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Predatory Profile of Myxobacteria Against Clinically Relevant Organisms

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Introduction

Abuse and overt use of antibiotics have resulted in multidrug resistance among pathogens which has troubled the medical world today. A renewed interest in novel antimicrobial agents has emerged within the research community which manifests from chemically modifying existing antibiotics to isolating natural compounds from potential producer organisms. Myxobacteria are predatory soil microbes studied extensively for their potential to produce natural products which can kill bacteria, fungi, viruses and parasites. Pharmaceutical researchers have isolated hundreds of myxobacterial secondary metabolites with potent antimicrobial properties, however very few enter even into pre-clinical trials. This is attributed to the technical difficulties in propagation of these organisms, the unstable nature of the isolated compounds and more importantly, lack of knowledge on their mechanisms of action. Therefore we employed a holistic approach of studying the predatory activity of 113 myxobacteria isolated from soil samples, against 10 clinically relevant organisms, to analyse their prey range.

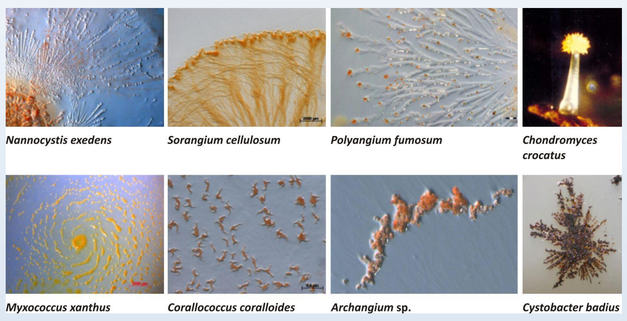


Fig.1. Colonies (swarming growth and fruiting bodies) of Myxobacteria

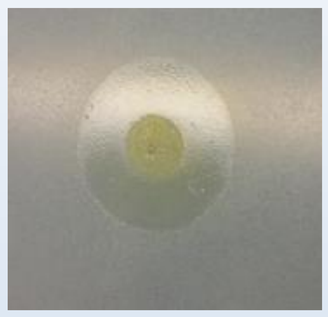


Fig.2. Myxobacteria showing a zone of predation on a prey lawn

Methods

Soil Sampling:

Soil samples from various habitats including woodlands, gardens, farmlands, streams and open fields were collected from the Aberystwyth and Carmarthen areas in West Wales. *E.coli* bait and filter paper methods of isolation were employed. Suspicious growth of myxobacteria typically seen as swarming growth or fruiting bodies (Fig.1) was purified for further identification and predation assays.

16SrRNA Sequencing and Analysis:

16S rRNA sequencing was performed using F27 and R1389 primers and the PCR products were sequenced. The assembled contigs were identified using the EzTaxon database and phylogenetic trees were constructed using MEGA 7 (Fig.5).

Predation Assay:

A lawn culture method was employed in this assay. 10 prey organisms (Fig. 3) were grown in Luria Bertani (LB) broth and centrifuged. The washed pellet was spread onto a WAT (non nutrient water agar) agar plate to form a uniform lawn. Myxobacterial isolates were grown in AMB broth and centrifuged. 10µl of the cell pellet was spotted onto the prey lawn and incubated. The diameter of the zone of swarming was recorded on day 4 as a measure of predatory activity (Fig2). Predatory activity data for the 10 prey organisms were clustered using the hierarchal clustering method in R (Fig.6).

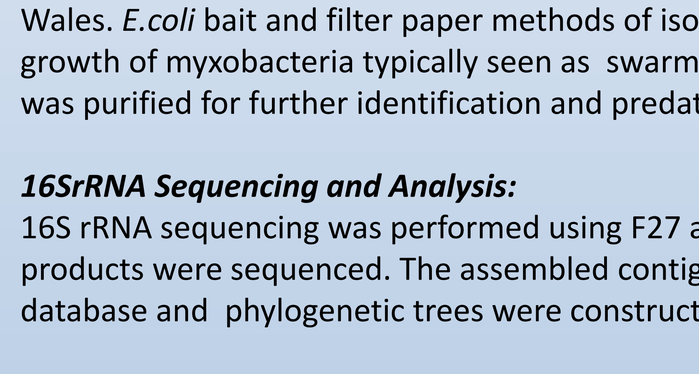


Fig.3. Box and whisker plots illustrating the predatory activity of Myxobacteria for the 10 prey organisms

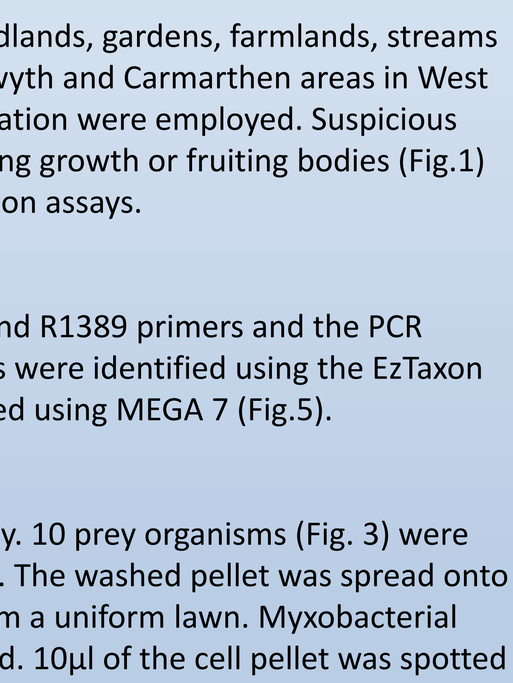


Fig.4. Cluster tree of the prey organisms according their susceptibility profile

Fig.5. 16S rRNA based Phylogenetic Tree showing 6 clusters

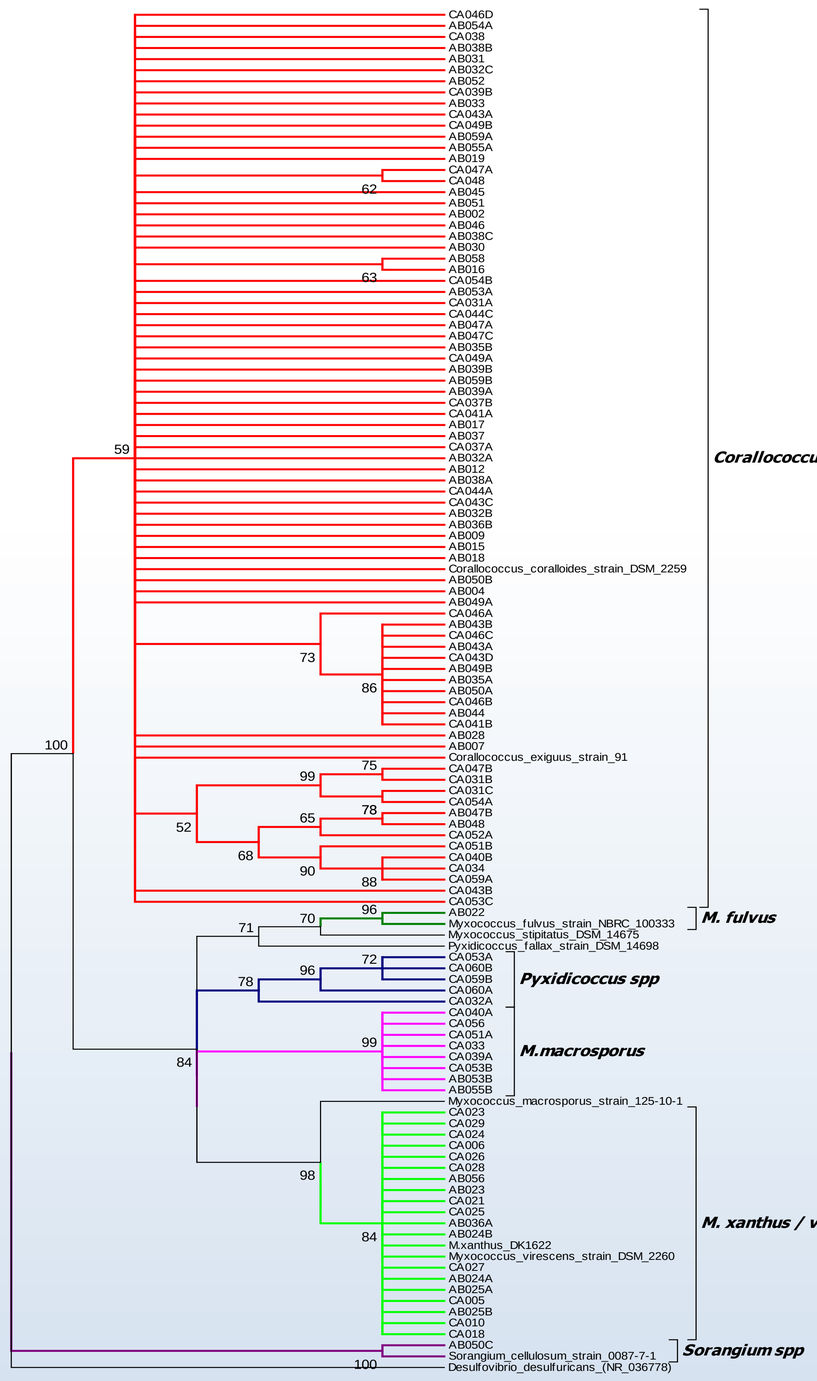
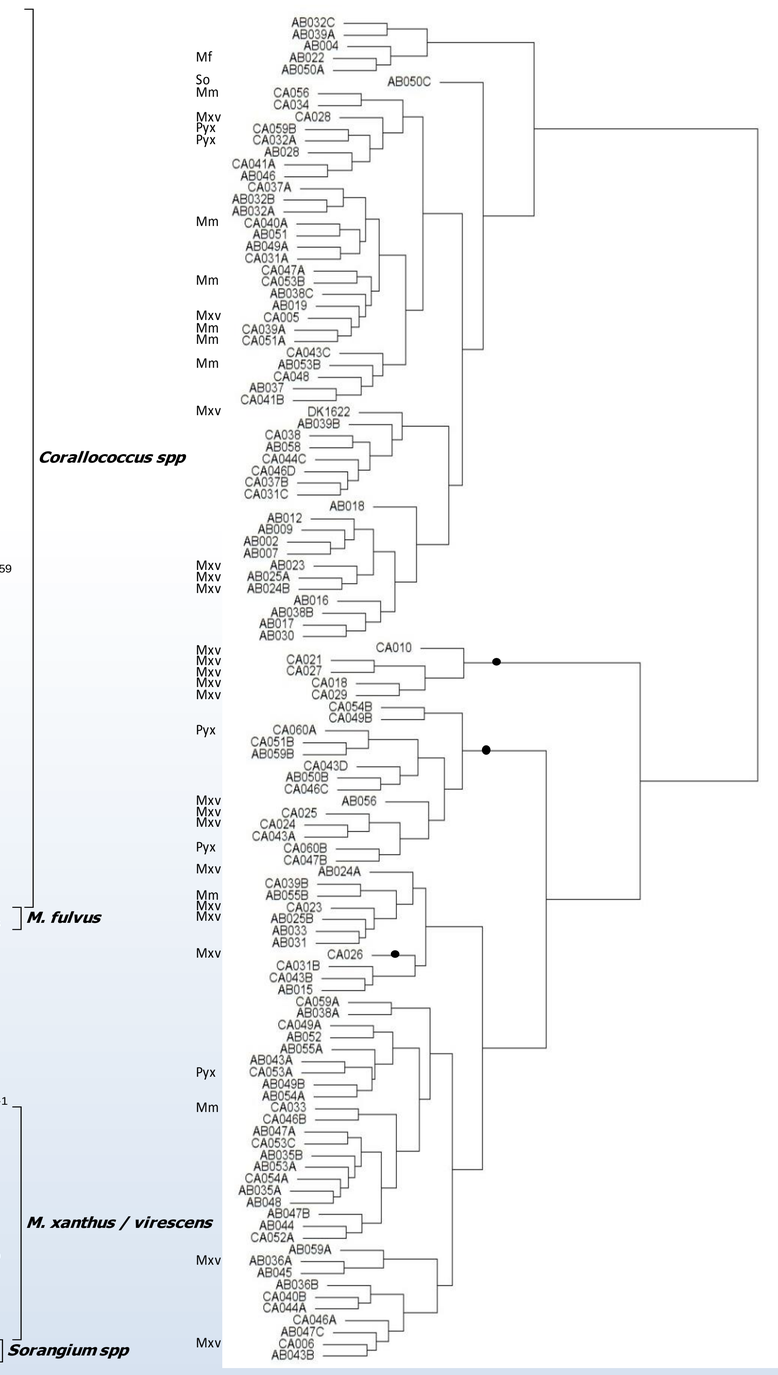


Fig.6. Predation Tree exhibiting good, moderate and poor groups



Results and Discussion

Novel Welsh Isolates

113 myxobacterial strains were isolated from 77 soil samples from Carmarthen and Aberystwyth areas. *Corallococcus spp.* were predominant (70%) followed by *Myxococcus spp.* (24%), *Pixicoccus spp.* (5%) and *Sorangium spp.* (1%).

Phylogenetic Clusters

Myxobacterial strains were grouped into 6 distinct phylogenetic clusters (Fig. 5) which were in accordance with EZTaxon assignments.

Broad Range of Predatory Activities

Cluster analysis (Fig. 6) grouped the isolates into 3 broad groups – good, moderate and poor predators. *K. pneumoniae, E. coli and P. mirabilis* were the best prey organisms while *P. aeruginosa*, *S. aureus, S. epidermidis* and *S. saprophyticus* were poor preys (Fig. 4).

Relationship Between Phylogeny and Predation

There was only partial congruence between phylogeny and predation from both the predators' and prey's perspective. This suggests that predation is mechanistically influenced by transferable genetic factors than a constitutive trait.

Conclusion

The novel isolates exhibited a broad predatory activity against clinically relevant organisms which can be explored for antibiotic discovery. Studying the predatory range of these novel organisms will also open doors in exploiting alternative therapeutic options against pathogenic organisms using the live organisms. Also, this study paves the way for a better understanding of predatory mechanisms, through genome wides studies which we are pursuing at the moment.

References

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