TRANSFER OF POLYMYXIN RESISTANCE FROM *V. CHOLERAE* 'ELTOR' TO *V. CHOLERAE* 'CLASSICAL' BY CONJUGATION

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ABSTRACT

Studies on transfer of polymyxin resistance factor between polymyxin resistant (pmx-r) eltor and sensitive (pmx-s) classical strains of *Vibrio cholerae* showed that the resistance factor was readily transferred to the sensitive cells with high frequency. The pmx-r factor was transferred independent of chromosomal genes both in the presence and absence of P factor (a plasmid known to confer sexuality on its host). The faster rate of pmx-r factor transfer, as compared to that of chromosomal markers in the presence of P factor and the lack of definite time schedule during conjugation, indicated the extra-chromosomal nature of the gene. The data also suggested that the resistance factor is an autotransferable extra-chromosomal element in *V. cholerae* which failed to bring maleness to its host.

INTRODUCTION

VIBRIO CHOLERAE has a stable pattern of susceptibility to polymyxin B, that is, eltor strains are resistant whereas classical strains are sensitive[1]. Transfer of resistance factors for various drugs such as sulphadiazine, sulfonamide, erythromycin, tetracycline, etc., between *Vibrio cholerae* and other bacterial cells was carried out by Prescott *et al.*[2]. A conclusion was drawn by Podosinnikova *et al.*[3] on the extra-chromosomal nature of tetracycline, levomycetin and penicillins, etc., in eltor *vibrios*. However, transfer of resistance to polymyxin B has not been described. Therefore, study was undertaken to investigate the nature of transfer of polymyxin resistance (pmx-r) in *V. cholerae* by mating eltor donor (pmx-r, str-s) and classical recipient (pmx-s, str-r) strains.

Data presented here demonstrate that pmx-r factor is transferred in *V. cholerae* independent of chromosomal genes and sex factor 'P' is not essential for its transfer which suggest that it may be an extra-chromosomal (cytoplasmic) genetic element.

MATERIALS AND METHODS

Bacterial strains

Of the strains used KB 9 and KB 408 were kindly supplied by Dr. K. Bhaskaran and strain No. 1957 was supplied by Institute of Preventive Medicine, Hyderabad (Govt. of A.P.). Some of the genetic markers of these strains are shown in Table I.

Culture media

DNB (Difco Nutrient Broth), BHI (Brain Heart Infusion : Difco laboratories), Semi-solid (sloppy) and Solid-media were prepared as described by Bhaskaran *et al.*[5].

Selective media

Selective minimal media, used for the isolation of arg^+, his^+ and ilv^+ recombinants, were prepared as described by Bhaskaran[6].

Isolation of pmx-r recipients

Difco nutrient agar (pH 7·4) + 50 μg/ml of polymyxin B + 500 μg/ml of streptomycin sulphate (CIBA, India) used for the isolation of polymyxin and streptomycin resistant recipient cells.

Test for P factor

For gross differentiation of P^+ and P^- strains, cross matching tests were performed in semi-solid agar as described by Bhaskaran *et al.*[5]. P^+ strain showed a growth thinning effect on indicator P^- strain whereas P^- strain had no effect on P^- strain.

Crosses

Crosses were performed on millipore membrane as described by Bhaskaran[6] and Bhaskaran *et al.*[5] with some modifications as described below :

One batch of donor broth cultures was exposed for 20 min at 46°C whereas the other batch was maintained at 37° C, and then 0·5 ml of each sample was transferred separately to 9·5 ml fresh BHI and this was further incubated at 37° C for one hour (a condition which has been shown to yield maximum number of recombinants[5]). At the end of one hour, donor viable count was determined. Equal volumes of donor and recipient cells were mixed. The mixtures were passed through millipore membrane filter and mating was allowed to take place for one hour without interruption at 37° C, the results of which are described in Table II.

Similarly, experiments were performed wherein conjugation was interrupted at 0, 30, 60, 90 and 120 min, to study the kinetics of teansfer of resistance factor (Figs. 1 and 2).

For the selective isolation of recombinants, mated mixtures were suspended in fluid minimal medium and plated on selective minimal agar. For the selection of pmx-r recipients, the above suspensions of mated cells were plated simultaneously on nutrient agar containing polymyxin and streptomycin,

TABLE I

Characteristics of Vibrio cholerae *strains used in crosses*

Strain	Bio-type	Sex factor	Arginine	Histidine	Isoleucine + valine	Streptomycin (500 μg/ml)	Polymyxin (50 μg/ml)
KB 9	classical	P^-	–	–	–	*r*	*s*
KB 408	eltor	P^+	+	+	+	*s*	*r*
No. 1957	eltor	P^-	+	+	+	*s*	*r*

– = absence of sex factor or nutritional requirement; *r* = resistant; *s* = sensitive.

TABLE II

Crosses between eltor donor and classical recipient strains of V. cholerae

Cross	Temp. in °C	Viable donors per ml	No. of recombinants per 10^8 donor cells			No. of pmx-r recipients per 10^8 donor cells
			arg^+	his^+	ilv^+	
$P^+ \times P^-$	37	$1{\cdot}42 \times 10^8$	0	0	0	$1{\cdot}00 \times 10^4$
(KB 408 × KB 9)	46	$9{\cdot}00 \times 10^7$	$1{\cdot}79 \times 10^4$	$2{\cdot}15 \times 10^4$	$1{\cdot}15 \times 10^4$	$8{\cdot}33 \times 10^5$
$P^- \times P^-$	37	$1{\cdot}00 \times 10^8$	0	0	0	$7{\cdot}58 \times 10^3$
(No. 1957 × KB 9)	46	$8{\cdot}54 \times 10^7$	0	0	0	$7{\cdot}30 \times 10^4$

Notes : 1. The donor strains were incubated at 37° C/46° C in a water bath (Tecam® Tempunit Techne Duxford-Cambridge, England and subsequently incubated at 37° C or one hour prior to mating.
2. Viability of the donor was estimated just before crossing with recipient cells.
3. Recipient strain was maintained at 37° C in all experiments and approximately 10^9 cells/ml of recipient cells were used in crosses.
4. Matings were performed for one hour on millipore membrane.
5. The donors and recipients when plated separately on the selective media did not grow.

Streptomycin was incorporated in the selective media for selective elimination of donor cells.

Recombination frequency was expressed as the number of recombinants per 10^8 donor bacteria present in the initial mating mixture[5]. Similarly, frequency of transfer of pmx-r factor was calculated per 10^8 donor cells present in the initial mating mixture[7].

Results and Discussion

The results of crosses between KB 408 (eltor, P^+, pmx-r, str-s) and KB 9 (classical, P^-, pmx-s, str-r) strains of *V. cholerae* are shown in Table II. The resistance to polymyxin was transferred to sensitive cells with a frequency of $1{\cdot}0 \times 10^4$ per 10^8 introduced viable donor cells, under normal conditions, *i.e.*, when donors were maintained at 37° C. Under these conditions arg^+, his^+ and ilv^+ genes were not transferred (Table II).

The effect of elevated temperature on the donor ability to transfer pmx-r factor was examined by incubating KB 408 cells at 46° C for 20 min prior to mating (as shown in materials and methods). The frequency of transfer of pmx-r factor was enhanced by 83·3 folds as compared to the frequency occurred at 37° C. Under these conditions, *i.e.*, when donors were pre-incubated at 46° C for 20 min arg^+, his^+, ilv^+ genes were transferred at a frequency lower than the frequency of pmx-r factor. The faster rate of pmx-r factor transfer as compared to that of chromosomal markers in the presence of P factor, indicated the extra-chromosomal nature of the gene. Similar observation was made in Enterobacteriaceae members[8].

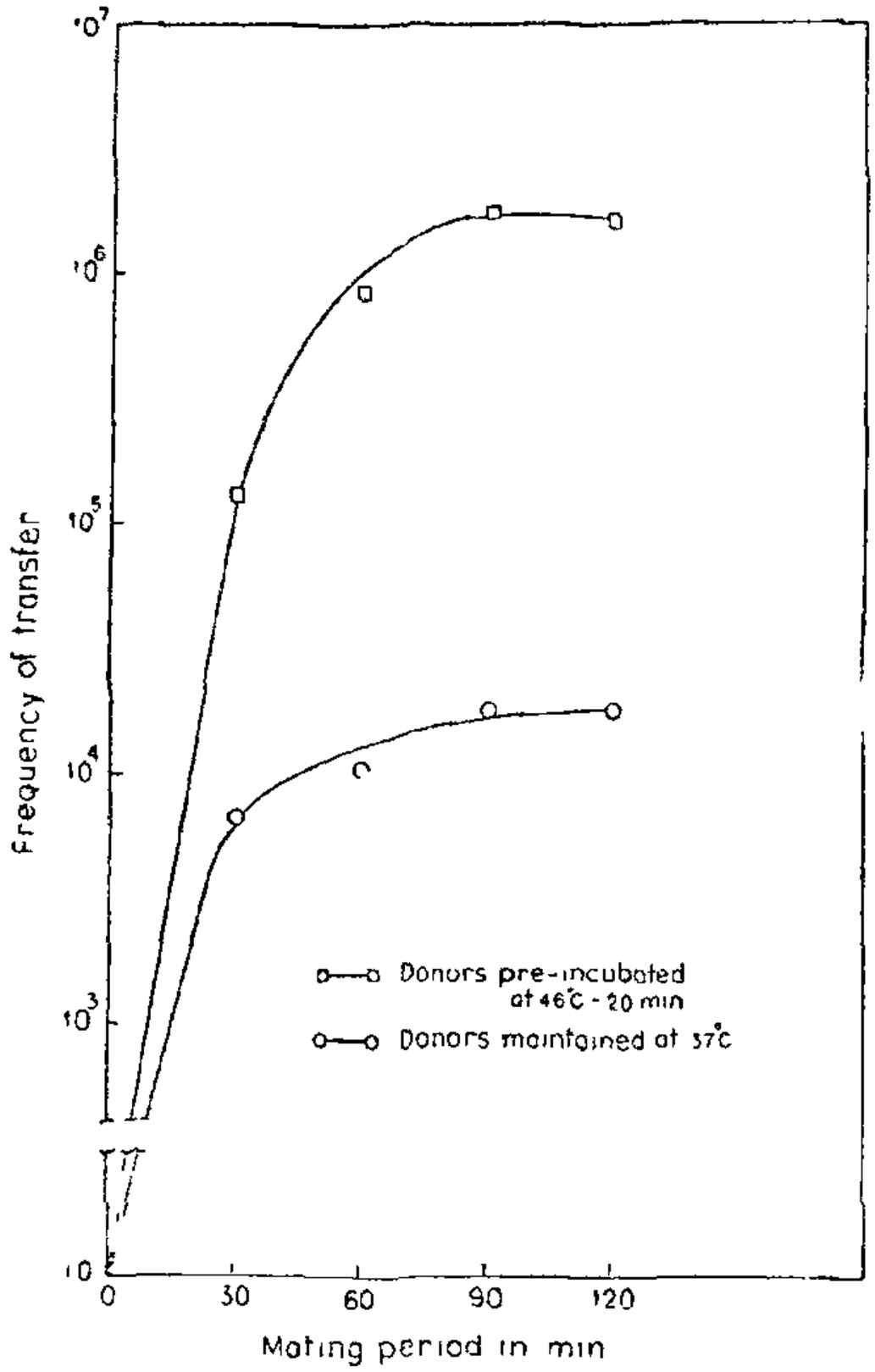

FIG. 1. Transfer of polymyxin resistant factor in crosses between eltor P^+ and classical P^- strains of *V. cholerae*.

To assess the role of P factor in the transfer of pmx–r factor, crosses between No. 1957 (P^-, pmx–r, str–s) and KB 9 (P^-, pmx–s, str–r) strains were carried out under normal conditions and also incubating the donors at 46° C for 20 min prior to mating. As shown in Table II, the pmx–r factor was transferred regardless of the presence of P factor suggesting that P factor was not essential for the transfer of resistant factor unlike for the transfer of chromosomal genes. A similar situation was found in *E. coli* strain where F factor was not necessary for the transfer of drug resistance[7,9]. It should be pointed out that recombinants of arg^+, his^+, and ilv^+ were not found in crosses between $P^- \times P^-$ even though transfer of pmx–r factor took place, indicating that pmx–r factor is an autotransferable extra-chromosomal element in *V. cholerae* which failed to bring maleness to its host. These results are in accordance with the observations made in members of Enterobacteriaceae[7–11].

An enhancement in the transfer of pmx–r factor was found when the resistant donors were pre-incubated at 46° C for 20 min (Table II). Such an enhancement in transfer of resistance factors was also reported in the case of Enterobacteriaceae members when the donor cells were u.v. irradiated prior to mating[12]. The precise molecular events which bring about enhancement in transfer are not known, however, it is suggested that some protein repressor (inhibitor) may be involved[13]. The repressor could be inactivated by heat or u.v. irradiation which in turn might enhance the process of transfer of resistance factors.

Conjugation between KB 408 (P^+) and KB 9 (P^-) and between No. 1957 (P^-) and KB 9 (P^-) were interrupted (by agitation of the mating mixture) at 0, 30, 60, 90 and 120 min of mating to determine the kinetics of transfer of pmx–r factor. The results of these experiments are presented in Figs. 1 and 2. There was a sharp increase in the frequency of transfer of pmx–r factor up to 90 min and no further change at 120 min of mating. About 3–4 colonies were always detected in all these crosses at zero time of mating which might be due to an additional mating on the plates after bacteria have been separated[7,14]. Thus lack of definite time schedule during conjugation as indicated in Figs. 1 and 2 also supports extra-chromosomal nature of this factor.

Further confirmation on the extra-chromosomal nature of pmx–r factor was obtained from Curing

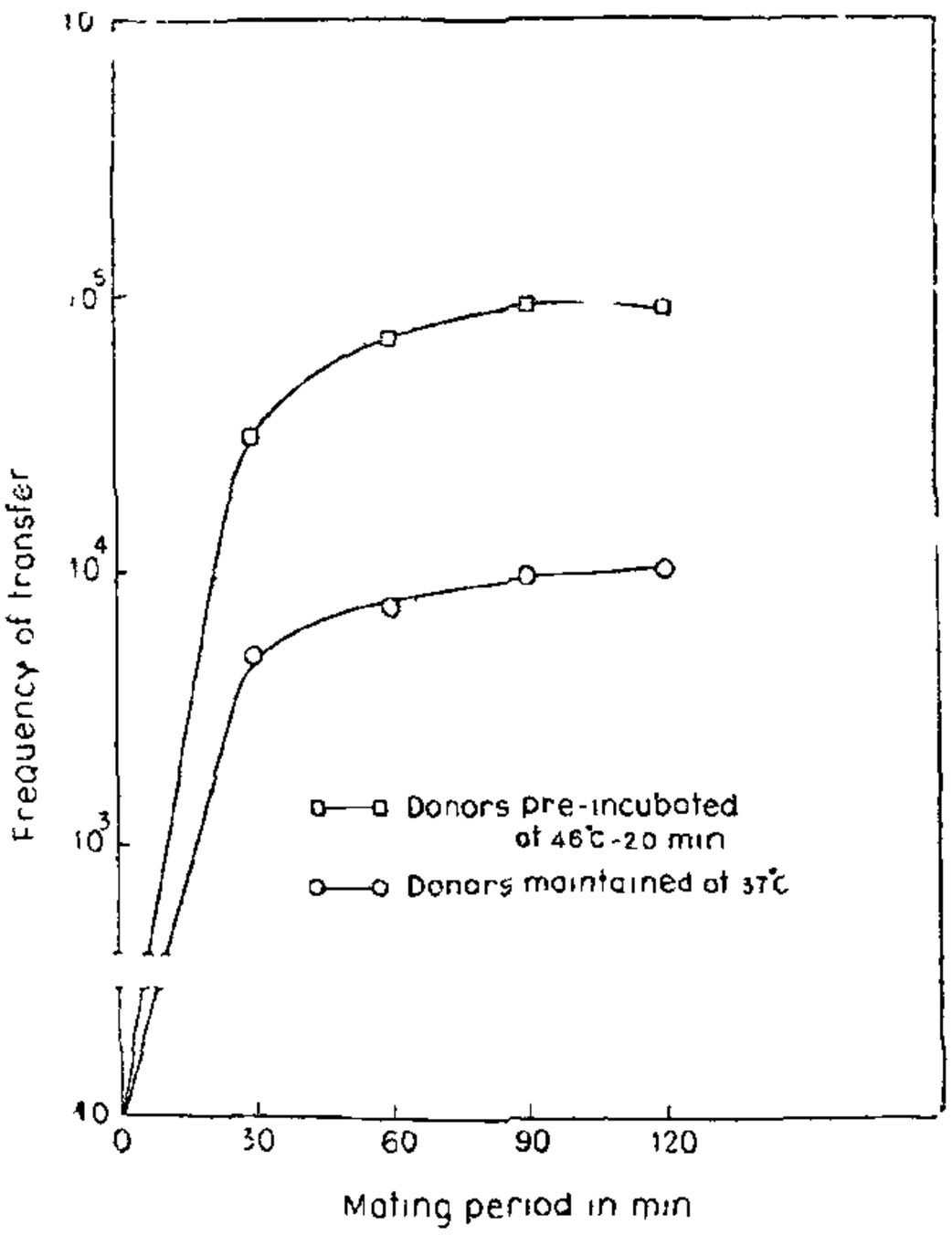

FIG. 2. Transfer of polymyxin resistant factor in crosses between eltor P^- and classical P^- strains of *V. cholerae*.

experiments, where pmx–r donors were treated with acridine orange and acriflavin, which resulted in elimination of this factor (under publication).

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CHANGES IN THE PROTEIN CONTENT OF THE MATERNAL AND EMBRYONIC TISSUES OF THE VIVIPAROUS SCORPION *HETEROMETRUS FULVIPES* DURING GESTATION PERIOD

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ABSTRACT

Changes in the protein content of the maternal and embryonic tissues of *Heterometrus fulvipes* have been followed during the gestation period.

The protein content of the hepatopancreas of the mother increases upto the 4th stage, followed by a decrease upto 6th, subsequent to which, a gradual increase is observed upto the time of parturition. The haemolymph protein also increases upto the 4th stage, beyond which a steady decrease continues upto parturition. The pedipalpal muscle shows no marked changes in the protein content.

A continuous increase of protein is noticed in the embryos throughout the gestation period. However, when expressed per gram wet weight a decline is recorded between the 4th and 5th stages. A comparison of the pattern of variations of proteins in the maternal tissues with that of the embryos lends no support for the supply of proteins from maternal stores to any significant extent. Embryonic requirements are suggested to be met by the dietary proteins of the mother directly.

INTRODUCTION

THE pattern of variations in the protein content of the maternal and embryonic tissues of viviparous forms is studied only in a few insects[1–5] and in mammals[6]. Storage of nitrogen by the maternal animal during pregnancy is known in mammals. Supply of proteins to the embryos in the form of albumin, transferrin and γ-globulin through yolk sac splanchnopleure is also well documented[7–9]. As in mammals, storage of proteins during pregnancy and utilization at times of increased embryonic requirements or maternal starvation is known in insects also[1–3].

Conversion of amino acids into other forms of storage material to sustain the embryonic development is also known amongst insects[5]. But for these few

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