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Genome Analysis of *Thermus caldophilus* GK24 and Thermostable Enzyme Engineering for Carbohydrates

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In studying carbohydrate biosynthesis of an extreme thermophile, *Thermus caldophilus* GK24, the genome DNA was analyzed through Shot-gun method with a large number of clones containing about 5 Kbp long genomic DNA fragments, and along with the cosmid clone analysis.

The resulting genome sequences were subject to the home-made DNA/protein database-analysis system at Linux platform (OSCA) (Fig. 1) (<http://glucose.kribb.re.kr>) to assemble contigs and ORF identifications, particularly carbohydrate-related enzyme genes. The OSCA, which was recently upgraded further by establishing the secondary structure database, carbohydrate enzyme database, and protein alignment database, showed high performance to find the series of carbohydrate enzyme genes in conjunction with the carbohydrate biosynthesis and sugar metabolic pathways, such as glycogen biosynthesis, glycolysis, pentose pathway, TCA cycle, vitamin B5 biosynthesis and others.

In order to understand the structure-reaction relationship of carbohydrate enzymes and the biosynthetic machineries, at first, the massive gene clonings and expressions of carbohydrate-related enzymes have been undertaken in *E. coli*. (Fig.2) Among the resulting clones, some recombinant carbohydrate enzymes were purified by heat treatment and chromatographic procedures. The enzyme reactions, particularly those related to glycolysis and glycogen biosynthesis, are being assayed through HPLC system.

Moreover, some 3-dimensional structures of the enzymes have been determined through computer simulation and x-ray analysis, not only for understanding structure-reaction relationship, but also for getting the structural motif for enzyme-enzyme recognition in the machinery assembly. The initial analysis showed that the structure motif for enzyme-enzyme interaction identified shows some homology, compared with those of other microbial enzymes.

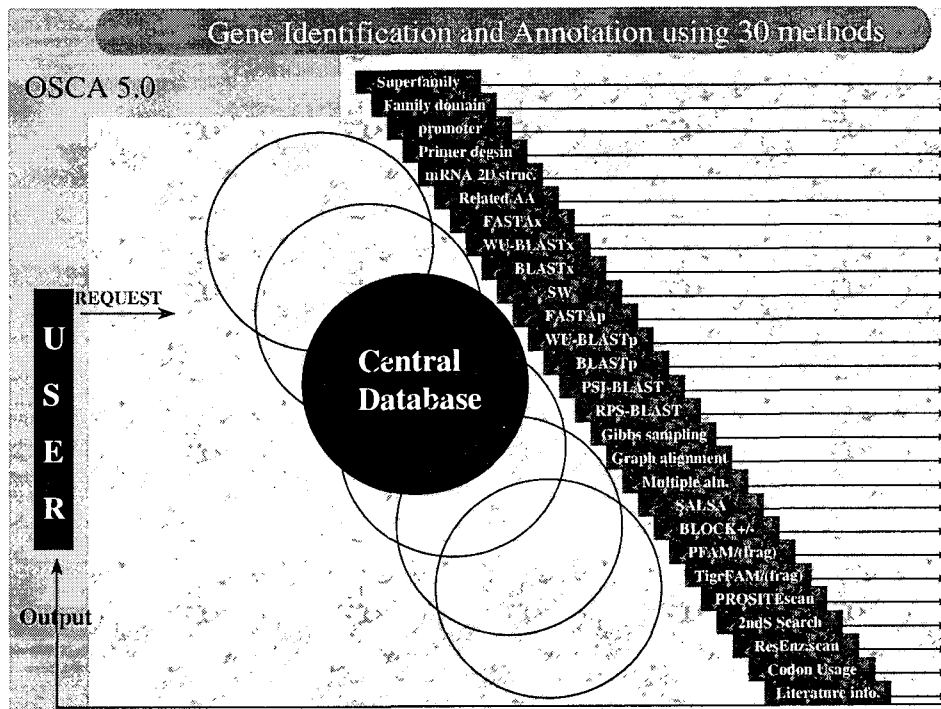


Fig. 1. The system outline of the home-made DNA/protein database-analysis system at Linux platform (OSCA)

α -GLUCAN BIOCHEMISTRY

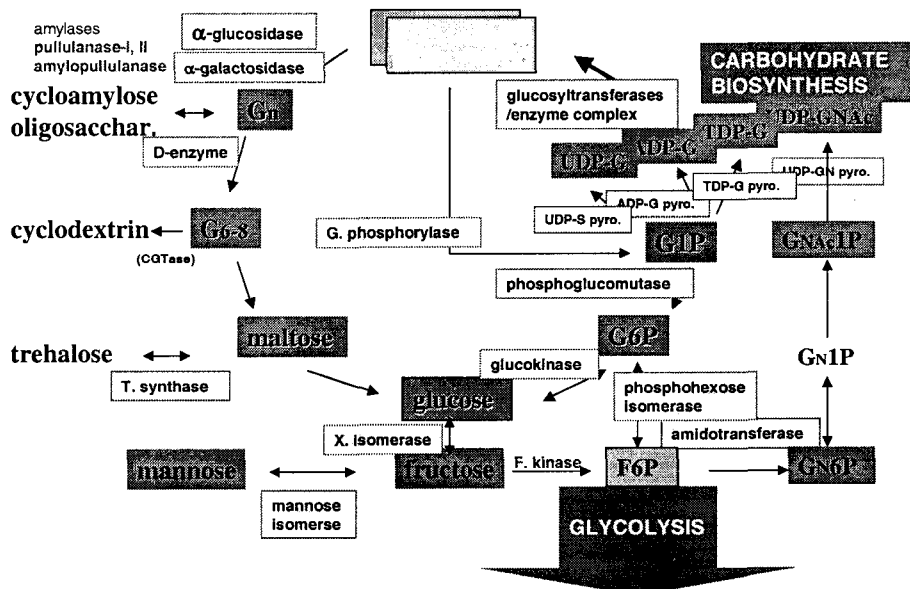


Fig. 2. The α -glucan biochemistry. All the enzymes from *Thermus caldophilus* GK24 in box were expressed in *E. coli*.