Cloning and sequencing of *Burkholderia cepacia* genes encoding esterase and lipase activities

Rashamuse, K and Cowan, D.

Department of Biotechnology,University of the western cape, Bellville,7535,CapeTown, South Africa.

Ferulic acid (FA) is a hydroxycinnamic acid present at relatively high concentrations in the cell walls of several plants. This molecule is of academic and industrial importance, because of its antioxidant properties and as a substrate for the synthesis of pharmaceutical products. However, FA is typically found covalently linked to ethyl groups (ethyl ferulate) and polysaccharides (feruloylated oligosaccharides) via ester bonds. The process for extraction of FA therefore requires the cleaving of the ester bonds.

We have screened and identified several microbial isolates with the ability to grow on phenolic ester ethyl ferulate (EF) as a sole carbon source. One isolate *Burkholderia cepacia* was selected on the basis of EF hydrolysis. A shotgun library of B. cepacia genomic DNA (prepared in *E. coli*/pUC18) was screened for lipase and esterase activities. Three positive clones were identfied and the inserts sequenced. Open reading frames (ORFs) from all three inserts showed classical GXSXG alpha/beta hydrolase motifs. A BLAST (blastp) search with clone pTEND5 ORF showed significant homology to *Ralstonia mannitolilytica* esterase and family S33 unassigned peptidase of *Burkholderia pseudomallei*, while pRASH23 ORF showed high homology to probable acyltransferase of *Burkholderia fungorum*. Clone pRASH14 ORF showed high homogy to *Pseudomonas cepacia* lipase and its chaperone. All these ORFs are currently being expressed in *E. coli* for further charasterisation.

Key words: Gene library; esterases; lipases; ferulic acid;