabolized PZQ given.

Statistics

One-way t-tests were run to determine the effect of substrate concentration on overall metabolite production, using R version 3.5.2, to see the significance of the differences between the quantitative HPLC results of unmetabolized PZQ during the dose comparisons, and to ascertain if those differences were significantly greater or lesser.

Results

When compared to both the control of PZQ, Fig 1A, and each fungi strain without PZQ, Fig 1 B-D, HPLC chromatograms demonstrate that both *C. elegans* species and *U. ramanniana*, ATCC 9628, were capable of metabolizing the parent molecule of PZQ. However, the metabolic activity *Y. lipolytica*, ATCC 20225 was negligible. The subsidiary peaks, assumed to be metabolites, were considered insignificant when proportionally compared to the parental praziquantel peaks, Fig 2. Thus, once the time courses were conducted *Y. lipolytica* was retired in order to conserve resources for the other more efficient strains. Additionally, no control was investigated either.

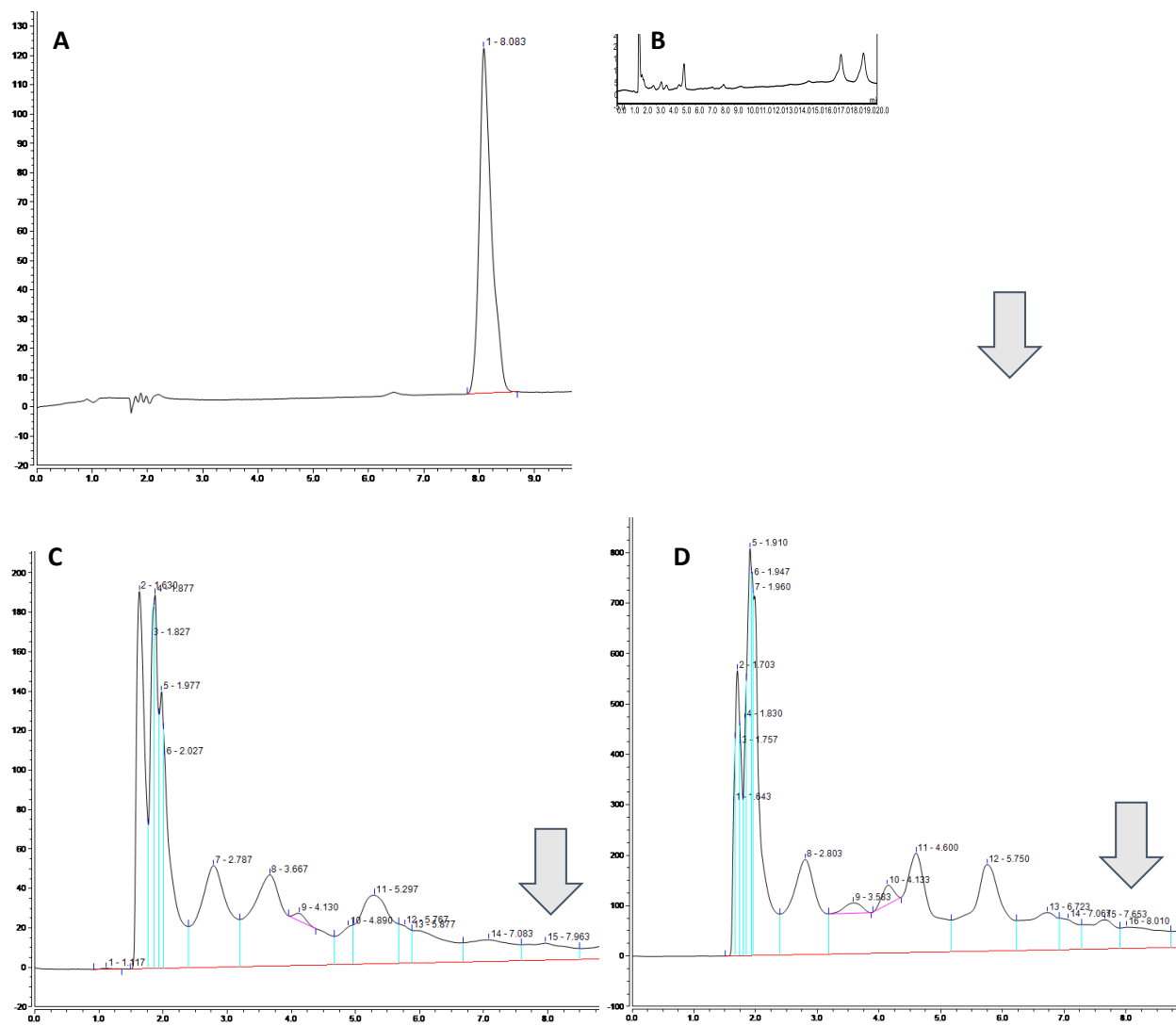


Figure 1: Chromatogram of controls A) Praziquantel, B) *C. elegans*, ATCC 8668a, C) *C. elegans*, ATCC 9245, D) *U. ramanniana*. Arrows indicate where PZQ would peak on the controls if present.

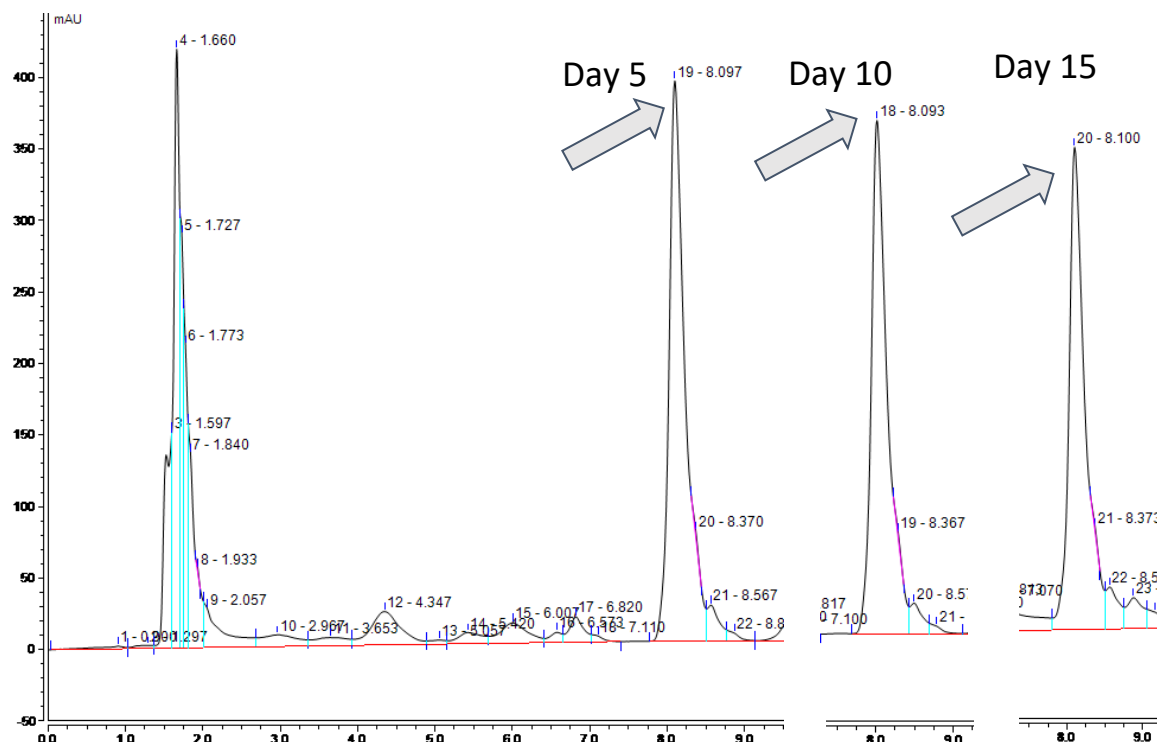


Figure 2: 5, 10, and 15 day chromatograms of *Y. lipolytica* cropped around the peak representing PZQ to show time course trends and comparison. Difference deemed negligible. Arrows indicate peak corresponding to unmetabolized PZQ.

Cunninghamella elegans, ATCC 8668a

HPLC chromatograms indicate that after ten days the peak indicating PZQ is near null, and on day 5 is considerably reduced, Fig 3. Thus, 1 days was identified as the most efficient metabolization time to yield maximum metabolites. Profiles of *C. elegans* ATCC 8668a show two major subsidiary peaks assumed to correspond to metabolites. These occur at 4.3 and 4.7 minutes, Fig 4, and are not seen in either of the controls, Fig 1A and 1B. comparing chromatograms from the dose courses, Fig 5, there was a significant difference, $p = 0.0063$, in the means of unmetabolized PZQ between single dose, 40mg/ml, and double dose, 80mg/ml,

groups. Double dose left an average of 0.4 mg/mL unmetabolized, compared to those cultures given a single dose, 0.9 mg/mL, Table 1.

Cunninghamella elegans, ATCC 9245

As with the previous strain, *C. elegans*, ATCC 9245, HPLC chromatogram profiles indicate two major subsidiary peaks also occurring at 4.3 and 4.7 minutes, Fig 6, and again are not seen in either of the controls, Fig 1A and 1C. Fifteen days was considered optimal with the peak indicating PZQ being considerably smaller when compared to the other days, Fig 7. When exposed to a double dose of PZQ the strain showed similar efficacy and metabolic activity producing the same metabolites as a single dose. Under both conditions the fungi left a similar mean of PZQ left unmetabolized, 0.299mg/mL for single dose and 0.195mg/mL for double, Table 1. Additionally, there are 3 further subsidiary peaks, at 5.8, 6.3 and 6.7 minutes, that become more pronounced at a double dose, when compared to the chromatogram of single dose, Fig 8. The statistics did show a significant variance between the means but not a significant difference, $p = 0.058$.

Umbelopsis ramanniana, ATCC 9628

U. ramanniana yielded similar results following the trends of both *C. elegans* strains. At a single dose of PZQ *U. ramanniana*, the peak for PZQ was reduced to the base line after 10 days, Fig 9, producing peaks assumed to be metabolites at 4.5 and 4.9 minutes, Fig 10. However, the fungi appeared to fail to respond to a double dose of PZQ, 11. The differences between the mean amount of unmetabolized PZQ between single dose and double dose groups was not shown to be significant, Table 1.

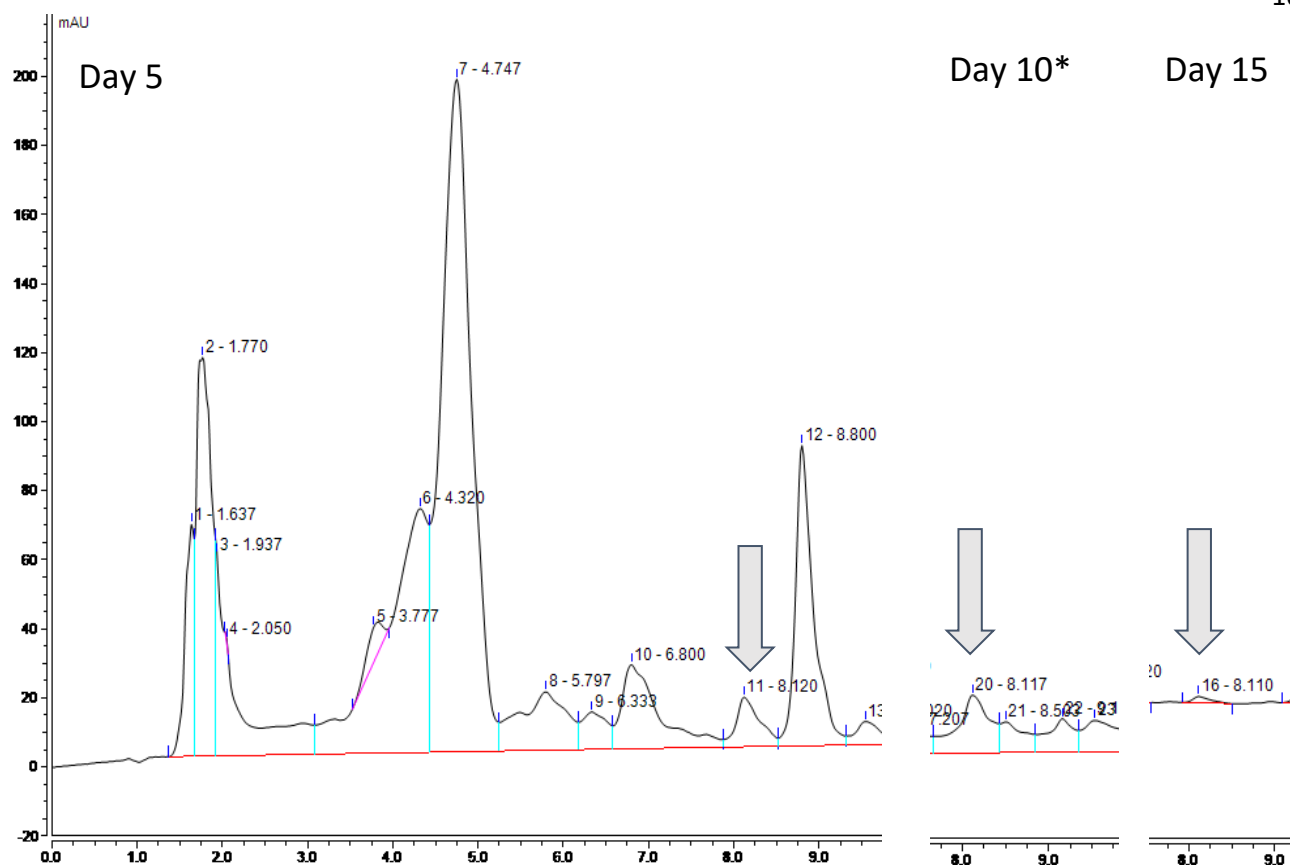


Figure 3: 5, 10, and 15 day chromatograms of *C. elegans*, ATCC 8668a, cropped around the peak representing PZQ to show time course trends and comparison. Day 10 shown to be optimal. Arrows indicate peak corresponding to unmetabolized PZQ

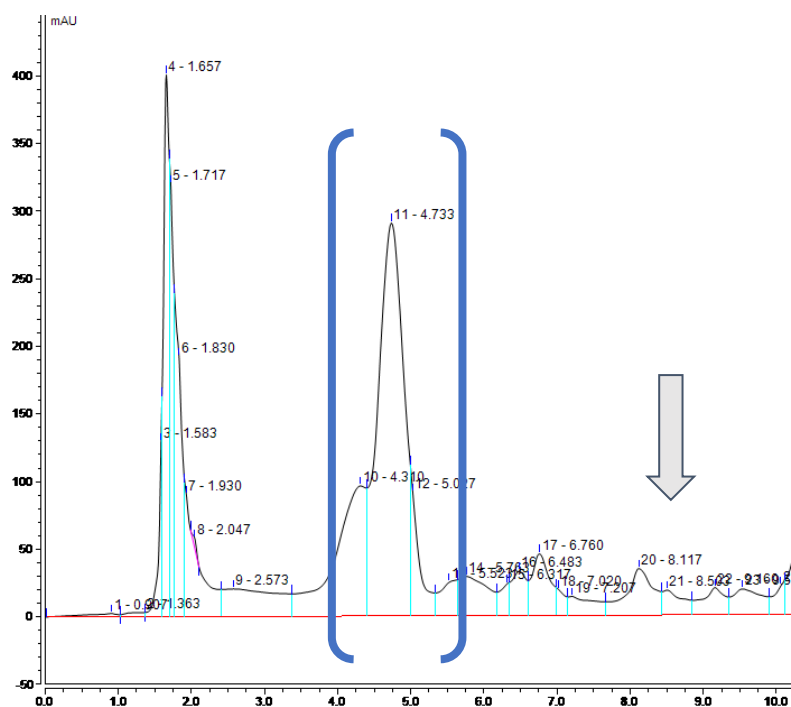


Figure 4: Full spectrum chromatogram for the metabolites produced after 10 days metabolism by *C. elegans*, ATCC 8668a. Arrows indicate peak corresponding to unmetabolized PZ, brackets surround major peaks assumed to indicate predominant metabolites, 4.3 and 4.7 minutes.

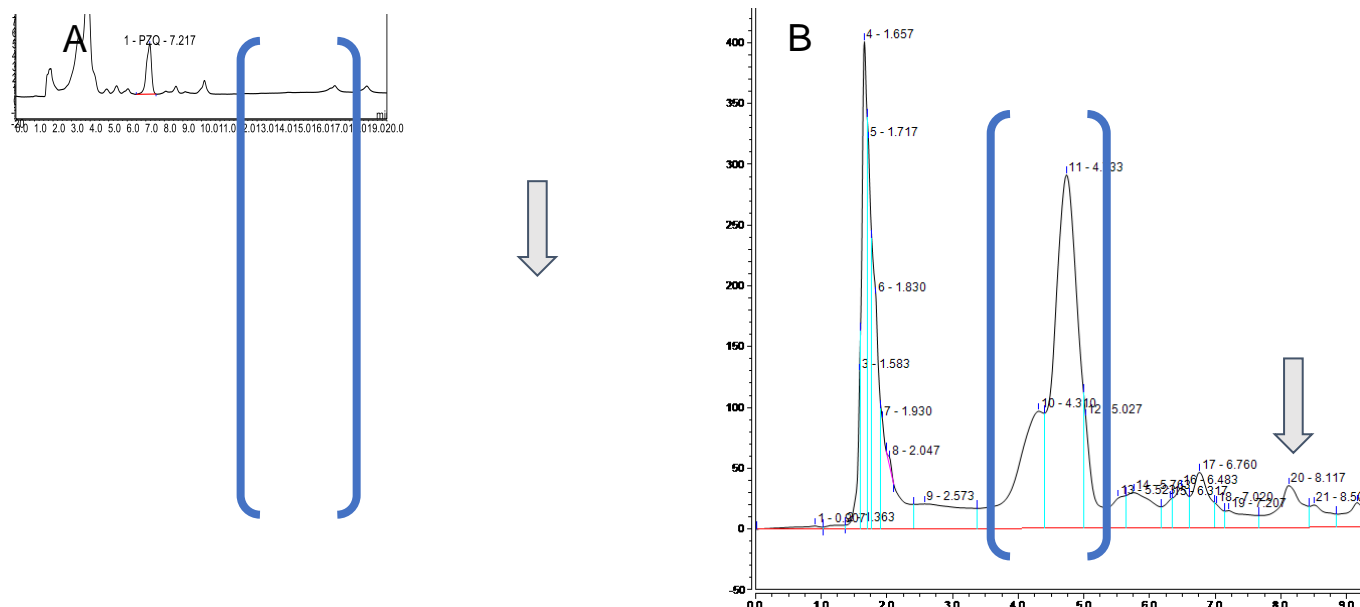


Figure 5: Chromatograms of the dose treatment for *C. elegans*, ATCC 8668a, A) double dose, 80mg/ml, B) single dose, 40mg/ml. Arrows indicate the peak representing the unmetabolized PZQ. The brackets highlight the area that the peaks assumed to be the major metabolites occur. Differing styles between chromatograms reflect analytical technique. A) tested strictly for PZQ, B) full spectrum of peaks.

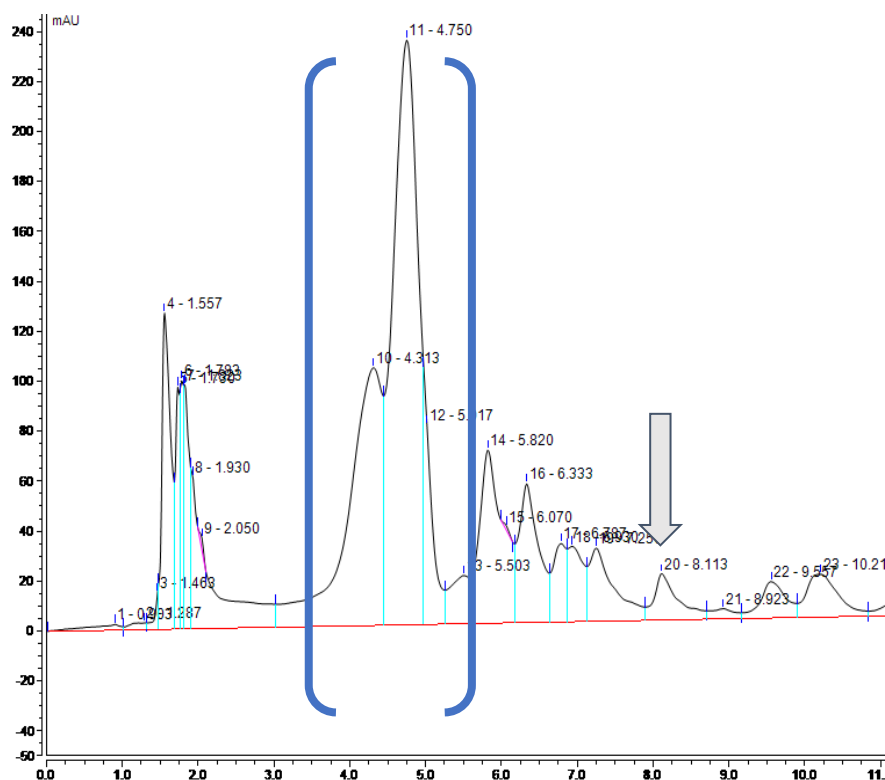


Figure 6: Full spectrum chromatogram for the metabolites produced after 15 days of metabolization by *C. elegans*, ATCC 9245. Arrows indicate peak corresponding to unmetabolized PZ, brackets surround major peaks assumed to indicate predominant metabolites, 4.3 and 4.7 minutes

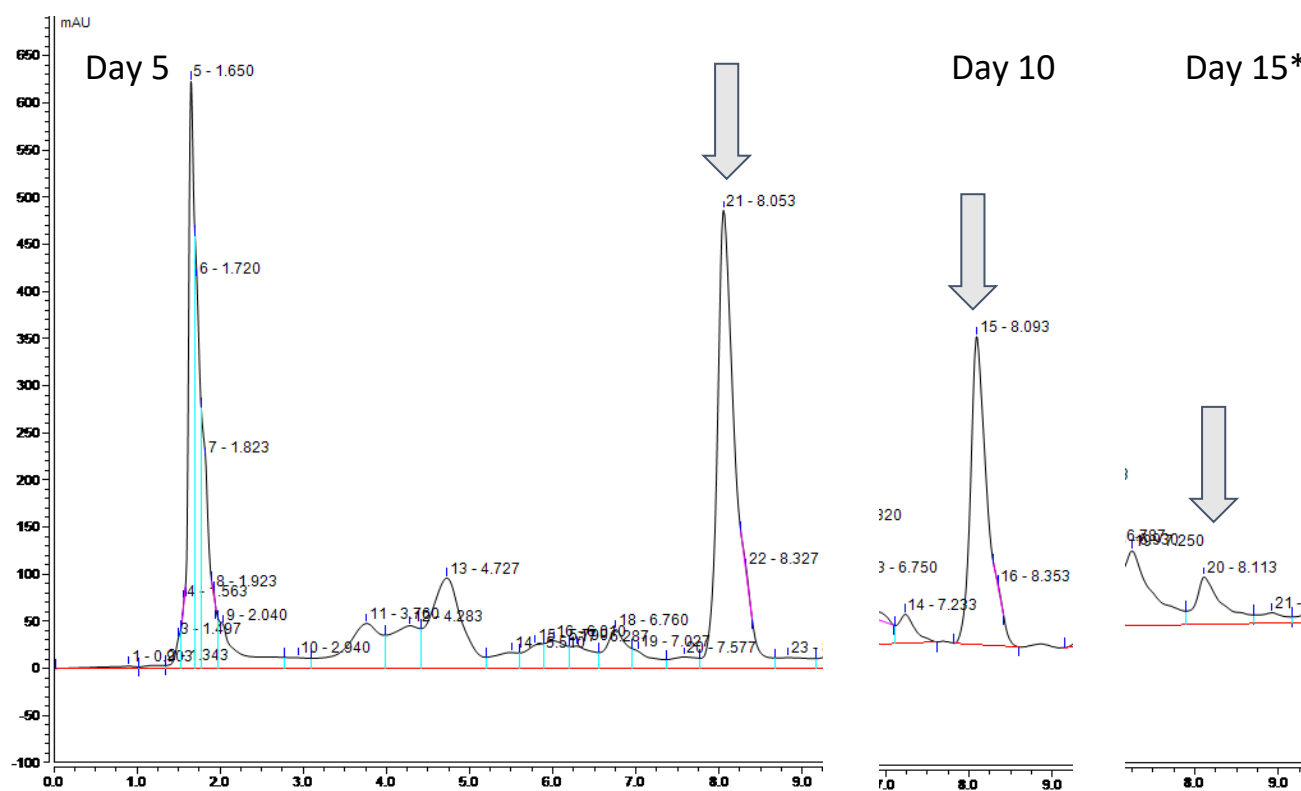


Figure 7: 5, 10, and 15 day chromatograms of *C. elegans*, ATCC 9245, cropped around the peak representing PZQ to show time course trends and comparison. Day 15 shown to be optimal. Arrows indicate peak corresponding to unmetabolized PZQ

Discussion

Both strains of *C. elegans* were able to fully degrade PZQ and produce metabolites. Cytochrome P450 enzymes are paramount to the metabolism of many medications including PZQ, (Lynch & Price, 2007). It is unanimous in mammals and found in many fungal strains such as *C. elegans*. Many investigations have demonstrated that humans, and more basal mammals such as rats, are able to metabolize PZQ, (Meier & Blaschke, 2001), therefore it was expected that the two *C. elegans* strains tested would also be able to metabolize PZQ.

Whilst both *C. elegans* strains produce major metabolites with the same peaks, 4.3 and 4.7 minutes, there are comparative differences in activity of the two strains. *C. elegans* ATC 8668a is able to fully metabolize PZQ in 10 days, in contrary to the 15 exhibited by *C. elegans*

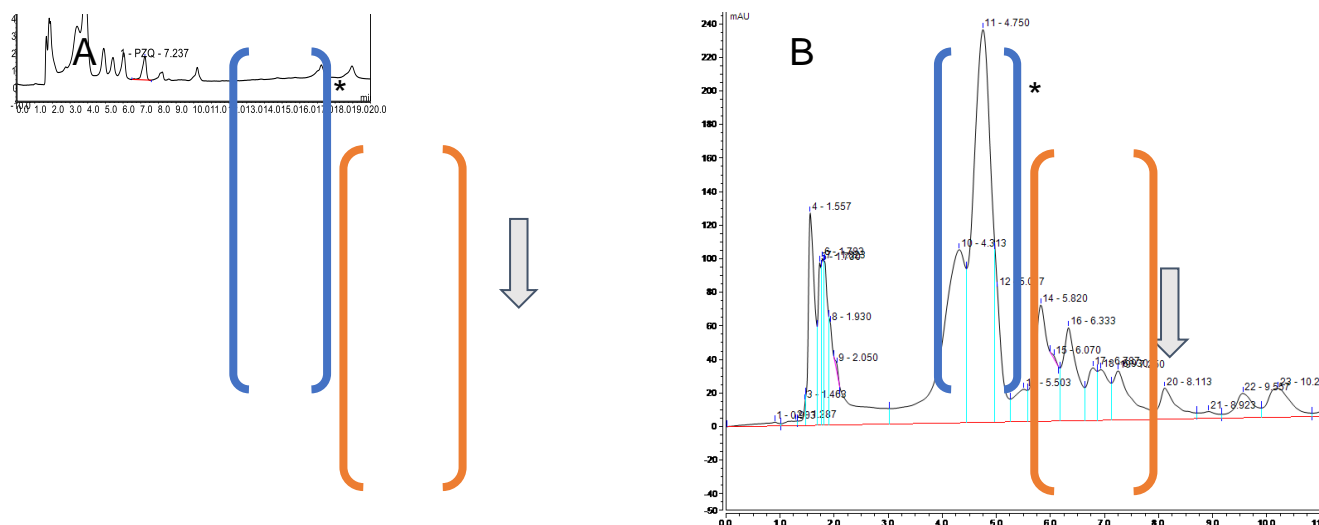


Figure 8: Chromatograms of the dose treatment for *C. elegans*, ATCC 9245, A) double dose, 80mg/ml, B) single dose, 40mg/ml. Arrows indicate the peak representing the unmetabolized PZQ. The brackets annotated with a *, highlight the area that the peaks assumed to be the major metabolites occur, the second set reflect the occurrence of a second set of metabolites occurring more prominently in A. Differing styles between chromatograms reflect analytical technique. A) tested strictly for PZQ, B) full spectrum of peaks.

ATC 9245. When considering the chromatograms of the time course for *C. elegans* ATC 9245 after 15 days the peak indicating PZQ is not completely flush to the base line. However, it is still deemed the optimal amount of time to allow for the most efficient metabolization. It is possible that leaving the strain for longer, such as 20 days, would yield a more complete metabolization of PZQ. However, when comparing peak height trends displayed by the chromatograms, Fig 7, that of day 15 is considerably smaller than the other two time points, specifically between day 10 and 15 where the greatest reduction is observed. Additionally, it is thought that the peak height is already nominal and that by allowing extra time for metabolism would serve to increase the overall timeline for metabolite production for minimal product gain. Therefore, in order to

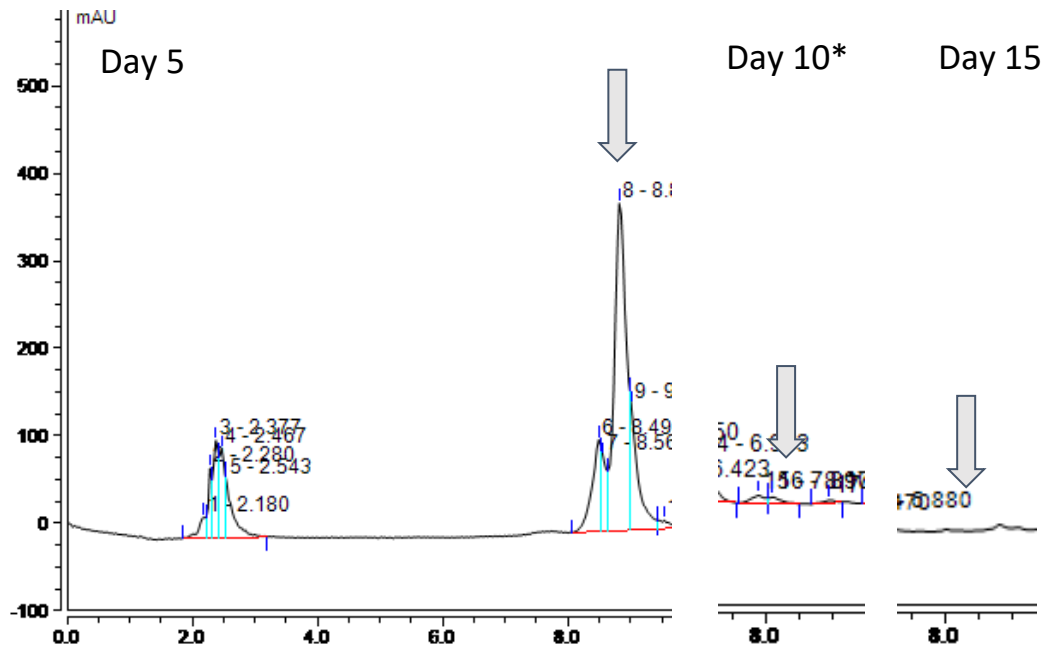


Figure 9: 5, 10, and 15 day chromatograms of *U. ramanniana*, cropped around the peak representing PZQ to show time course trends and comparison. Day 10 shown to be optimal. Arrows indicate peak corresponding to unmetabolized PZQ

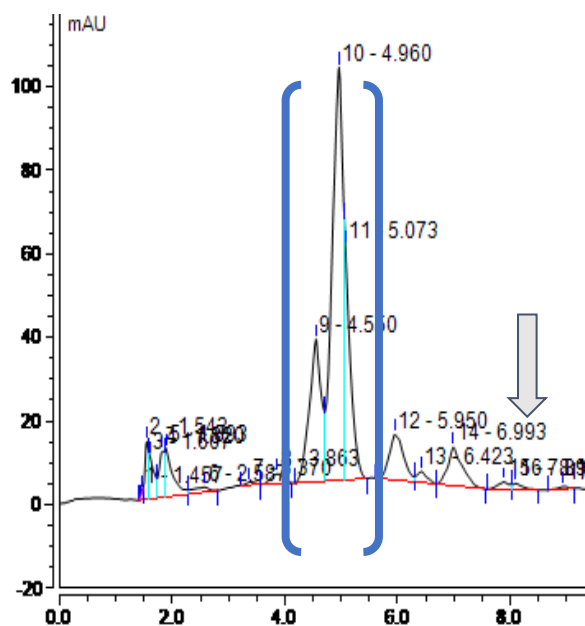


Figure 10: Full spectrum chromatogram for the metabolites produced after 15 days metabolization by *U. ramanniana*. Arrows indicate peak corresponding to unmetabolized PZ, brackets surround major peaks assumed to indicate predominant metabolites, 4.5 and 4.9 minutes

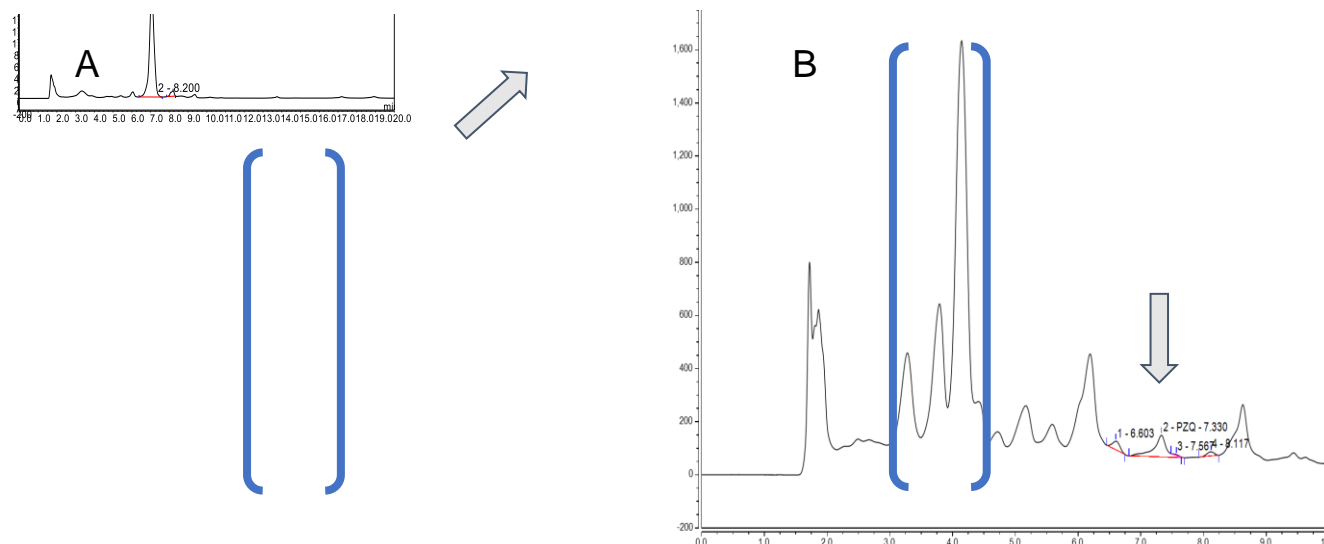


Figure 11: Chromatograms of the dose treatment for *C. elegans*, ATCC 8668a, A) double dose, 80mg/ml, B) single dose, 40mg/ml. Arrows indicate the peak representing the unmetabolized PZQ. The brackets highlight the area that the peaks assumed to be the major metabolites occur.

Table 1: Summary table of statistical analysis demonstrating the means between the single and double dose treatments of each fungi as well as the P value demonstrating significance of the differences

Fungal Strain	Single Dose	Double Dose	P-Value
Cunninghamella elegans, ATCC 8688a	0.973	0.44	0.006
Cunninghamella elegans, ATCC 9245	0.299	0.195	0.058
Umbelopsis ramanniana, ATCC 9628	0.404	3.857	Insignif.

preserve methodic efficiency 15 days was decided to be optimal. Another interesting observation was that occurrence of three additional subsidiary peaks on the chromatogram at double dose. Whilst these metabolite peaks are seen under single dose conditions, they are far more pronounced and distinct from the surrounding “noise” under double dose. This implies that these

peaks represent additional minor, in the terms of this study, metabolites. Moreover, proportionally these peaks appear to increase greater than the major metabolic peaks with an increase in dose. This may indicate that *C. elegans*, ATCC 9245, may be able to produce more metabolites than *C. elegans*, ATCC 8668a. This may mean that it produces more drug analogues which may make it the preferable *C. elegans* strain despite longer needed for optimal metabolization.

In the dose course assays, *C. elegans* ATCC 8668a shows a significant difference between the single and double doses administered. These differences suggest that at a double dose *C. elegans* ATCC 8668a is more efficient. Presenting a lower mean of unmetabolized PZQ suggests it is able to utilize and metabolize at a higher rate than at a single dose. This was an unexpected result; one possible explanation is the increased dose saturates the culture medium more efficiently than at a single dose allowing higher surface area contact between mycelia and drugs allowing them to metabolize a greater volume of PZQ. The means for *c. elegans*, ATCC 9245, were not shown to be significantly different and it can be concluded that, based on the similar means the averages of two groups are significantly similar. It is therefore likely that the strain is just as efficient at metabolizing a double dose of the drug solution as a single dose.

At a single dose of PZQ *U. ramanniana*, acted similar to *C. elegans*, ATCC 8668a, reducing PZQ effectively after ten days. This result was also expected as many biotransformation investigations use the two strains in comparative studies, usually yielding similar results. Additionally, although not explicitly studied, *U. ramanniana* is thought to contain the cytochrome P450 based on an study conducted by Antoniou, et al., 1994. The investigation studied azole inhibition concluded that this provides evidence that the hydroxylases are cytochromes P-450. Under a double dose of PZQ *U. ramanniana*, appeared to not produce any

metabolites or reduce the amount of PZQ. Additionally, the mean amount of PZQ unmetabolized was both higher than the single dose and higher than the original amount added to the cultures. Initially this appears to be an anomalous result. However, when comparing the chromatograms from the fungi to the control of PZQ the peaks representing PZQ from the fungi appear to be much wider, Fig 12. Doubling the dose of PZQ also includes doubling the volume of solvent added to the culture medium. This increase in acetone may not be conducive to organism growth and retard mycelial metabolism. Thus, yielding metabolites which do not represent a complete degradation of the parent model. Such metabolites maybe considered incomplete and not be distinct in their polarity form the original structure. Thus, do not differentiate themselves enough to form their own distinct HPLC peak. This would cause a false positive and the resulting chromatogram peak to widen and the analysis to calculate a greater amount of PZQ.

Previous experiments examining the biotransformation of PZQ have not directly used *Yarrowia lipolytica*, therefore it was unknown how it would react. *Yarrowia lipolytica* presents both oxygenases and hydroxylases, which along with other enzymes degrades hydrophobic substrates very efficiently, (Bankar, et al., 2009), and give the fungi great potential for the production of various biochemicals and intermediates, (Spagnuolo, et al., 2018). However, in this investigation *Y. lipolytica* was not able to significantly metabolize PZQ or produce metabolites; surprising given the hydrophobic nature of PZQ, (Mainardes & Evangelista, 2005). However, studies utilizing the strains often focus on the byproducts of metabolic processes rather than the metabolites. Those that are targeting the bio transformation or degradation of a compound often use lipids and other organic oils and fatty acids as targets, (Bankar, et al., 2009). It is therefore possible that the lack of success with PZQ is due to utilizing the fungi for the incorrect mechanisms and focusing on the wrong products. Additionally, investigations that have used *Y.*

lipolytica as a tool for screening its efficacy with certain compounds often cite that peak metabolic efficiency is correlated to specific medium parameters, particularly those that are nutrient limited. Most conclude that there is considerable selection of suitable strains with the desired properties needed to find the most suitable strain for the needs, (Kim, et al., 2007). Therefore, again it could be that despite its proven efficacy with other compounds and its popularity in industry the strain might have not been as appropriate for this study as initially thought.

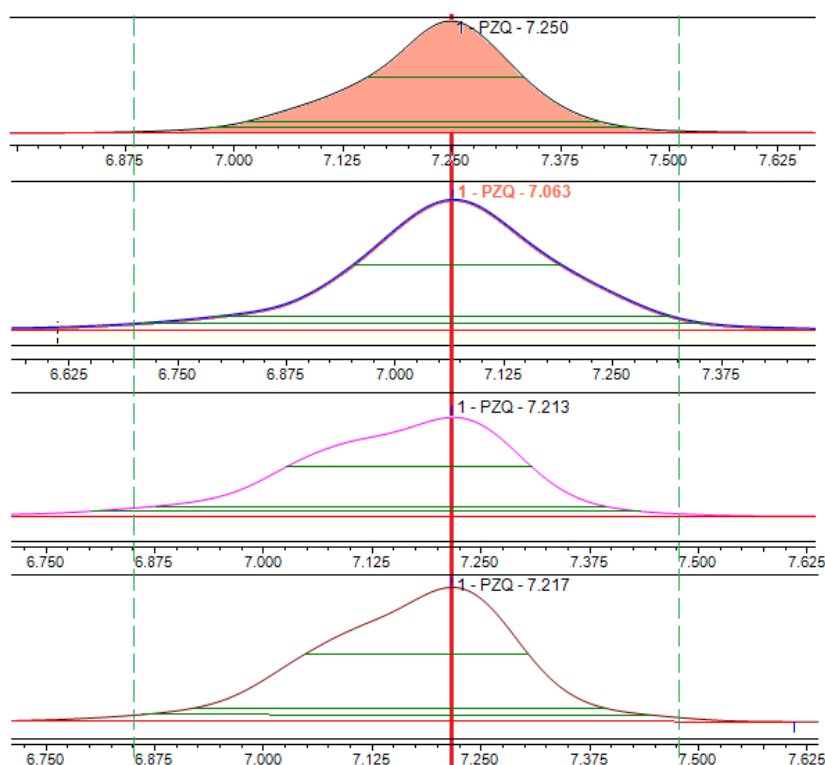


Figure 6: Comparison of PZQ peaks across dose treatments after 10 days of metabolism by *U. ramanniana*. Peaks aligned on solid red line by the maximum height of peaks. The top chromatogram indicates *U. ramanniana* dosed with 40mg/mL, the proceeding chromatograms were dosed with 80mg/mL. Dashed green lines indicate the upper and lower bounds of the PZQ peak at a single dose demonstrating the difference in widths between the different dose treatments.

Conclusion

The results of this study further provide evidence of fungal strains practicality in the biotransformation of compounds and drugs. In addition to providing a targeted methodology for specific strains when used in conjunction to PZQ; demonstrating the efficacy of both *C. elegans* and *U. ramanniana*. Those strains which were successful in their metabolization are thought to have introduced modifiable moiety increasing the opportunity for derivatization of the original structure. However, in order to verify this the chemical structures of the derivatives must be studied through further testing, such as NMR and mass spectrometry.

References

- Alsaqabi, S., & Lofty, W. (2014). Praziquantel: A Review. *Journal of Veterinary Science & Technology*, 5(200), Published online.
- Antoniou, T., Curran, B., & Smith, K. (1994). Is mRNA sequestration involved in the regulation of progesterone 14 α -hydroxylase cytochrome P-450 expression in *Mucor hiemalis*? . *Microbiology Society*, 1633-1640.
- Bankar, A., Kumar, A., & Zinjarde, S. (2009). Environmental and industrial applications of *Yarrowia lipolytica*. *Applied Microbiology and Biotechnology*, 84(5), 847-865.
- Bankar, A., Kumar, A., & Zinjarde, S. (2009). Environmental and industrial applications of *Yarrowia lipolytica*. *Appl Microbiol Biotechnol*, 847-865.
- Chitsulo, L., Engels, D., Montresor, A., & Savioli, L. (2000). The global status of schistosomiasis and its control. *Acta Trop*, 77(1), 41-51.
- Cioli, D., Pica-Mattoccia, L., & Archer, S. (1993). Drug resistance in schistosomes. *Parasitol Today*, 9, 162–166.
- Couto, F., Coelho, P., Araujo, N., Kusel, J., Katz, N., Jannotti-Passos, L., & Mattos, A. (2011). *Schistosoma mansoni*: a method for inducing resistance to praziquantel using infected *Biomphalaria glabrata* snails. *Memórias do Instituto Oswaldo Cruz*, 106(2), 153-157.
- Dube, A., & Kumar, M. (2017). Biotransformation of bromhexine by *Cunninghamella elegans*, *C. echinulata* and *C. blakesleeana*. *Brazilian Journal of Microbiology*, 48(2), 259-267.

- Fickers, P., Benetti, P., Wache, Y., Marty, A., Mauersberger, S., Smit, M., & Nicaud, J. (2005). Hydrophobic substrate utilisation by the yeast *Yarrowia lipolytica*, and its potential applications. *FEMS Yeast Research*, 5(6), 527-543.
- Gryseels, B., & Polderman, A. (1991). Morbidity, due to schistosomiasis mansoni, and its control in Subsaharan Africa. *Parasitol Today*, 7, 244-248.
- Gryseels, B., Polman, K., Clerinx, J., & Kestens, L. (2006). Human schistosomiasis. *Lancet*, 368(9541), 1106-18.
- Hotez, P., Alvarado, M., Basáñez, M., Bolliger, I., Bourne, R., Boussinesq, M., . . . Carabin, H. (2014). The global burden of disease study 2010: interpretation and implications for the neglected tropical diseases. *PLoS neglected tropical diseases*, 8(7), Published online.
- Huang, J., Bathena, S., & Alnouti, Y. (2015). Metabolite Profiling of Praziquantel and its Analogs During the Analysis of in vitro Metabolic Stability Using Information-Dependent Acquisition on a Hybrid Triple Quadrupole Linear Ion Trap Mass Spectrometer. *Drug Metab Pharmacokinet.*, 25(5), 487-499.
- Kang, S., Kang, S., & Hur, H. (2008). Identification of fungal metabolites of anticonvulsant drug carbamazepine. *Applied Microbiology Biotechnology*, 79(4), 663-669.
- Kim, J., Kang, S., Woo, J., Lee, J., Chul, B., & Kim, S. (2007). Screening and its potential application of lipolytic activity from a marine environment: characterization of a novel esterase from *Yarrowia lipolytica* CL180. *Applied Microbiology and Biotechnology*, 820-828.

- King, C., & Dangerfield-Cha, M. (2008). The unacknowledged impact of chronic schistosomiasis. *Chronic Illn*, 4, 65–79.
- King, C., Dickman, K., & Tisch, D. (2005). Reassessment of the cost of chronic helminthic infection: a meta-analysis of disability-related outcomes in endemic schistosomiasis. *Lancet*, 365(9470), 1561-1569.
- Lopez, A., Mathers, C., EzzatiM, Jamison, D., & Murray, C. (2006). *Global burden of disease and risk factors*. New York/Washington: Oxford University Press/The World Bank.
- Lynch, T., & Price, A. (2007). The Effect of Cytochrome P450 Metabolism on Drug Response, Interactions, and Adverse Effects. *Am Fam Physician*, 391-396.
- Mainardes, R., & Evangelista, R. (2005). PLGA nanoparticles containing praziquantel: effect of formulation variables on size distribution. *Int J Pharm*, 137-144.
- Meier, H., & Blaschke, G. (2001). Investigation of Praziquantel metabolism in isolated rat hepatocytes. *Journal of Pharmaceutical and Biomedical Analysis*, 409-415.
- Meister, I., Ingram-Sieber, K., Cowan, N., Todd, M., Robertson, M., Meli, C., . . . Keiser, J. (2014). Activity of praziquantel enantiomers and main metabolites against *Schistosoma mansoni*. *Antimicrobial Agents Chemotherapy*, 58(9), 5466-72.
- Moody, J., Freeman, J., Fu, P., & Cerniglia, C. (2002). Biotransformation of mirtazapine by *Cunninghamella elegans*. *Drug Metabolism Disposition*, 30(11), 1274-1279.
- Parshikov, A., Freeman, J., Lay Jr, J., Beger, R., Williams, A., & Sutherland, j. (1999). Regioselective transformation of ciprofloxacin to N-acetylciprofloxacin by the fungus *Mucor ramannianus*. *FEMS Microbiology Letters*, 177(1), 131-135.

- Parshikov, I., Freeman, J., Lay Jr, J., Beger, R., Williams, A., & Sutherland, J. (2000). Microbiological transformation of enrofloxacin by the fungus *Mucor ramannianus*. *Applied and Environmental Microbiology*, 66(6), 2664-2667.
- Parshikov, I., Netrusov, A., & Sutherland, J. (2012). Microbial transformation of azaarenes and potential uses in pharmaceutical synthesis. *Applied Microbiology and Biotechnology*, 95(4), 871-889.
- Pothuluri, J., Sutherland, J., Freeman, J., & Cerniglia, C. (1998). Fungal Biotransformation of 6-Nitrochrysene. *Applied and Environmental Microbiology*, 64(8), 3106-3109.
- Renganathan, E., & Cioli, D. (1998). An international initiative on praziquantel use. *Parasitol Today*, 14, 390-391.
- Ross, A., Bartley, P., Sleigh, A., Olds, G., Li, Y., Williams, G., & McManus, D. (2002). Schistosomiasis. *N Engl J Med*, 346, 1212–1220.
- Rui, L., Reardon, K., & Wood, T. (2005). Protein engineering of toluene ortho-monooxygenase of *Burkholderia cepacia* G4 for regiospecific hydroxylation of indole to form various indigoid compounds. *Applied Microbiology and Biotechnology*, 6(4), 422-429.
- Schepmann, D., & Blaschke, G. (2001). Isolation and identification of 8-hydroxypraziquantel as a metabolite of the antischistosomal drug praziquantel. *Journal of Pharmaceutical and Biomedical Analysis*, 5(6), 791-799.
- Spagnuolo, M., Hussain, M., Gambill, L., & Blenner, M. (2018). Alternative Substrate Metabolism in *Yarrowia lipolytica*. *Front Microbiol.*

- Steinmann, P., Keiser, J., Bos, R., Tanner, M., & Utzinger, J. (2006). Schistosomiasis and water resources development: systematic review, meta-analysis, and estimates of people at risk. *The Lancet*, *6*, 411-425.
- Sutherland, J., Cross, E., Heinze, T., Freeman, J., & Moody, J. (2005). Fungal biotransformation of benzo[f]quinoline, benzo[h]quinoline, and phenanthridine. *Applied Microbiology and Biotechnology*, *67*(3), 405-411.
- Tanaka, H., & Tsuji, M. (1997). From discovery to eradication of schistosomiasis in Japan: 1847–1996. *Int. J. Parasitol*, *27*, 1465-1480.
- Taylor, A., Roberts, K., Cao, X., Clark, N., Holloway, S., Donati, E., & Polcaro, C. (2017). Structural and enzymatic insights into species-specific resistance to schistosome parasite drug therapy. *Journal of Biological Chemistry*, *292*(27), Published online.
- Utzinger, J., Keiser, J., Shuhua, X., Tanner, M., & Singer, B. (2003). Combination chemotherapy of schistosomiasis in laboratory studies and clinical trials. *Antimicrob Agents Chemother*, *47*, 1487–1495.
- Vale, N., Gouveia, M., Rinaldi, G., Brindley, P., Gärtner, F., & Correia da Costa, J. (2017). Praziquantel for Schistosomiasis: Single-Drug Metabolism Revisited, Mode of Action, and Resistance. *Antimicrobial Agents Chemotherapy*, *61*(5), Published online.
- Valentim, C., Cioli, D., Chevalier, F., Cao, X., Taylor, A., & Holloway, S. (2013). Genetic and Molecular Basis of Drug Resistance and Species-Specific Drug Action in Schistosome Parasites. *Science*, *342*(6164), 1385-1389.

- Wang, W., Wang, L., & Liang, Y. (2012). Susceptibility or resistance of praziquantel in human schistosomiasis: a review. *Parasitology Research*, *111*(5), 1871-1877.
- Weerakoon, K., Gobert, G., & McManus, D. (2015). Advances in the Diagnosis of Human Schistosomiasis. *Clin Microbiol Rev*, *28*(4), 939-967.
- WHO Expert Committee. (2002). *Prevention and control of schistosomiasis and soil-transmitted helminthiasis*. . Geneva: World Health Organisation.
- Woelfle, M., Seerden, J., de Gooijer, J., Pouwer, K., Oliaro, P., & Todd, M. (2011). Resolution of Praziquantel. *PLoS Neglected Tropical Disease*, *5*(9), Published online.
- World Health Organisation. (1996). *Schistosomiasis Fact Sheet*.
<http://www.who.int/mediacentre/factsheets/fs115/en/>.
- Zhang, D., Freeman, J., Sutherland, J., Walker, A., Yang, Y., & Cerniglia, C. (1996). Biotransformation of chlorpromazine and methdilazine by *Cunninghamella elegans*. *Applied and Environmental Microbiology*, *62*(3), 798-803.
- Zhang, W., & Wong, C. (2003). Evaluation of the 1992–1999 world bank schistosomiasis control project in China. *Acta Trop*, *85*, 303–313.