Development Of A Recombinant Organism For Production Of 2-Keto-L-Gulonic Acid

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Since humans and some animals lack l-gulono-¡ -lactone oxidase, the key oxidising enzyme in liver for L-ascorbic acid biosynthesis, they have to consume vitamin C from exogenous source. The main symptom of L-ascorbic acid deficiency, mainly due to a dietary lack of fresh fruit and vegetables are general weakness, fatigue and restlessness followed by a shortage of breath and aching in the bones. As the illness known from ancient times as scurvy-progresses, the skin becomes dry and rough followed by a swelling of the gums, easy bleeding and loss of teeth. The synthesis of L-ascorbic acid (Vitamin C) has received considerable attention over many years due to its relatively large market volume and high value as a specialty chemical. At present most Vitamin C is produced by a modification of the Reichstein-Grussner synthesis, first developed in 1934. The process is a somewhat lengthy and capital intensive route. The use of microbial processes to produce L-ascorbic acid has recently become attractive, because plant investment costs are lowered and ecological problems of organic solvent disposal is eliminated.

The goal of the present study was towards the development of a recombinant organism capable of producing increased amounts of 2-KLG in a single fermentation step. In light of the above, the present investigation was focussed on identification, cloning and characterization of the genes of sorbitol metabolism pathway, which lead to the biosynthesis of 2-KLG in *Erwinia herbicola* ATCC 21998.

During the present investigation we were successful to clone and partially characterise Sorbitol permease and Sorbitol dehydrogenase genes, the two most important key enzymes of this pathway. Both the genes were found to be located on the chromosomal DNA and not plasmid borne unlike glucose dehydrogenase gene which has been reported as plasmid borne in this organism by Koul et al. 1995. The sorbitol dehydrogenase in this organism seems to be cytoplasmic and was found to be NAD^{+} / $NADP^{+}$ dependent unlike the glucose dehydrogenase which is membrane bound

and NAD^+ /$NADP^+$ independent (Koul et al. 1995). Besides this an efficient method of electro-transformation was developed in this organism. With the help of this electro-transformation protocol a mutation strategy based on homologous recombination insertion was developed which proved a successful tool for raising various gene specific mutations/auxotrophs in this commercially important microorganism. During the present study we were able to raise three different mutants Ehpvq1, Ehpvq2 and Ehpvq3. The first two are mutants of Sorbitol dehydrogenase and sorbose dehydrogenase respectively while the third is an NAD^+ mutant.