The Sulfate Activation Locus of *Escherichia coli* K12: Cloning, Genetic, and Enzymatic Characterization*

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Thomas S. Leyh†, John C. Taylor, and George D. Markham

From the Institute for Cancer Research, Fox Chase Cancer Center, Philadelphia, Pennsylvania 19111

The sulfate activation locus of *Escherichia coli* K12 has been cloned by complementation. The genes and gene products of this locus have been characterized by correlating the enzyme activity, complementation patterns, and polypeptides associated with subclones of the cloned DNA. The enzymes of the sulfate activation pathway, ATP sulfurylase (ATP:sulfate adenylyltransferase, EC 2.7.7.4) and APS kinase (ATP:adenosine-5'-phosphosulfate 3'-phosphotransferase, EC 2.7.1.25) have been overproduced ~100-fold. Overproduction of ATP sulfurylase requires the expression of both the cysD gene, encoding a 27-kDa polypeptide, and a previously unidentified gene, denoted cysN, which encodes a 62-kDa polypeptide. Purification of ATP sulfurylase to homogeneity reveals that the enzyme is composed of two types of subunits which are encoded by cysD and cysN. Insertion of a kanamycin resistance gene into plasmid or chromosomal cysN prevents sulfate activation and decreases expression of the downstream cysC gene. cysC appears to be the APS kinase structural gene and encodes a 21-kDa polypeptide. The genes are adjacent and are transcribed counterclockwise on the *E. coli* chromosome in the order cysDNC, cysN and cysC are within the same operon and cysDNC are not in an operon containing cysHIJ.

The metabolic assimilation of sulfur from inorganic sulfate requires that sulfate be chemically activated (1, 2). The known forms of activated sulfate are adenosine 5'-phosphosulfate (APS)1 and 3'-phosphoadenosine 5'-phosphosulfate (PAPS). APS and PAPS contain a mixed phosphoric-sulfuric acid anhydride bond (3) which has an unusually high ΔG° of hydrolysis of approximately ~19.5 kcal/mol (1). Formation of APS is catalyzed by ATP sulfurylase (ATP:sulfate adenylyltransferase, EC 2.7.7.4) (Reaction 1):

\[
\text{ATP} + \text{SO}_4^{2-} \rightleftharpoons \text{APS} + \text{PPi}
\]

APS formation is extremely energetically unfavorable, with an equilibrium constant of ~10⁻²⁴ for reaction 1 (4). The combined actions of APS kinase (ATP:adenosine-5'-phosphosulfate 3'-phosphotransferase, EC 2.7.1.25) (Reaction 2) and inorganic pyrophosphatase (Reaction 3) allow accumulation of PAPS (1):

\[
\text{ATP} + \text{APS} \rightleftharpoons \text{ADP} + \text{PAPS}
\]

\[
\text{PPi} + \text{H}_2\text{O} \rightleftharpoons 2 \text{P}_i
\]

Despite the importance of activated sulfate as an obligate intermediate in sulfate metabolism, little is known of the detailed enzymatic mechanisms which catalyze its formation. ATP sulfurylase has not been purified from a procaryotic organism, and the partial purification of APS kinase activity from *Escherichia coli* has only recently been reported (3). The genetics and metabolism of activated sulfate have been characterized in *E. coli* and *Salmonella typhimurium*. In *E. coli*, cysD mutants are devoid of ATP sulfurylase activity, whereas cysC mutants lack APS kinase activity (6). These mutants were isolated by virtue of their growth requirement for cysteine (7). The cysC and cysD genes are tightly clustered along with cysG, cysI, and cysJ between 59 and 60 min on the *E. coli* K12 chromosomal map (8). The cysH and cysI genes are responsible for APS reductase and sulfite reductase activities, respectively (8). Although the genes of the cysCDHIJ cluster show positive and coincident regulation by the cysB gene product (6, 9), information regarding gene organization and operon structure(s) within this cluster is lacking in *E. coli*.

In studies directed toward characterization of the genes and proteins involved in sulfate activation, we have cloned the *E. coli* ATP sulfurylase structural genes and purified the enzyme to homogeneity. In so doing, a previously unidentified gene, denoted cysN, was discovered. ATP sulfurylase is composed of two types of subunits which are encoded by cysD and cysN. We have also cloned the *E. coli* cysC gene which appears to be the structural gene for APS kinase. In addition, an operon which includes cysNC and excludes cysHIJ has been identified.

**MATERIALS AND METHODS**

**RESULTS**

Cloning of cysC and cysD—The cysC and cysD mutations present in strains JM81A and TSL1, respectively, prevent the anabolic utilization of sulfate (18). Growth of these strains on media containing sulfate as the only sulfur source provides a
positive selection for cys' transformants. cysD was cloned by transforming strain TSL1 to a cys' phenotype with a bacteriophage λ library of HindIII digested E. coli K12 chromosomal DNA. The isolated transforming phage, XTL1, complemented both the cysC and cysD mutations of strains JM81A and TSL1, but did not complement the cysH mutation of strain JM96. Analysis of HindIII digested XTL1 DNA revealed that the phage DNA contained a 9.4-kb insert.

Gene and Gene Product Characterization—The strategy employed in mapping and characterizing the cloned sulfate activation genes and gene products involved correlating the complementation patterns, ATP sulfurylase and APS kinase activities, and polypeptides associated with various subclones of the 9.4-kb insert. Thus, the presence or absence of a particular cloned gene product may be associated with that of a specific enzyme activity, known genetic allele, and region of the 9.4-kb insert. The plasmids used in these studies are illustrated in Fig. 1. The associated complementation and enzyme activity data are presented in Tables I and II. Lanes 1-6 of Fig. 2 show the results of studies in which [35S]methionine was incorporated in vivo into the polypeptides expressed from the plasmids pTL1-pTL6 depicted in Fig. 1.

![Fig. 1. Plasmids used for characterization of the cys genes.](image)

The plasmids listed in the left-hand column are composed of the insert shown and either vector pT7-5 (contained in pTL1,2,3,5,7, and 8) or pT7-6 (found in pTL4,6, and 8) (see “Materials and Methods”). The proximity and orientation of the T7α10 promoter with respect to the cys genes is indicated by a right angle arrow at an insert terminus. The approximate coding regions of the cys genes are shown as labeled black rectangles. The positions and sizes of the coding regions are estimated from restriction mapping and apparent molecular weights of the gene products. The 5’-3’ direction of the genes are defined by the arrows beneath the coding regions of pTL1. A circle at an insert terminus indicates that that terminus was generated by BAL3I exonuclease. The transcriptional orientation and point of insertion of the kanamycin gene in pTL1 are depicted by the triangle and its associated arrow. The shaded region of pTL5 is a fragment of pBR325. The restriction sites, labeled on pTL1, are indicated by tic marks. The restriction enzyme abbreviations shown in pTL1 are: H, HindIII; P, PstI; E, EcoRI; S, Sall; B, BamHI; F, PvuII; Hc, HincII and C, CiaI. The detached restriction sites at the left-hand termini of pTL5 and pT71 are found in the vector.

### Table I

| Plasmid | Polypeptides expressed | Activity | Complementation |
|---------|------------------------|----------|-----------------|
|         |                        | ATP sulfurylase | APS kinase | TSL3 (cysD) | JM81A (cysC) |
| pT7-5* | + + + + + + + + + + + + | 0.2 | 0.5 | - | - |
| pT7L3 | + + + + + + + + + + + + | 13 | 16 | + | + |
| pT7L4 | + + + + + + + + + + + + | 27 | 62 | - | - |
| pT7L5 | + + + + + + + + + + + + | 27 | 62 | - | - |
| pT7L6 | + + + + + + + + + + + + | 27 | 62 | - | - |
| pT7L7 | + + + + + + + + + + + + | 27 | 62 | - | - |
| pT7L8 | + + + + + + + + + + + + | 27 | 62 | - | - |

* Determined by [35S]methionine incorporation into plasmid-encoded polypeptides (see Fig. 2 and associated text).

### Table II

The effects of plasmid or chromosomal insertion of pkn into cysN on the expression of the genes and enzymes of the sulfate activation locus

| Plasmid | Activity | Complementation |
|---------|----------|-----------------|
|         | ATP sulfurylase | APS kinase | TSL2 (cysD) | JM81A (cysC) | DM62 (cysNC) |
| pT7-5 | 0.032 | 0.088 | - | - | - |
| pT7L3 | 0.077 | 0.067 | + | + | + |
| pT7L4 | 3.4 | 8.3 | + | + | + |
| pT7L5 | + | + | - | - | - |
| pT7L6 | + | + | - | - | - |
| pT7L7 | + | + | - | - | - |
| pT7L8 | + | + | - | - | - |

* ATP sulfurylase and APS kinase activities were determined using the radioactive assays described in the text. The extracts assayed were prepared from the strain CL510, the precursor of DM62, harboring the indicated plasmid and pGP1-2. The activity units are expressed as nmol/min/mg extract protein.

The incorporation of radioactive amino acids was accomplished using the pT7/pGP1-2 dual vector system (see “Materials and Methods”).

Some peptides were identified using the radioactive assays described in the text. The extracts assayed were prepared from the strain CL510, the precursor of DM62, harboring the indicated plasmids. The activity units are expressed as nmol/min/mg extract protein.

The complementation protocol is described under "Materials and Methods."
cysD, cysC proteins and proteins 2-6 are on the same strand of the 9.4-kb insert; whereas, protein 1 is encoded on the complementary strand. Thus, the protein 1 coding region is transcribed antiparallel to that of the coding regions for the other labeled polypeptides. Maxicell labeling techniques were used to map protein 1 to the 3.2-kb HindIII-EcoRI fragment of the plasmids (see Fig. 1 and "Materials and Methods"). Cell extracts of the in vivo labeled proteins were loaded onto a polyacrylamide gel, electrophoresed, and autoradiographed. The standards used to determine the apparent molecular mass of the labeled peptides are shown in lane 7. The left most column indicates the structural gene encoding a specific labeled polypeptide. In cases where the structural gene is not known the polypeptides are numbered.

Mapping, Gene Product Identification, and Characterization of CysC—To map the position of cysC on the cloned 9.4-kb fragment, a series of subclones were constructed in pT7 vectors. The subclone inserts share a common PstI endpoint and were progressively deleted from the ClaI site of the PstI-ClaI fragment of the 9.4-kb clone using Bal31 exonuclease (see Fig. 1 and "Materials and Methods"). Two such plasmids, pTL3 and pTL4 (see Fig. 1), differ in that the insert of pTL4 is ~800 base pairs shorter than that of pTL3. [35S]Methionine labeling of the proteins encoded by these plasmids demonstrates that pTL4 expresses five of the six polypeptides expressed by pTL3 (see lanes 3 and 4 of Fig. 2). The polypeptide not expressed by pTL4 is of apparent molecular mass 21 kDa. Thus, some fraction of the coding region for the 21-kDa polypeptide has been deleted in pTL4. Table I shows that some part of the ~800 base pairs removed from pTL3 is required for complementation of the cysC allele of JM81A. The concomitant loss of ~800 base pairs of DNA, expression of the 21-kDa polypeptide, and the ability to complement cysC suggests that the 21-kDa polypeptide is the cysC gene product. Plasmids pTL1, containing the entire cysC coding region, and pTL3 appear to express the same 21-kDa polypeptide (see Figs. 1 and 2). This indicates that the 21-kDa protein is not artificially truncated due to Bal31 deletion of the cysC coding region of pTL3. Extracts of cells harboring pTL3 exhibit a ~32-fold increase in APS kinase specific activity as compared to control extracts (see Table I). Extracts of cells harboring pTL4 show approximately wild type levels of APS kinase activity (see Table I). In light of the mapping and complementation studies, the activity data suggest that cysC is the structural gene for APS kinase and that the 21-kDa protein is the APS kinase proteme (see "Discussion").

Mapping, Gene Product Identification, and Characterization of cysD—Plasmid pTL5 was constructed for the characterization of cysD (see "Materials and Methods"). This plasmid is composed of a ~1.1-kb cysD complementing fragment of the 9.4-kb clone inserted into pT7-5 (see Fig. 1). [35S]Methionine incorporation studies indicated that the pTL5 insert encodes a single polypeptide which is of apparent molecular mass 27 kDa (see Fig. 2, lane 5). Plasmid pTL6 neither complements strain TSL3 nor expresses the 27-kDa polypeptide at levels detectable by [35S]methionine labeling. These data map cysD within a ~1.1-kb region of the 9.4-kb clone and strongly suggest that the 27-kDa polypeptide is the cysD gene product.

The levels of ATP sulfurylase activity in extracts of cells containing plasmid-encoded cysD protein differ by as much as two orders of magnitude (see Tables I and II). Although cysD protein is not visible in extracts of control strains electrophoresed on SDS polyacrylamide gels and stained with Coomassie Brilliant Blue, it is detected in extracts of strains containing plasmid-expressed cysD. The plasmids pTL5 and pJT1 (Fig. 1) express detectable levels of cysD protein, yet the ATP sulfurylase activity in extracts of strains containing these plasmids approximate the levels found in extracts of control strains (Tables I and II). Plasmid pTL1, which contains the full 9.4-kb insert, expresses nearly a ~100-fold increase in ATP sulfurylase activity over control (Table II). Thus, the entire insert contains the genetic determinants necessary for the overproduction of ATP sulfurylase activity and expression of cysD alone is inadequate for this purpose.

In attempting to isolate the sequences sufficient for overproduction of enzyme activity, numerous subclones of the 9.4-kb insert were constructed, all of which contained cysD, the presumed ATP sulfurylase structural gene (18) (see "Materials and Methods"). Plasmid pTL4 (Fig. 1), isolated from these constructions, is our smallest isolate which overproduces ATP sulfurylase activity. Extracts of strains containing pTL4 show a 60 fold increase in ATP sulfurylase activity over the pT7-5 control (Table I). Plasmid pTL4 expresses only the cysD protein and the 62-kDa polypeptide. To verify that the 62-kDa polypeptide was not solely responsible for the enhanced enzyme activity associated with pTL4, pTL6 was constructed (Fig. 1). Plasmid pTL6 differs from pTL4 in that part of the cysD coding region has been deleted. Although extracts of cells harboring pTL6 show no apparent increase...
in ATP sulfurylase activity (Table I), expression of the 62-kDa protein is easily detected by Coomassie Brilliant Blue staining of acrylamide gels containing these extracts. Thus, the enhanced expression of both the cysD and 62-kDa proteins is required for overproduction of ATP sulfurylase activity. These observations do not discriminate between regulatory versus catalytic roles for these proteins; however, it is clear that the 62-kDa protein plays a critical role in the overproduction of ATP sulfurylase activity.

Identification of cysN, a Second Gene Required for ATP Sulfurylase Overproduction—To further investigate the function of the 62-kDa protein in the expression of ATP sulfurylase activity, a strain harboring a mutation in the 62-kDa coding region was constructed. In this strain, DM62, the gene which confers kanamycin resistance (kan) has been inserted into the 62-kDa coding region of the chromosome by homologous recombination using the PvuII fragment of pJT1 (see “Materials and Methods” and Fig. 1). Strain DM62 is a cysteine auxotroph. The complementation studies, which characterize DM62 and address the essential role of the 62-kDa protein in sulfate activation, are shown in Table II. The plasmids used in these studies express different subsets of the cloned genes (see Fig. 1 and “Materials and Methods”). Plasmid pTL8, which complements DM62 encodes only the cysCD and 62-kDa proteins (see lane 4, Fig. 2). Plasmid pTL7 which contains intact cysC and 62-kDa coding regions and a partially deleted cysD also complements DM62. Strain DM62 is not complemented by pTL6 which encodes only the 62-kDa protein (see lane 6, Fig. 2). Plasmid pTL8, containing the cysC coding region and lacking part or all of the cysD and 62-kDa coding regions, does not complement DM62. Taken together, these data indicate that both the cysC and the 62-kDa coding regions are necessary and sufficient for the complementation of DM62. That pTL8 complements the cysC allele of JM83A but does not complement DM62 verifies that the 62-kDa polypeptide is essential for sulfate activation in E. coli. As such, the gene encoding the 62-kDa polypeptide was previously unidentified in the sulfate activation locus of E. coli. The mnemonic cysN has been chosen to represent this gene.

The insertion of kan into chromosomal cysN inactivated cysC (Table II). This kan-mediated polar inactivation of cysC suggested that cysN and cysC reside within the same operon. To exclude the possibility that cysC inactivation was an artifact of strain construction, enzyme activity and complementation studies were performed using strains containing pJT1 (see Table II and Fig. 1). Plasmid pJT1, derived from pTL1 by insertion of kan in cysN, was the plasmid used in the construction of DM62. Restriction mapping showed that the transcriptional orientation of kan in pJT1 is parallel to that of cysDNC. The enzyme assays demonstrate that kan insertion into cysN reduces the expression of APS kinase activity at least 127-fold compared to that associated with pTL1 (see Table II). The complementation studies (see Table II) show that pJT1, which carries an intact cysC coding region, cannot complement the cysC allele of JM83A. The activity and complementation data attest a strong polarity between cysN and cysC. This polarity indicates that cysN and cysC lie within the same operon (35). It is significant that plasmid pTL8, which does not contain most or all of the cysD and cysN coding regions, complements the cysC allele of JM83A (see Fig. 2 and Table II). This suggests that either an internal cysC promoter exists within the operon or that transcription of cysC initiates at promoter(s) within the pT7-5 vector of pTL8. That pJT1 does not complement JM83A argues against the possibility of a promoter located between cysN and cysC.

**cysD and cysN Proteins Are Subunits of ATP Sulfurylase—** Having established an essential role for the cysN protein in sulfate metabolism and the interdependence of the cysD and cysN proteins in overproducing ATP sulfurylase activity, it was of interest to more acutely probe the functional relationship of these polypeptides. The results presented thus far suggested that these polypeptides could be subunits of ATP sulfurylase. To address this possibility, and for future mechanistic studies, the enzyme was purified to apparent homogeneity (see “Materials and Methods”). The enzyme purification involved streptomycin sulfate and ammonium sulfate precipitation followed by gel filtration and anion exchange chromatography. The procedure resulted in a 40-fold increase in ATP sulfurylase activity (Table I), expression of the 62-kDa protein is easily detected by Coomassie Brilliant Blue staining of acrylamide gels containing these extracts. Thus, the enhanced expression of both the cysD and 62-kDa proteins is required for overproduction of ATP sulfurylase activity. These observations do not discriminate between regulatory versus catalytic roles for these proteins; however, it is clear that the 62-kDa protein plays a critical role in the overproduction of ATP sulfurylase activity.

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| Table III Purification of ATP sulfurylase |
|------------------------------------------|
| Step | Purification | Protein | Activity | Specific Activity | Yield | Puri |
|------|-------------|---------|----------|------------------|-------|------|
|      |            | mg          | µmol/min | µmol/min/mg | %      | fold |
| Crude extract | 52 | 842 | 4.8 | 0.0057 | 100 | 1 |
| Streptomycin sulfate | 50 | 800 | 5.8 | 0.0073 | 120 | 1.3 |
| Ammonium sulfate | 49 | 63 | 4.8 | 0.076 | 100 | 13 |
| Sephacryl S-300 | 75 | 12 | 2.6 | 0.21 | 54 | 37 |
| Q-Sepharose | 48 | 8 | 2.2 | 0.23 | 46 | 40 |

* Determined by Bradford analysis (see “Materials and Methods”).

a ATP sulfurylase activity was determined using the continuous assay (see “Materials and Methods”).

b The crude extract was prepared from 12 g (wet weight) of *E. coli* JM83 containing pGP1-2 and pTTL3 (see Fig. 1).
cluster suggest a genetically silent region between cysCD and cysHJJ (18), similar to that identified in S. typhimurium (44). The 39-kDa polypeptide expressed from pTL2 lies within this region and is transcribed antiparallel to cysDNS. Thus, cysHJJ, while clearly part of a regulon which includes cysCNDHJJ, is not within the operon which includes cysNC. Separate operons encoding cysCD and cysHJJ have been identified in S. typhimurium (45).

Purification of ATP sulfurylase has revealed that the enzyme is composed of two types of subunits. Furthermore, we have demonstrated that the polypeptides encoded by cysD and cysN are the subunits of ATP sulfurylase. These studies have defined a functional role for the cysN protein and verified that cysD and cysN are the structural genes for ATP sulfurylase. cysD and cysN mutants do not require plasmids containing both cysD and cysN for complementation. This indicates that the subunit interactions required for ATP sulfurylase activity occur when the wild type polypeptides are expressed from separate mRNAs. In contrast to the dissimilar subunit structure of ATP sulfurylase from E. coli, the enzyme from Penicillium chrysogenum appears to be composed of identical 56-kDa subunits (46). The Saccharomyces cerevisiae ATP sulfurylase appears to be the product of a single gene consistent with identical subunits (47).

Plasmids which complement cysC confer high levels of APS kinase activity and express a 21-kDa peptide. These data suggest that cysC is the structural gene for APS kinase; however, they are also consistent with cysC encoding a trans activator of APS kinase. Although numerous mutants in the cysCNDHJJ cluster exist in S. typhimurium and E. coli, genetic and biochemical studies have failed to reveal an APS kinase regulatory gene in this cluster (18, 48). APS kinase has been partially purified from E. coli (5). The authors suggest that the apparent molecular mass of the APS kinase subunit is 40 kDa and quote a specific activity of ~600 nmol of PAPS produced/min/mg for the purified enzyme. Recently, the 21-kDa cysC polypeptide has been purified to near homogeneity and shown to have an APS kinase specific activity of ~10^4 mol of PAPS produced/min/mg, suggesting that cysC is the structural gene for APS kinase.

DISCUSSION

The current study offers considerable information regarding the genetic organization of the cysDCHIJ cluster located at ~59 min on the E. coli chromosomal map (8). Previous studies of the cys cluster failed to identify cysN (18). It is likely that this was due to a paucity of well-defined mutants and the relative inaccuracy of traditional mapping techniques. Our studies map cysN between cysD and cysC thus redefining the cluster as cysDNSC. Construction and characterization of a cysN mutant has revealed that cysN is essential for the utilization of sulfate as a sole sulfur source. The relative positions and transcriptional orientations of cysDNSC have been determined. From the known positions of cysD and cysC on the E. coli chromosome (8), it is apparent that cysDNSC is transcribed in a counterclockwise orientation on the chromosome. Insertion of kan into cysN dramatically decreases expression of cysC. This demonstration of polarity between cysN and cysC indicates that these genes belong to the same operon. The contiguous, parallel structure of cysDNSC, the functional relatedness of cysDNSC and cysDNA, and the coincident metabolic regulation of ATP sulfurylase and APS kinase suggest the possibility that cysDNSC lies within the operon containing cysN and cysC. Although the literature generally supports the likelihood that enzyme subunit structural genes are contained within the same operon (37-41), exceptions exist (42, 43). Classical mapping studies of the cysCNDHJJ gene

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SYPHILIS ACTIVATION LOCUS (SLT) OF ESCHERICHIA COELI: GENES AND ENZYMES

**SYNOPSIS**

The Sulfate Activation Locus of *Escherichia coli*: Cloning, Genetic and Enzymatic Characterization

Theodore E. Lohf, John C. Taylor and George W. Raefeld

**MATERIALS AND METHODS**

*E. coli* strain TSL2 was cultured in LB media and grown to an OD660 of 1.0. The strain was isolated from a natural source. *E. coli* strain TSL2 was transformed by electroporation with 200 µg/mL of plasmid DNA. The transformed strain was selected for growth on LB agar plates containing 30 mg/mL of ampicillin.

- **Phenotypes:**
  - The Sulfate Activation Locus of *Escherichia coli*: Cloning, Genetic and Enzymatic Characterization
  - *E. coli* strain TSL2 was cultured in LB media and grown to an OD660 of 1.0. The strain was isolated from a natural source.
  - *E. coli* strain TSL2 was transformed by electroporation with 200 µg/mL of plasmid DNA. The transformed strain was selected for growth on LB agar plates containing 30 mg/mL of ampicillin.

- **Results:**
  - The strain was isolated from a natural source.
  - *E. coli* strain TSL2 was cultured in LB media and grown to an OD660 of 1.0.
  - The strain was isolated from a natural source.

- **Discussion:**
  - The strain was isolated from a natural source.
  - *E. coli* strain TSL2 was cultured in LB media and grown to an OD660 of 1.0.
  - The strain was isolated from a natural source.

- **Conclusion:**
  - The strain was isolated from a natural source.
  - *E. coli* strain TSL2 was cultured in LB media and grown to an OD660 of 1.0.
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- **Acknowledgments:**
  - The strain was isolated from a natural source.
  - *E. coli* strain TSL2 was cultured in LB media and grown to an OD660 of 1.0.
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**References**

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The Sulfate Activation Locus of E. coli: Genetics and Enzymology

Phasmid Construct

**Phasmid pTL11** was constructed by ligation of **pTL12** digested with BamHI and EcoRI with **pBR325** cut with BamHI and SalI. Phasmid **pTL12** was constructed by ligation of **pTL11** digested with SalI and BglII with **pBR325** cut with BglII and SalI. Phasmid **pTL11** was isolated from a train DL2 rhxh *as* asef..sdf in CY and rsristmce to chloramphenicol and upiclllin following lig. tim  artura S. Tabor, personal communication. Salutian 01 Bulfer B vhich  contained 32 d Na,ATP, 30 d YgCl, 1.3 d N&I, Typically, 3 pl of the extract was added to 12 pl of a purified APPL kxnase. After incubation at 21*2°C, the reaction was stopped by placing the reaction vials into a boiling water bath for 1 min. The samples were then collected and assayed for hy an action to ATP sulfurylase. The APS kinase reaction was initiated by the addition of 100 pl (2.5 ml/ml) of reaction buffer containing 0.1 ml of [32P] ATP and 1.0 ml of partially purified enzyme. The reaction was stopped by heating the samples at 100°C for 5 min. The samples were then subjected to electrophoresis on a 0.8% agarose gel. The APS kinase reaction was then performed using the purified enzyme. The samples were then subjected to electrophoresis on a 0.8% agarose gel. The ATP sulfurylase reaction was then performed using the purified enzyme. The samples were then subjected to electrophoresis on a 0.8% agarose gel. The reaction was followed by monitoring the release of 32P and ATP. The reaction was carried out under the ATP sulfurylase reaction to study the Superzyme 12 chromatographic fractions.

Radioligand APS Sulfturylase Activity - APS sulfurylase activity was assayed by following the incorporation of [32P]PAP into PAPS, produced in the APS sulfurylase catalyzed reaction, was converted to 32P by adding APS kinase. The APS kinase was partially purified from **Am** containing pAB2-2 and pAB2. Phasmid pAB2 was derived from pAB2 by deletion of the 0.8 fragment internal to the 0-4 kbp band, which was cloned into pBR325. Phasmid pAB2 was derived from pAB2 by deletion of the 0.8 fragment internal to the 0-4 kbp band, which was cloned into pBR325. The APS kinase reaction was performed on a 32P buffer containing 0.1 ml of [32P] ATP and 1.0 ml of partially purified enzyme. The reaction was stopped by heating the samples at 100°C for 5 min. The samples were then subjected to electrophoresis on a 0.8% agarose gel. The APS kinase reaction was then performed using the purified enzyme. The samples were then subjected to electrophoresis on a 0.8% agarose gel. The reaction was followed by monitoring the release of 32P and ATP. The reaction was carried out under the ATP sulfurylase reaction to study the Superzyme 12 chromatographic fractions.
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Table 1

| E. coli 812 Strains and Restriction Maps |
|----------------------------------------|
|                                        |
| **Strain**                             | **Genealogy/Description** | **Source** | **Ref.** |
|                                        |                           |            |          |
| JS201                                  | E. coli B 15, C 13, C 14,  | D. Buckner  | 38       |
|                                        | E. coli 812               |            |          |
| JS405                                  | E. coli B 15, C 13, C 14,  | D. Buckner  | 3       |
|                                        | E. coli 812               |            |          |
| JS211                                  | E. coli B 15, C 13, C 14,  | D. Buckner  | 38       |
|                                        | E. coli 812               |            |          |
| JS320                                  | E. coli B 15, C 13, C 14,  | D. Buckner  | 38       |
|                                        | E. coli 812               |            |          |
| GL100                                  | E. coli B 15, C 13, C 14,  | J. W. Smith | 39       |
|                                        | E. coli 812               |            |          |
| PK23                                   | E. coli B 15, C 13, C 14,  | J. W. Smith | 20       |
|                                        | E. coli 812               |            |          |
| TE11                                   | E. coli B 15, C 13, C 14,  | This paper  | 20       |
|                                        | E. coli 812               |            |          |
| TE12                                   | E. coli B 15, C 13, C 14,  | This paper  | 20       |
|                                        | E. coli 812               |            |          |
| TE51                                   | E. coli B 15, C 13, C 14,  | This paper  | 20       |
|                                        | E. coli 812               |            |          |
| JS201                                  | E. coli B 15, C 13, C 14,  | D. Buckner  | 38       |
|                                        | E. coli 812               |            |          |
| JS405                                  | E. coli B 15, C 13, C 14,  | D. Buckner  | 3       |
|                                        | E. coli 812               |            |          |
| GL100                                  | E. coli B 15, C 13, C 14,  | J. W. Smith | 39       |
|                                        | E. coli 812               |            |          |
| PK23                                   | E. coli B 15, C 13, C 14,  | J. W. Smith | 20       |
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|                                        | E. coli 812               |            |          |
| TE12                                   | E. coli B 15, C 13, C 14,  | This paper  | 20       |
|                                        | E. coli 812               |            |          |
| TE51                                   | E. coli B 15, C 13, C 14,  | This paper  | 20       |
|                                        | E. coli 812               |            |          |

* References describing observations used in this paper are: E. coli 812
  rudiment: 1, heat shock 2, temperature 3, type II restriction
  endonuclease inhibitors 4.

1. Department of Human Genetics, Tufts University School of Medicine, New
  Bedford, CT 01290.

2. Vannevar Institute, Vannevar.

3. Biology Department, Massachusetts Institute of Technology, Cambridge, MA 02139.

4. The Institute for Cancer Research, Temple University School of Medicine, Philadelphia, PA 19142.

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Fig. 3. Schematic of pT7-7 Deletion Constructions. The thick arc of pT7-
represents the vector pT7-7. The thin arc of pT7-7 represents the 0.4 kb clone.
Shaded rectangles are 0.4 kb bands. The vertical arrow indicates the direction of
igetion. The horizontal arrows indicate the approximate range of E. coli strain derivatives included in the
construction. The labeled arcs indicate restriction enzyme analysis
clones. The restriction enzyme abbreviations are: E. coli, F. coli, B. coli.

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Fig. 2. Gel filtration chromatography of purified ATP sulfurylase. The solid line is the
profile of the enzyme, stained during chromatography of purified ATP sulfurylase on the gel
filter column. The arrow indicates the position of the gel filtration column. The arrows indicate
the boundaries of the enzyme associated with elution fractions.