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Screening of genes required for decolorization of triphenylmethane dye in *Citrobacter* sp.

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We screened dye-nondegrading mutants using transposon mutagenesis to identify genes involved in the decolorization of crystal violet, triphenylmethane dye, in crystal violet-decolorizing bacterium, *Citrobacter* sp. The resulting mutant bank yielded 25 mutants with complete defect in color removal capability of crystal violet. Southern hybridization with a Tn5 fragment as a probe showed a single hybridized band in the mutants and these mutants appeared to have insertions at different sites of the chromosome. Tn5-inserted genes were isolated and the DNA sequence flanking Tn5 was determined. From comparison with a sequence database, putative protein products encoded by *ctg* genes were identified as follows. Ctg1, Ctg15, Ctg23 and Ctg29 are oxidoreductase; Ctg2 is transcriptional regulator gadX; Ctg3 is glagella protein FlhB; Ctg4 is FimH protein homolog; Ctg5 is maltose permease (malG); Ctg6 is protein interacting with RecR and RecF; Ctg7 is mannitol-specific enzyme II component; Ctg8 is tryptophan permease homolog: Ctg10 is C4-dicarboxylate transport protein homolog; Ctg11 is lysophospholipase L; Ctg13 is RhsC core protein; Ctg14 is RhsF protein; Ctg22 is Ankyrin-like regulatory protein; Ctg24 is guanosine pentaphosphatase homologs; Ctg32 and Ctg33 are ABC-type transport protein, ydbA.2. The sequences deduced from four *ctg* genes, *ctg*9, *ctg*12, *ctg*20 and *ctg*30, did not show a significant similarity to any DNA or proteins in the public sequence databases. Therefore, these results indicate that these four *ctg* genes encode unidentified proteins responsible for decolorization of crystal violet.