Discovery of novel enzymes with potential of antimicrobialactivity

Mtimka S.[1,2], Kwezi L[1], Pooe O.J[.2] and Tsekoa T.L.[1]

[1]Technology Demonstration Group, Biomanufacturing Technologies, Future Production: ChemicalsCluster, Council for Scientific and Industrial Research, P O Box 395, Pretoria 0001, South Africa

[2]Discipline of Biochemistry, School of Life Sciences, University of KwaZulu-Natal, Private Bag X54001,Durban 4000, South Africa

Over the past 20 years, the rise and spread of bacterial resistance to known and existing antibiotics has increased at an alarming rate. Further compounded by the misuse of antibiotics, this has resulted in an increased global challenge. Finding novel alternatives that can be used to disrupt the increase in antibiotic resistance is crucial. A number of alternatives have been identified and these include phage lytic proteins amongst others. Phage lytic proteins are enzymes that have the ability to degrade bacterial cell wall components. These enzymes are classified as; endolysins and virion-associated peptidoglycan hydrolases (VAPGHs). Indigenous microbial hotspots are a treasure trove for novel function discovery, soil sample from Kwa-Zulu Natal region was sampled and library constructed with novel genes. The gene bank with sequences was analysed with the aid of bioinformatics tools such as BioEdit and NCBI BLAST. Endolysins are a class of lytic protein, with the aid of metagenomics (sequence-based) function, a search revealed a number of endolysins relatedgenes and based on homology studies and sequence identity, and four genes were selected for further studies. The genes were synthesised and cloned and recombinantly expressed asa histidine fusion protein. All selected genes (designated Lytic1, Lytic2, Lystic3 and Lytic4) were produced in *Escherichia coli* BL21-(AI) cells and were successfully purified by immobilized metal affinity chromatography (IMAC). The same target proteins were also successfully transiently expressed in *Nicotiana. benthamiana.* The use of phage lytic proteins against bacterial pathogens is advantageous as they are highly specific towards the target and do not have noted adverse effects on the host. Preliminary biological activity revealed some of the lytic proteins to have some antimicrobial activity against *Pseudomonas aeruginosa*. Discovering endolysins can be channelled back into combating drug resistance is crucial for future containment of infections with resistance.

Keywords: Antibiotic resistance, Endolysin, Enzybiotics, Phage Lytic Protein