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THIOPHENE METABOLISM BY *E. COLI*

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DISCLAIMER

EXECUTIVE SUMMARY

The manuscript by Martha J. Juhl and David P. Clark entitled "Thiophene - Degrading *Escherichia coli* Mutants Possess Sulfone Oxidase Activity and show Altered Resistance to Sulfur-Containing Antibiotics" has been published in Applied and Environmental Microbiology (Vol 56: pp 3179-3185, October 1990). The manuscript by Kiswar Alam and David P. Clark entitled "Cloning and Sequencing of Genes Involved in Thiophene Oxidation by *E. coli*" has been extensively revised and resubmitted to the Journal of Bacteriology. In particular, our sequence has been matched to neighboring sequences on the *E. coli* chromosome. Our *thdF* gene is 2.1 kb long and encodes a protein of approximately 48,000 daltons which is preferentially expressed in late exponential to early stationary phase. A large stem and loop structure, which may specify this regulatory pattern lies in front of the *thdF* gene. We will attempt to subdivide the regulatory and structural elements of the *thdF* gene for further analysis by the polymerase chain reaction (PCR).

The sulfone using strain of *Acinetobacter* has been characterized for its growth characteristics, on both carbon and sulfur sources. It can use many alcohols and acids as carbon source but grows poorly on sugars. For sulfur source, it uses a wide range of sulfonic acids, sulfones and sulfoxides. We are preparing two cloning vectors, one each of high and low copy, in order to clone sulfur genes from *Acinetobacter*.

BACKGROUND

The objective of this project is to investigate the mechanism of degradation of sulfur containing heterocyclic molecules by mutants of *Escherichia coli* K-12. We previously isolated multiple mutants of *E. coli* which were selected for improved oxidation of furan and thiophene derivatives. We have focussed on the *thdA* mutation in our subsequent research as it appears to be of central importance in thiophene oxidation. We hope that analysis of the *thd* genes of *E. coli* will lead to improvement of our thiophene metabolizing bacterial strains.

PUBLICATIONS

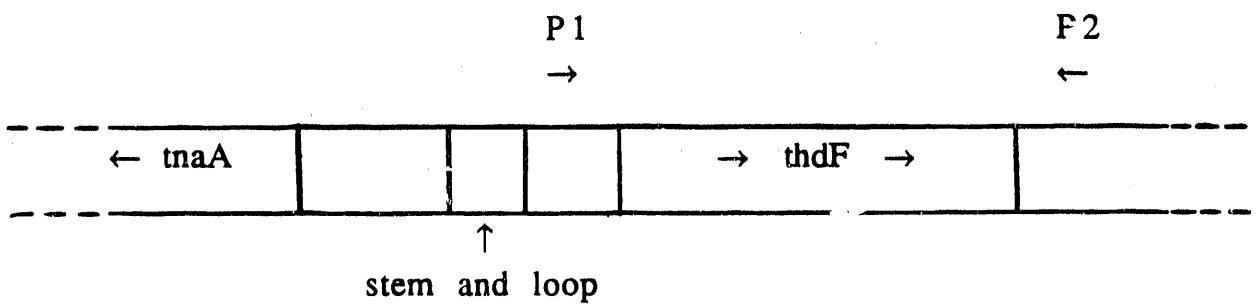
(a) The paper by M.J. Juhl and D.P. Clark which was previously accepted has now been published in Applied and Environmental Microbiology. Reprints are attached.

(b) The manuscript by K.Y. Alam and D.P. Clark concerning the cloning and sequencing of *thdF* has been revised and new material added. This has been resubmitted to the Journal of Bacteriology. A copy of the manuscript is attached.

RESULTS

The *thdF* Gene

See the attached manuscript (b) above for recent results regarding the *thdF* gene. In order to subdivide the *thdF* gene into a regulatory segment and the structural gene sequence we are using the PCR (polymerase chain reaction) approach.



The diagram shows the arrangement of the *tnaA* and *thdF* genes on the *E. coli* chromosome. Just in front of *thdF* is a large stem and loop structure. The arrows P1 and P2 represent PCR primers. We have synthesized two PCR primers corresponding to the known DNA sequence at the places indicated. Both primers possess artificial restriction sites designed to complement those of the vector to be used.

P1 = front of thd (ZAG) 27 mer (1024 - 1050)

(NcoI)

5' C CAC T CC ATG G AA RGG CAA GAC GAC AC 3'

| |

A A

P2 = back of thd (ZIG) 27 mer (2336 -2362)

(Hind III)

5' TGC CT A AGC TT C GCT ACG ACG AGT ATC 3'

| |

C G

We will use these to PCR out the *thdF* structural gene away from the regulatory region. The *thdF* gene will then be inserted into the Nco/Hind site of expression vector pKK233-2. This vector allows induction of the inserted gene by addition of IPTG as the insert is under the control of a hybrid *trp/lac* promoter. This should allow us to express *thdF* before the cells enter stationary phase.

Sulfone Degrading Acinetobacter

We have screened this strain for its ability to use various carbon sources. We found that many alcohols and carboxylic acids support vigorous growth but that this organism grows poorly on most sugars (Table 1). We also surveyed the ability to use a wide range of sulfur compounds (Table 2). Many low molecular weight sulfonic acids and sulfones and some sulfoxides can be used. We selected mutants of *Acinetobacter* for resistance to selenate, a toxic analog of sulfate. Such mutants lose the ability to take up sulfate and hence require some other sulfur source. In the case of *E. coli* such mutants require sulfide or cysteine. In the case of *Acinetobacter* we found that selenate mutants still grew, albeit more slowly, with sulfate when provided with certain carbon sources, implying that they may have a second sulfate transport system. However when grown on ethanol, the selenate resistant derivatives of *Acinetobacter* were quite unable

to grow with sulfate and showed a clear requirement for some other sulfur source. Thus the second sulfate transport system is apparently not expressed under these conditions, and the ability of various organosulfur compounds to permit growth can be easily checked (Table 2).

We are making DNA of the low copy cloning vector pHSG575 in order to clone sulfur source genes from *Acinetobacter*. This plasmid has a multiple cloning site and specifies resistance to chloramphenicol. Since it is only present in about 10 copies per cell, it is necessary to grow and process much larger cultures than normal to obtain sufficient plasmid DNA for cloning experiments. We have just completed a plasmid prep and hope to attempt the cloning experiment soon.

Table 1: Growth Properties of Acinetobacter.

(a) Substances Used as Carbon Source.

Glucose (poorly), Gluconate, Glucarate

Acetate, Succinate, Malate, Butyrate, Ethanol, n-Propanol, n-Butanol, n-Pentanol, n-Hexanol, n-Heptanol, n-Octanol, 2,3-Butanediol.

Benzoate, p-Hydroxybenzoate, 3,4-Dihydroxybenzoate, 2-Aminobenzoate.

(b) Substances Not Used as Carbon Source.

Fructose, Maltose, Galactose, Mannose, Lactose Rhamnose, Trehalose, Xylose, Fucose, Mannitol, Sorbitol, Glucuronate.

Glycollate, Glycerol, Ethylene glycol, Methanol, 1,2 Propanediol, 2,4 Pentanediol, 2-Propanol, Isoamyl alcohol, Acetone.

2-Furoate, 2-Thiophene carboxylate, p-Toluate, 2,5 Dihydroxybenzoate, Benzyl alcohol, Naphthalene, Biphenyl.

Table 2: Sulfur Sources for Acinetobacter.

(a) Substances Used as Sulfur Source.

Sulfoacetate, Isethionate, Ethane sulfonate, Butane sulfonate

Methyl sulfone, Ethyl sulfone, Tetramethylene sulfone, Phenyl sulfone (slow), Methylsulfonyl ethanol.

Dimethyl sulfoxide

1-Naphthalene sulfonate (slow)

1,4-Butane sultone

(b) Substances Not Used as Sulfur Source.

p-Toluene sulfonic acid, DBT-sulfone.

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