The haloacid operon of *Burkholderia sp.* MBA4 is catabolically repressed

J. S. H. Tsang, H. F. Yuen and K. F. Kong

School of Biological Sciences, The University of Hong Kong, Pokfulam Road, Hong Kong SAR, China

Degradation of haloacids such as monochloroacetate (MCA) in *Burkholderia sp.* MBA4 is mediated by the expression of a haloacid operon producing a dehalogenase (Deh4a) and an associated permease. When MBA4 was cultivated in a defined medium containing succinate and MCA a biphasic growth pattern was demonstrated. The release of chloride was witnessed apparently after succinate was depleted (Fig. 1).

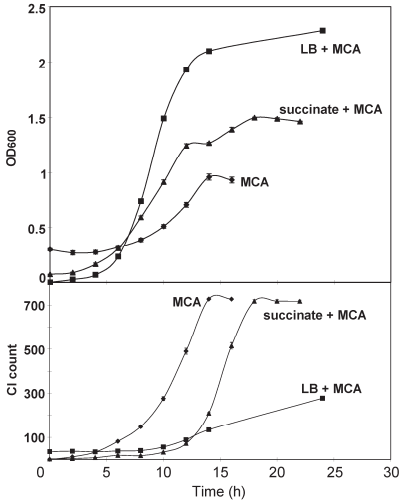

Fig. 1. Growth and release of chloride from chloroacetate by *Burkholderia sp.* MBA4. Growth was monitored by a spectrophotometer and chloride amount was determined by a chloride counter. MCA, chloroacetate, LB, Luria-Bertani broth.

Quantitative reverse-transcriptase PCR was used to quantify the expression levels of the dehalogenase gene (*deh4a*). When MCA was the only carbon source and the expression level of *deh4a* was treated as 100, then the relative expressions were 16, 42, 40 and 23%, respectively, when Luria-Bertani broth without sodium chloride (LB), succinate, pyruvate and glucose, were supplemented in the medium (Fig. 2). This showed that the haloacid operon was catabolically repressed.

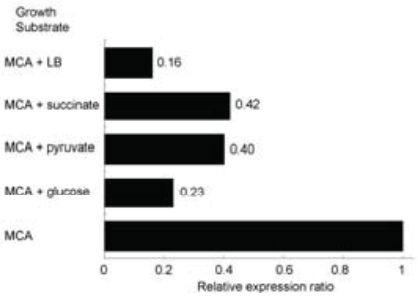

Fig. 2. Relative expression levels of dehalogenase *deh4a* in various substrates. Quantitative RT-PCR was used to determine the transcript levels. The level in cells grown in MCA was used as 1.

A transposon carrying plasmid, pOT182, was conjugated from *E. coli* to MBA4 to generate a library with randomly disrupted genes. Transconjugants were isolated for relieved degradation of MCA in LB^- by a colorimetric method. A transconjugant, 131M04, was found to express *deh4a* to 42% of the MCA-induced level. The DNA sequence of the disrupted gene was determined and was found to encode for a putative branched-chain amino acid (BCA) transporter of 638 residues with two bacterial-protein-dependent-transport-2 domains. Cloning and expression of this gene in *E. coli*, strain B7634, defective in BCA transport systems, helped the cell to grow in medium with low isoleucine concentration. When leucine, isoleucine and valine were supplemented to defined medium containing MCA, the expression of *deh4a* in MBA4 was repressed 50%. Under similar growth conditions, the expression level of mutant 131M04 was relieved to 80% of the induced level. These results indicated that the expression of the haloacid operon was catabolically repressed and BCA is one of the inhibitors.

Keywords Burkholderia; catabolite repression; amino acid