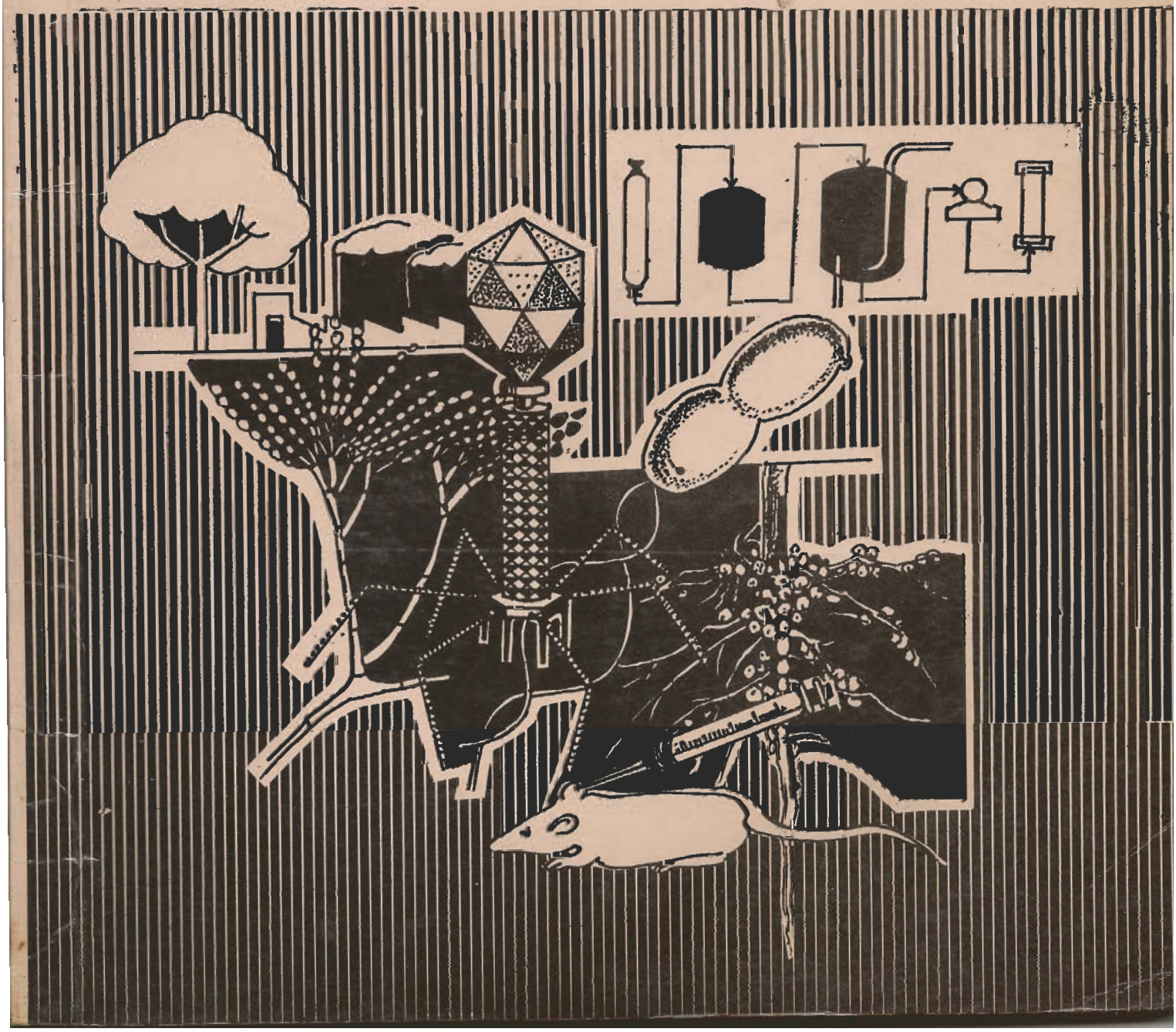
ASSOCIATION OF MICROBIOLOGISTS OF INDIA

27th ANNUAL CONFERENCE

Nagpur, 20-22nd December, 1986

RESEARCH ABSTRACT BOOK



BB-5

CLONING arg F GENE OF PSEUDOMONAS AERUGINOSA IN ESCHERICHIA COLI

A. A. M. Kunhi*, V. Karunakaran, and R. E. Drew**

*Discipline of Microbiology and Sanitation, CFTRI, Mysore-570013.

**Institute for Biotechnological Studies, C/o. Polytechnic of Central London, 115, New Cavendish Street, London WIM 8JS, U. K.

Dept. of Biochemistry, University College London, Gower Street, London, U. K.

Ornithine carbamoyl transferase (OTCase) is one of the enzymes in the arginine biosynthetic pathway of *Pseudomonas aeruginosa*. This is the only enzyme in the arginine biosynthetic pathway which is under strong feed back inhibition. Hence, it is expected that increasing the gene number by introducing it to the organism through a high copy number plasmid vector would mop up the repression and allow the organism to overproduce the amino acid. With this aim as well as to study the regulatory mechanism of the gene arg F coding for OTCase, work was taken up.

In *P. putida* arg F gene is located on the large resident plasmid pCRI. A large scale preparation of this plasmid was carefully made and after its fragmentation (with Bam HI or Pst 1) it was ligated to the *E. coli* vectors pAT153 and pBR322. Transformation of arg-*E. coli* W4100 was successfully carried out with the recombinant vectors (after one passage through *E. coli* HB101 for enrichment of vectors carrying pCRI DNA fragments, as primary transformation of the arg–*E coli* W4100 strain with low levels of recombinant DNA was known to be not conducive for transformation). The recombinant vectors carrying pCRI DNA at the Bam HI site complimented arginine deficiency in the host. Although the presence of the recombinant vector was further confirmed by sensitivity to tetracycline, resistance to ampicillin and ability to grow on a media deficient in arginine of the recombinant strains, efforts to isolate the vector carrying the arg F gene from the transformants were not fruitful.

Kunhi,A.A.M., Karunakaran,V., and Drew,R.E. 1986. Cloning of argF gene of *Pseudomonas aeruginosa* in *Escherichia coli*. **XXVII Annual Conference of Association of Microbiologists of India**, 20-25, 1986, Nagpur, India