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Abstract
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In an effort to isolate the aro cluster-"ene (Gaertner and Cole 1977 Biochem. Biophys. Res. Commun. 75: 259) from N. crassa, we prepared a total BamHI digest of chromosomal DNA, ligated into the Saccharomyces shuttle vector pYE13 (Broach et at. 1979 Gene 8: 121), and amplified the resultant genomic library in E. coli (Procedure I). The amplified gene-bank was used to attempt complementation in four mutants of E. coli (see E. coli strains lacking aro activities by transformation (Procedure II). Only the E. coli mutant SK288a (aroD), which lacks DHQase, was complemented. Isolation of plasmid DNA (Procedure III), and subsequent restriction of the DNA with BamHI, showed that the complementing activity was present on a 3.2 kbp BamHI fragment. This fragment in pYE13 also complements the qa-2 gene of N. crassa (Case and Giles, personal communication), but does not complement DHQase minus strains of Saccharomyces cerevisiae (our unpublished results). Subcloning of the 3.2 kbp insert was performed to obtain both orientations within the pYE13 shuttle-vector. Complementation was observed in the E. coli, aroD, mutant in both orientations, indicating that promoter activity was inherent to the fragment and independent of plasmid promoter functions. Enzyme preparations from the complemented E. coli aroD mutant also confirmed the existence of the qa-2 gene on the 3.2 kbp fragment. Thus, we concluded that the 3.2 kbp BamHI fragment from Neurospora crassa carried the structural and promoter information of the qa-2 dehydroquinase gene.

Complementing activities for other E. coli aro mutations relevant to the aro cluster-gene have not been observed, suggesting either that BamHI cuts and inactivates these genes, or that some incompatibility exists between E. coli and N. crassa with respect to these genetic elements.

Procedure I - Library Formation and Plasmid Amplification

Ligation of 4.8 micrograms of BamHI digested N. crassa genomic DNA with 1.6 micrograms of BamHI digested, alkaline phosphatased, pYE13 DNA was done in a total volume of 800 microliters, also containing T4 DNA ligase buffer (1x T4 DNA ligase buffer: 50 mM Tris-Cl 7.8, 10 mM $MgCl_2$, 2 mM DTT, 1 mM ATP and 50 micrograms per ml of BSA) and 1600 U of T4 DNA ligase. The ligation was carried out for 18 h in a 15°C water bath. The 800 microliter ligation was then added to 16 ml of competent E. coli HB101 cells and our usual transformation was performed. The entire transformation mixture was added to 500 ml of LB-medium containing 25 micrograms per ml of ampicillin and grown to a stationary phase overnight in an air shake-incubator at 37°C. These cells were then spun down and resuspended in 10 ml of LB-medium plus 25 micrograms per ml of ampicillin. Enough of this resuspension was added to 500 mls of M-g medium to bring the initial O.D. 600 to 0.16 using a 1 cm pathlength. The culture was then grown in an air shake-incubator at 37°C until the O.D. 600 reached 0.8, at which time chloramphenicol was added to a final concentration of 200 micrograms per ml and the culture grown for an additional 18 h. The plasmid DNA was then recovered as described below in Procedure III.

Procedure II Bacterial Transformation

Bacterial strains were made competent according to the following protocol. A single colony isolate was started in 20 ml of LB-medium and grown at 37°C overnight. The following day, a 560 microliter aliquot of the overnight culture was added to 56 ml of LB-medium in a 250 ml side-arm flask with a 1 cm pathlength. The culture was then grown to an O.D. 600 of 0.16 and the flask placed in ice-water for 10 minutes. The cells were then spun down in sterile 30 ml oak-ridge tubes, resuspended in 28 ml of C.M.T. buffer (1x C.M.T. buffer: 0.1 M $CaCl_2$, 5 mM $MgCl_2$, 10 mM Tris-Cl 7.8), and incubated on ice for 25 minutes. The cells were spun down again and resuspended in 1.4 ml of C.M.T. The transformation procedure was begun by the addition of DNA to the competent cells in a ratio of 5-10 microliters of aqueous DNA per 200 microliters of competent cells, and incubated on ice for 15 minutes. After a 5 minute heat shock in a 37°C water-bath, 800 microliters of LB-medium was added per 200 microliter transformation aliquot, and the culture vigorously shook in a 37°C water-bath for 60 minutes. The transformation mixture was then plated on selectable medium or amplified directly.

Procedure III - Plasmid Purification

Plasmid purification was done according to the alkaline lysis protocol in the Cold Spring Harbor laboratory manual "Molecular Cloning," T. Maniatis, E.F. Fritsch and J. Sambrook (1982), without modification.

LB-Medium (1000 ml)	M-9 Medium (1000 ml)	10x M-9 Salts (500 ml)
10 g tryptone 100 ml	10x M-g salts (see below)	54.7 g $Na_2HPO_4,7H_2O$
5 g yeast extract	5 g casaminoacids	15.0 g KH_2PO_4
5 g NaCl		2.5 g NaCl
0.5 ml 5 N NaOH		5.0 g NH_4Cl

E. coli Strains

The "SK" strains of E. coli were a generous gift from Sidnev R. Kushner (The Universitv of Georgia, Athens).
SK494: F^-, gal^-, thi^-, aroE, spc^R, hsr-4, $endA^-$, sbcB15, (DH5 reductase)
SK890: F^-, gal^-, thi^-, aroB, str^R, ton^R hsr-4, $endA^-$, sbcB15, (DHQ synthase)
SK2881: F^-, gal-, lac^-, srl-, leuC, aroD6$^-$, tonA, hsdR4, recA1, sbcB15, $endA^-$, (DHQase)
SK3337: F^-. qal-K2, acvl, mH-1, xyl-5, his-4, pro-A, thi-1, tetS, λ^- aroA2, hsdR4, (ESP synthase)
HBlOl: $hsdR^-$, $hsdM^-$, recAB, supE44, 1ac24, leuB6, proA2, thi-1, Sm^R

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