The Use Of ^{1}H NMR Metabolic Profiling In The Differentiation Of Clinical And Environmental Isolates of *Acanthamoeba*

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Introduction

Acanthamoeba are opportunistic protozoan pathogens that can cause a sight-threathening keratitis in healthy individuals and granulomatous amoebic encephalitis in immunocompromised individuals.[1] Early diagnosis of pathogenic *Acanthamoeba* is a pre-requisite in the successful treatment of *Acanthamoeba* infections. Current methods of distinguishing between pathogenic and non-pathogenic isolates involve *in vitro* assays using human cells and *in vivo* vertebrate models, which are cumbersome, time-consuming and lack sensitivity. Here we employ ^{1}H NMR spectroscopy to metabolically profile extracts of clinical and environmental isolates of *Acanthamoeba* and to use principal components analysis (PCA) software to compare resulting profiles.

Materials and Methods

Cultures of parasites: The following *Acanthamoeba* isolates were used; (i) a clinical isolate of *Acanthamoeba* (T4 genotype), isolated from a keratitis patient (American Type Culture Collection, ATCC 50492), (ii) a clinical isolate of *Acanthamoeba* (T1 genotype), isolated from an encephalitis patient (ATCC 50494), and (iii) an environmental isolate of *Acanthamoeba* (T7 genotype), isolated from soil (ATCC 30137). All amoebae isolates were grown without shaking in PYG medium [15ml, proteose peptone 0.75% (w/v), yeast extract 0.75% (w/v) and glucose 1.5% (w/v)] in T-75 tissue culture flasks at 30°C as previously described.[2] In addition, clinical and non-clinical isolates of *Escherichia coli* (a CSF isolate, O18:K1:H7 from meningitis patient and a K12 laboratory strain, HB101) were studied. Washed cells were homogenised, centrifuged and the supernatan lyophilised and reconstituted in 0.750 ml D_2O with sodium trimethylsilylproprionate-d_4 as an internal chemical shift reference and quantification standard. The solutions were placed into 5mm o.d. NMR tubes for NMR.

NMR spectroscopy: NMR spectra were obtained on an 11.74T Varian Unity Plus operating at 500 MHz for protons. Spectra were collected into 32k data points with a sweep width of 6 kHz, a pulse width of 45° and at a temperature of 25°C. The acquisition time was 2.73 s and a relaxation delay of 2.5 s was employed. The residual water resonance was attenuated by irradiation at the water frequency during the relaxation delay. FID's were zero filled to 64k and an exponential line broadening of 0.2 Hz applied prior to Fourier transformation. Spectrum intensities were divided into 213 buckets of 0.04 ppm width covering a range of -0.02 - 8.50ppm. Principal Components Analysis (PCA) was carried out using the software package SIMCA (Umetrics Ltd, Windsor, UK).

Results and Discussion

Typical ^{1}H NMR spectra of *Acanthamoeba* extracts are shown in Figure 1. It is evident that clinical isolates of *Acanthamoeba*, i.e., T1 and T4 genotypes exhibit distinct NMR spectra compared with that from non-pathogenic environmental isolate, i.e., T7 genotype (Figure 1), resulting from differences in metabolism between the genotypes. Also it is interesting to note that both the encephalitis (T1) and keratitis isolates (T4) exhibit differences in their metabolic profiles (data not shown). The data from the bucketed ^{1}H MR metabolic profiles of both *Acanthamoeba* and *Escherichia coli* were analysed using PCA to produce a scatter plot showing the metabolome of all the bacterial isolates (Figure 2).

Metabonomic profiling has been extensively used for evaluating physiological differences in organisms[3] with pattern recognition methods facilitating the comparison of information-rich spectra. Figure 2 shows that there is a clear separation between the metabolic profiles of the T1, T4 and T7 genotypes. It is also interesting to note that the NMR spectra for the invasive and non-invasive *E. coli* bacterial isolates, K1 *versus* K12 are also separated from *Acanthamoeba* but overlap each other.

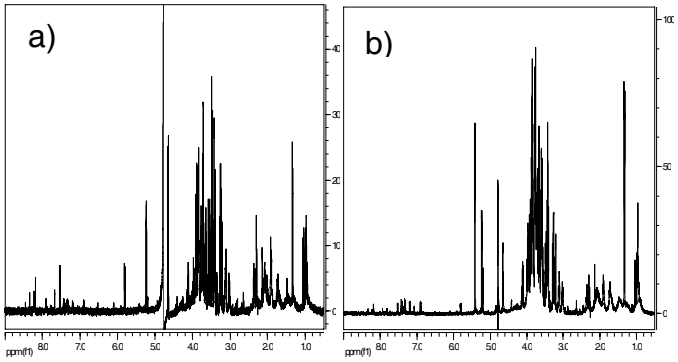

Figure 1. ^{1}H NMR spectra of a) T4 pathogenic and b) T7 non-pathogenic *Acanthamoeba* genotypes.

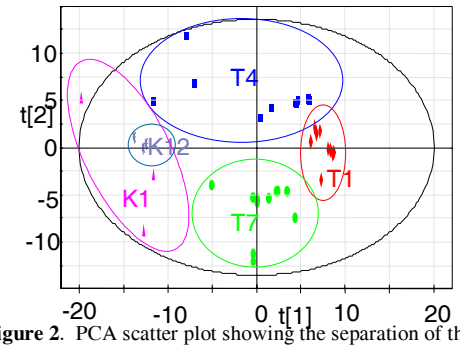

Figure 2. PCA scatter plot showing the separation of the T1, T4 and T7 *Acanthamoeba* genotypes. There is also a clear separation for *E. coli* genotypes K1 and K12. Hotelling T^2 with a 95% confidence interval is presented as an ellipse

Conclusions

This is the first report demonstrating the application of ^{1}H NMR metabolic profiling in the differentiation of pathogenic *Acanthamoeba* genotypes (and from *Escherichia coli)*. This chemometrics approach to metabolic profiling, enables the discernment of pathogenic from non-pathogenic genotypes, enabling the development of a rapid diagnostic assay for assessment of pathogenicity.

References

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