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# Cytogenetic Assays of the Mutagenic Potential of the Bacterium, *Pseudomonas aeruginosa* in Five Species of Experimentally Treated Fish

G. K. Manna and S. Biswas

Department of Zoology, University of Kalyani,
Kalyani 741 235, India

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Among over 140 species belonging to *Pseudomonas*, *P. aeruginosa* (*Bacillus pyocyaneous*) was isolated first more than a century ago as a causative factor of the bluish-green discolouration of surgical dressing (Eykyn 1985). It was found to produce chloroform soluble characteristic pigment "Pyocyanin" and to be pathogenic to man (Cruickshank 1970) and few congeneric species to fish (Richards and Roberts 1978). *P. aeruginosa* is present in small number in normal intestinal flora and on skin of man and animals. Some mutant strain has been found important in oil industry. The antibiotic, Pyocyanin (Brathwaite and Cunningham 1982) and the antifungal activity of the fluorescent pigment (Misaghi *et al.* 1981) of *P. aeruginosa* were also reported. *Pseudomonas* could be found in sewage and they cause plant diseases (Paton 1960). *P. aeruginosa* infection in mouse (Chatterlee 1987) and in fish (Richards and Roberts 1978, Pal and Pal 1986) was claimed to produce tumour. They are very adaptive and can utilize over 80 different organic compounds for growth. A number of enzymes and toxins including endotoxins in addition to the slime seemed to produce pathological effect. Three exotoxins, A, B, and C, lethal to mice and dogs have been identified. However, the genotoxic active principle of *P. aeruginosa* remained unknown.

Some genetical studies on *P. aeruginosa* have been carried out (Brown *et al.* 1979). Studies of clastogenic, mutagenic and teratogenic potential of this species on mice as experimental model not only in directly treated specimens but also in their $F_1$ and $F_2$ generations by a battery of mutagenicity testing protocols (Manna 1980, 1986a, 1989, Dey 1981, Chatterjee 1987) yielded very positive results. We reported earlier (Manna and Biswas 1986) by the micronucleus test the genotoxic potential of *P. aeruginosa* in four species of treated fish while the results of elaborate studies on mutagenic potential of this bacterial species obtained from a battery of tests have been presented hereunder indicating its wide range of mutagenic potential in terrestrial mice and aquatic fish.

## Materials and methods

The bacterium, *Pseudomonas aeruginosa* belonging to family Pseudomonadaceae and section 4—gram negative bacillus measures between 0.5 $\mu$ and 1.5 $\mu$, and moves by a single polar flagellum and produces pigments—pyocyanin and fluorescin in culture medium (Holt 1984). A loopful pure stock of solid slant peptone culture of *P. aeruginosa* was first subcultured in 5 ml sterile liquid peptone medium and after second subculturing a sample of 0.1 ml was put into 50 ml medium. The log phase of growth reached after 5 hr contained approximately $17 \times 10^7$ cells per ml as determined by dilution plating method. Living sepcimens of the fish, the female mouth brooding freshwater tilapia, *Oreochromis mossambicus* (Trewavas 1982); the climbing perch, *Anabas testudineus*; three species of Indian major carps, *Labeo rohita*, *Catla catla* and *Cirrhinus mrigala* were individually injected intraperitoneally with this

*P. aeruginosa* sample at the rate of 1 ml per 100 g body weight in all cases, while for the study of the dose-dependent effect 1/2 ml, 1 ml and 2 ml doses were injected individually @ 1 ml per 100 g b. w. to different sets. Parallely normal specimens of these five freshwater species individually injected intraperitoneally with same rate of sterile peptone medium served as controls.

(a) *Somatic chromosome preparation*: Gill epithelia of both *O. mossambicus* and *A. testudineus* and kidney cells of the latter species were collected and suspended in 1 % sodium citrate solution from individual specimen at 2 hr after injecting 0.1 % colchicine solution @ 1 ml per 100 gm b. w. The cell suspension was fixed in acetic alcohol (1 : 3), spread on slides by flame-drying and stained by diluted Giemsa stain (1 : 20) following mammalian method.

(b) *Germinal chromosome preparation*: The cell suspension of testes of the same colchicine pretreated male *O. mossambicus* of control and *P. aeruginosa* series prepared separately in sodium citrate solution was subjected to acetic acid-flame drying-Giemsa staining schedule like that of somatic cells.

(c) *Preparation for sperm head abnormality*: The method developed and described by us (Manna and Biswas 1988) has been followed in control and *P. aeruginosa* treated male *O. mossambicus* only.

(d) *Preparation for micronucleus test* (*MNT*): Cells of peripheral blood of all the five species of fish and in addition cell suspension of gill and kidney of *O. mossambicus* and *A. testudineus* of control and *P. aeruginosa* treated specimens were smeared separately on slides and air-dried over-night followed by a dip into methanol, air-dried and then stained with Wright's stain (a double stain made up of oxidized methylene blue and eosin y) for 5 min, rinsed in water and restained in diluted 1 % Giemsa (1 : 20) for 10 min, rinsed in water, air dried and finally mounted in DPX for scoring of data.

(e) *The lethal test*: Following Manna and Sadhukhan (1986, 1992) peptone medium injected control and *P. aeruginosa* treated male *O. mossambicus* were mated separately with normal virgin females. The individual mother after 48 hr of mouth brooding were made to release eggs into a petridish (Fig. 23). On release of the entire brood, the number of unfertilized (whitish in texture) and fertilized eggs was recorded and after 72 hr of development the number of hatched out larvae and the dead ones was recorded. In the pooled data, the mutagenic index (M. I.) and the induced lethal (I. L.) frequencies for unfertilized eggs and dead embryos of control and treated series were determined in $F_1$.

For brevity abbreviations used in the text, tables and figures are: GEC=Gill Epithelial Cell; KC=Kidney Cells; PE=Peripheral Erythrocytes; M=Marker and NM=Nonmarker chromosomes; CB=Chromatid/Chromosome Break; AF=Acentric Fragment; TR=Translocations like; CF=Centric Fusion; R=Ring and TA=Terminal Association/Fusion; PSA=precocious separation of autosomes; C=Constriction; G=Gap; NUM=Polyploidy/Aneuploidy; MIS=Stickiness/Pycnosis; MN=Micronucleus; SHA=Sperm Head Abnormality; MI=Mutation Index %; IL=Induced Lethal %. The five species-wise mutagenicity testing results of peptone medium control and *P. aeruginosa* treated series have been presented in the following.

## Results and comments

1. *Oreochromis mossambicus*: (Fam. Cichlidae)

This species has most extensively been used by us (Manna 1986b) as experimental model because of the advantages like (i) small size, low cost and easy availability; (ii) thrived well when introduced in India; (iii) live in fresh water but hardy to withstand experimental stress and water quality; (iv) consumed in many countries and used in mass culture and model in genetic

study (Wohlfarth and Hulata 1981); (v) karyotype of 2n=44 chromosome includes a pair of very large marker chromosomes; (vi) can be bred in aquarium, 2–3 broods per year; (vii) mouth brooding habit very suitable for lethal test etc.

(a) *Chromosome aberrations in gill epithelial cells (GEC)*: In spite of some differences in morphological details claimed by different workers, the diploid number of 44 chromosomes in both sexes with no sex chromosomal demarcation but with an outstandingly large pair of subtelocentric M and 21 NM pairs having variable morphology and size (Figs. 1, 2) was agreeable (Manna and Som 1982). An assessment of 200 metaphase plates of GEC each of 1/2 ml, 1 ml and 2 ml doses of peptone medium control and *P. aeruginosa* injected specimens yielded variable number and types of aberrations (Table 1), such as CB (Figs. 3, 9), ICB (Fig. 5), AF (Fig. 9), TR as TA (Fig. 4), R (Fig. 6) and CF (Figs. 8, 9), C (Fig. 7), G, polyploidy (Fig. 10),

Table 1. Frequency of chromosome aberrations in gill epithelia of peptone control (data in brackets) and *P. aeruginosa* treated *O. mossambicus* assessed at 24 hr

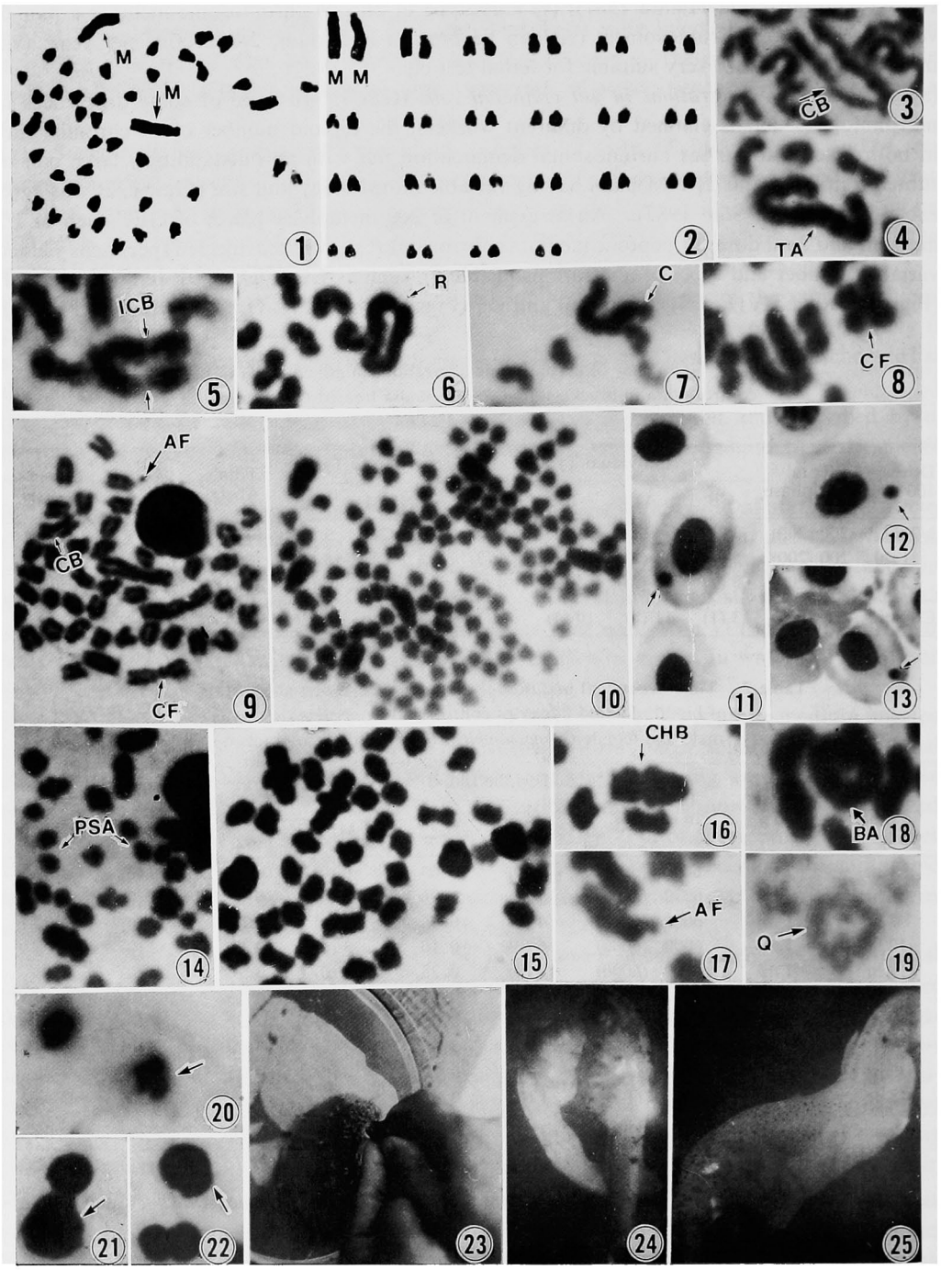
| Dose/ 100 ml | Total metaphase | Individual Type | | | | Gross Type | | Total Aberr | % | Significance |
|---|---|---|---|---|---|---|---|---|---|---|
| | | CB | AF | TR | C & G | NUM | MIS | | | |
| 1/2 ml | 200 (200) | 3 (—) | 2 (2) | 1 (1) | 2 (2) | 3 (2) | 9 ( 7) | 20 (14) | 10.0 (7.0) | P>0.05 |
| 1 ml | 200 (200) | 5 (—) | 4 (1) | 4 (1) | 4 (3) | 3 (2) | 15 (10) | 35 (17) | 17.5 (8.5) | P<0.005 |
| 2 ml | 200 (200) | 5 (1) | 5 (2) | 5 (2) | 4 (2) | 3 (2) | 16 ( 9) | 38 (18) | 19.0 (9.0) | P<0.005 |
| Combi. | 600 (600) | 13 (1) | 11 (5) | 10 (4) | 10 (7) | 9 (6) | 40 (26) | 93 (49) | 15.5 (8.1) | P<0.001 |

Table 2. Time-dependent and dose-dependent effect assessed by MNT in cells of blood, gill and kdney of control and *P. aeruginosa* treated male and female *O. mossambicus* (Control data in brackets)

| Fixa. time | Dose/ 100 g | Total cells in each tis. | Periphe Blood | | Gill | | Kidney | |
|---|---|---|---|---|---|---|---|---|
| | | | MN | % net incre | MN | % net incre | MN | % net incre |
| 1 hr | 1 ml | 6000 (6000) | 2 (0) | 0.03 | 0 (0) | 0 | 0 (0) | 0 |
| 3 hr | 1 ml | 6000 (6000) | 4 (0) | 0.06 | 2 (0) | 0.03 | 1 (0) | 0.01 |
| 8 hr | 1 ml | 6000 (6000) | 4 (0) | 0.06 | 3 (0) | 0.05 | 1 (0) | 0.01 |
| 12 hr | 1 ml | 6000 (6000) | 6 (0) | 0.10 | 3 (0) | 0.05 | 2 (0) | 0.03 |
| 18 hr | 1 ml | 6000 (6000) | 17 (0) | 0.28 | 16 (0) | 0.26 | 8 (0) | 0.13 |
| 24 hr | 1/2 ml | 6000 (6000) | 7 (0) | 0.11 | 8 (0) | 0.13 | 5 (0) | 0.08 |
| | 1 ml | 6000 (6000) | 19 (0) | 0.31 | 20 (1) | 0.32 | 8 (0) | 0.13 |
| | 2 ml | 6000 (6000) | 20 (0) | 0.33 | 20 (2) | 0.30 | 8 (0) | 0.13 |
| 30 hr | 1 ml | 6000 (6000) | 15 (0) | 0.25 | 9 (0) | 0.15 | 4 (0) | 0.06 |
| 48 hr | 1 ml | 6000 (6000) | 1 (0) | 0.01 | 0 (0) | 0 | 0 (0) | 0 |
| Combi. | 1 ml | 48000 (48000) | 68 (0) | 0.14 | 53 (1) | 0.10 | 24 (0) | 0.05 |

stickiness etc. The data (Table 1) showed that the treatment of *P. aeruginosa* induced significantly high frequency of chromosome aberrations than that of control in 1 ml, 2 ml and combined data of 3 doses but it was not significant in 1/2 ml dose. The aberration frequencies increased from lower to higher doses of treatment but it was not strictly linear and the peptone medium seemed to have milder genotoxic potential as compared to normal fish. Further, the M pair was more vulnerable to genotoxic effect of *P. aeruginosa*. Out of 23 CB, C and G, 11 were observed in M pair against the expected number of only 1 as the proportion of M to NM was 1: 21. Thus the breaks appeared to be nonrandomly distributed.

(b) *MNT in cells of blood, gill and kidney*: The genotoxic potential of *P. aeruginosa* in four




Figs. 1–25. 1, normal metaphase (2n=44) of *O. mossambicus*. 2, karyotype including 1 M and 21 NM pairs of chromosomes in gill from *O. mossambicus*. 3–25, various cytogenetic effects in *O. mossambicus* treated with *P. aeruginosa*: 3–8, parts more magnified. 9–10, full metaphase complements of gill showing various representative types of chromosome aberrations—3, a CB in a M. 4, TA between two Ms. 5, ICB in a M. 6, R formed by a M. 7, C in a M. 8, CF between two nonmarker chromosomes. 9, full plate showing a CF, a CB and an AF. 10, a hexaploid metaphase indicated by the 6 Ms. 11–13, micronucleus indicated by arrows in cells of peripheral

species of fish by MNT was reported by us (Manna and Biswas 1986) which has more elaborately been studied in *O. mossambicus* presented here. Qualitatively the MN in PE (Fig. 11), GE (Fig. 12) and KC (Fig. 13) did not differ in any demarcable way among three tissues of control and *P. aeruginosa* treated specimens but it differed in quantitative data (Table 2).

The MNT data (Table 2) revealed that peptone medium injected control *O. mossambicus* had no MN cell in blood and kidney and meagrely 1 in a total of 48000 cells of gill; for time-dependent effect the treatment of 1 ml dose of *P. aeruginosa* 24 hr was the peak hr in all the three tissues and the effect was practically the same in PE and GE which was more than double the frequency in KC. The MN cells were found between 3 hr and 30 hr in all the 3 tissues but in PE it was found even at 1 hr and 48 hr. In the combined data of 8 intervals, out of the total 48000 cells of control and treated specimens, the MN frequency was signicantly high ($p<0.001$) in PE, GE and KC of treated specimens and among these three tissues no significant differential tissue sensitivity was found in the frequency of MN cells of blood and gill ($p>0.05$) but it was highly significant ($p<0.001$) between blood and kidney and also between gill and kidney cells. Therefore, the MNT for time-dependent effect rendered support to the mutagenic potential of *P. aeruginosa* which was also found for chromosome aberrations in GE and also revealed that MN might not necessarily be formed after 1st mitosis as suggested in mammals. It seemed in some case MN was formed by the disrupted metaphase plates after treatment. MNT could be studied in any form of cell which undergoes mitosis as found here in PE, GE and KC but their sensitivity might differ. Further, MNT data of dose-dependent effect in blood, gill and kidney for the treatment of 1/2 ml, 1 ml and 2 ml doses of *P. aeruginosa* to *O. mossambicus* assessed at 24 hr (Table 2) showed that the increase in frequency of MN cells was very striking from 1/2 ml to 1 ml but from 1 ml to 2 ml dose the increase was negligible in PE, decreased in GE and unaltered in KC indicating that perhaps 1 ml dose was the optimum one. Anyhow, the data of dose-dependent effect also supported the mutagenic potential of *P. aeruginosa*, the KC were least susceptible while PE and GE were almost equally and more susceptible among the three tissues tested for the treatment of three doses.

(c) *Chromosome aberrations in first spermatocyte metaphase*: Since chromosome aberrations in meiotic cells induced by a mutagenic agent have special significance connected with reproduction, it was studied in testes of control and *P. aeruginosa* treated male *O. mossambicus* at 24 hr after injection. An assessment of 200 plates of metaphase I revealed significantly high frequency of chromosome aberrations ($P<0.005$) than that of control with 55 plates comprising 8 break type, 4 physiological type, 30 numerical changes, 4 miscellaneous types and 11 cases of precocious separation of autosomes (Figs. 14–19) making a total of 57 or 28.5% aberrations in treated males against 35 plates affected in control comprising 2 break type, 25 numerical changes, 3 miscellaneous and 5 cases of PSA making a total of 35 or 17.5% aberrations. Because of the overcondensed condition of the bivalents, the chromosome aberrations were sometimes not so clear like that of somatic metaphase. Most of the polyploid metaphase I originated possibly by cell fusion because the marker bivalents served as a good indicator (Fig. 15). Rarely they formed a quadrivalent structure (Fig. 19). The frequency of chromosome aberrations was relatively high in meiotic cells than that of GE of both control and treated specimens but as the data were limited, we did not consider the difference was due to the differential sensitivity of the two tissues. Anyhow the data of chromosome aberrations

blood, gill and kidney respectively. 14, metaphase I showing PSA. 15, a tetraploid metaphase I indicated by two M bivalents. 16–19, more magnifiied parts of metaphase I showing in 16, a chromosome break in M pair, 17, an AF, 18, BA (bivalent association) between M and NM bivalents and 19, a quadrivalent-like structure (Q) in Ms. 20–22, sperm with abnormal head morphology shown by arrows. 23, method of releasing eggs from mouth cavity. 24, a siamese twin fused ventrally. 25, an abnormal embryo with defective skeleton.

in metaphase I also supported the mutagenic potential of *P. aeruginosa.*

(d) *Sperm head abnormality (SHA)*: The technique and the data of SHA in normal, control and *P. aeruginosa* injected male *O. mossambicus* have already been presented elsewhere by us (Manna and Biswas 1988) which revealed in the form of irregular contour (Fig. 20), polyploid constitution (Figs. 21, 22) etc., the frequency of which did not show significant difference. It seemed that 24 hr treatment had no effect on SHA as compared to other tests mentioned above. Therefore, SHA could not be considered as supportive evidence to mutagenic potential of *P. aeruginosa* as found in mice also at 24 hr (Chatterjee 1987). Possibly the data indicated that mature sperm were not vulnerable to the effect of the treatment of *P. aeruginosa* like mice but the timetable of spermatogenesis in *O. mossambicus* has not yet been worked out as done in mouse. It was reported that about 11.5 days were taken from leptotene to reach the end of spermatogenesis in male *O. mossambicus* by radioautographic study (Ghosal *et al.* 1983) but the result needed verification.

Table 3. Lethal test data combined of 20 females each after mating to 8 control and 8 *P. aeruginosa* treated male parents for 4 weeks

| Mati week | Series | Mothers | Total | | M.I. % | I.L. % | Total | | | M.I. % | I.L. % |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | Brood | Un E | | | Fe E | Ha L | De E | | |
| | Cont | 20 | 4484 | 10 | 0.22 | | 4474 | 4463 | 11 | 0.24 | |
| 1 wk | | | | | | 0.98 | | | | | 0.58 |
| | Trea | 20 | 4635 | 56 | 1.20 | | 4579 | 4541 | 38 | 0.82 | |
| | Cont | 20 | 4401 | 10 | 0.22 | | 4391 | 4382 | 9 | 0.20 | |
| 2 wk | | | | | | 0.45 | | | | | 0.39 |
| | Trea | 20 | 4527 | 31 | 0.68 | | 4496 | 4469 | 27 | 0.60 | |
| | Cont | 20 | 4783 | 12 | 0.25 | | 4771 | 4760 | 11 | 0.23 | |
| 3 wk | | | | | | 0.32 | | | | | 0.27 |
| | Trea | 20 | 4362 | 25 | 0.57 | | 4337 | 4315 | 22 | 0.50 | |
| | Cont | 20 | 4014 | 7 | 0.17 | | 4007 | 4003 | 4 | 0.09 | |
| 4 wk | | | | | | 0.26 | | | | | 0.22 |
| | Trea | 20 | 4087 | 18 | 0.44 | | 4069 | 4056 | 13 | 0.31 | |
| | Cont | 80 | 17682 | 39 | 0.22 | | 17643 | 17608 | 35 | 0.19 | |
| Combi. | | | | | | 0.52 | | | | | 0.37 |
| | Trea | 80 | 17611 | 130 | 0.73 | | 17481 | 17381 | 100 | 0.57 | |

Abbreviation: M.I.=Mutation Index; I.L.=Induced Lethal; Un E=Unfertilized eggs; Fe E=Fertilized eggs; Ha L=Hatched out larvae; De E=Dead embryos.

Formulae Used: $\text{M.I. (Eggs)} = \frac{\text{Unfertilized eggs}}{\text{Total brood}} \times 100$; $\text{M.I. (Embryos)} = \frac{\text{Dead embryos}}{\text{Fertilized eggs}} \times 100$

$$\text{I.L. (Eggs)} = \left(1 - \frac{\text{Fert. eggs/Total brood in treated series}}{\text{Fert. eggs/Total brood in control series}}\right) \times 100$$

$$\text{I.L. (Embryos)} = \left(1 - \frac{\text{Total hatched out larvae/fertilized eggs in treated series}}{\text{Total hatched out larvae/fertilized eggs in control series}}\right) \times 100$$

(e) *Lethal test*: One set of 8 mature males in peptone medium control and another set of 8 mature males in *P. aeruginosa* treated series were individually injected intraperitoneally 1 ml dose per 100 g b. w. and each male was mated with 4 normal virgin females. Each day impregnated females were individually removed to separate aquarium and the vacancy was replaced by the fresh normal virgin females so as to enable the same male to mate consecutively for 4 weeks. Following the method stated elsewhere (Manna and Sadhukhan 1986, 1992), collection of the brood after 48 hr of mouth incubation, determination of fertilized and unfertilized eggs on releasing the brood into pertridish and the determination of the number

of hatched out larvae and the dead embryos after 72 hr of development in water into petridish were made and the week-wise data of 20 each of control and treated series mothers were combined and presented.

An analysis of the lethal data (Table 3) would reveal that the frequency of unfertilized eggs as well as the dead embryos was always higher in treated series than that of control in each of the 4 weeks but the difference fluctuated. In order to have larger sample the data of four weeks were combined, which showed that the frequency of dead embryos as well as unfertilized eggs between control and treated series was significantly high ($p<0.001$) in *P. aeruginosa* treated series. Therefore, it could be suggested that the mutagenic factor induced by the treatment of *P. aeruginosa* was transmitted to $F_1$ leading to significantly high rate of fertilization failure forming unfertilized eggs and dead embryos during development of the fertilized eggs. As 80 mothers of control and treated series yielded average number of eggs per mother $221.02\pm5.02$ and $220.13\pm5.58$ respectively, it seemed that there was no difference in the egg laying potentiality in two series. Lastly, though we recorded the number of dead embryos during development of the fertilized eggs, no critical attempt was made to evaluate the frequency of abnormal larvae. They were likely to be present as some abnormal larvae were encountered during the study (Figs. 24, 25).

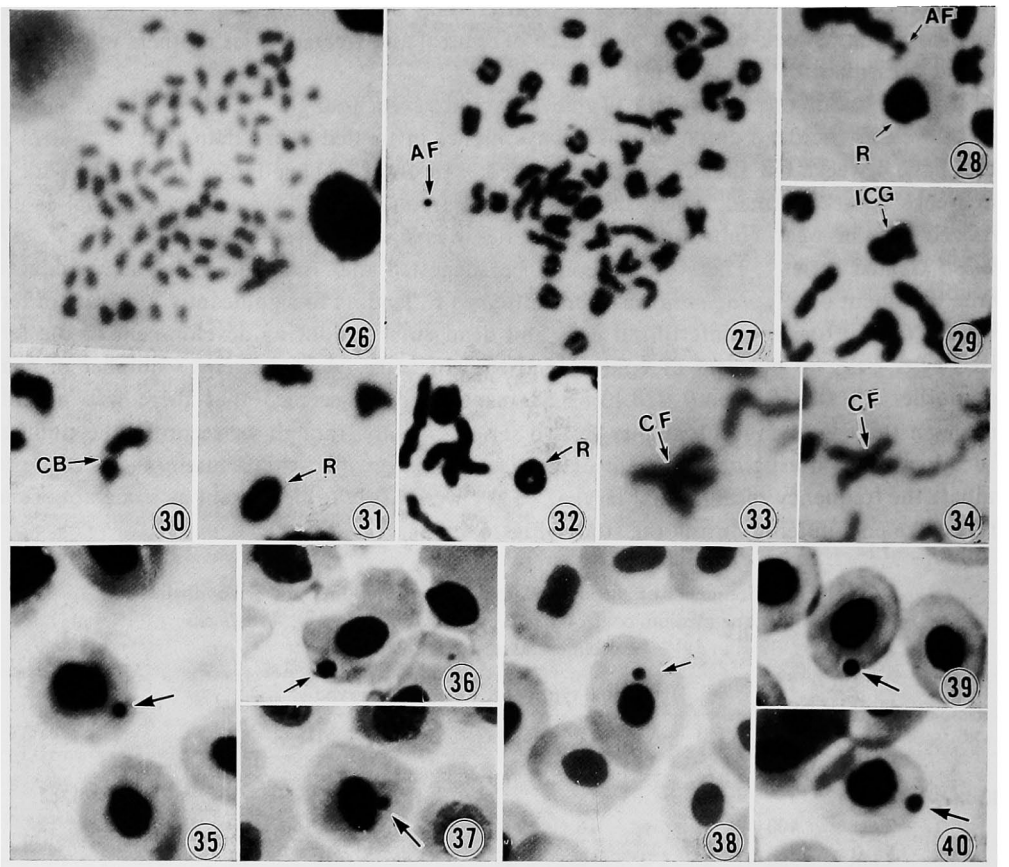
Table 4. Frequency of chromosome aberrations in kidney and gill epithelia of peptone medium control and *P. aeruginosa* injected *A. testudineus* specimens assessed at 24 hr

| Tissue | Series | No of metap | Individual type | | | | Gross type | | Total Aberr | % | Net incr % in Trea |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | CB | AF | TR | C and G | NUM | MIS | | | |
| Kidney | Cont | 400 | 1 | — | — | 6 | — | 14 | 21 | 5.2 | 12.2 |
| | Trea | 400 | 8 | 6 | 16 | 17 | 1 | 22 | 70 | 17.5 | |
| Gill | Cont | 300 | 2 | 2 | 2 | 1 | — | 10 | 17 | 5.6 | 8.7 |
| | Trea | 300 | 7 | 4 | 6 | 5 | 2 | 19 | 43 | 14.3 | |

2. *Anabas testudineus*: (Fam. Anabantidae)

The live-fish climbing perch, *A. testudineus* possess $2n=46$ rodlike chromosomes of more or less gradual seriation (Manna and Prased 1973). Adult specimens were individually injected intraperitoneally at the rate of 1 ml per 100 g b. w. sterile peptone medium to serve as control and log culture of *P. aeruginosa*. The mutagenic potential of *P. aeruginosa* was tested by somatic chromosome aberrations and MNT in *A. testudineus* from the preparations made following the methods described before.

(a) *Somatic chromosome aberrations*: The chromosome aberrations in kidney cells at 8 intervals between 15 min and 7 day in specimens intraperitoneally injected with the dose of 1 ml per 100 g b. w., of *P. aeruginosa* were reported by us (Biswas and Manna 1989) but presently it was studied in gill epithelia of *P. aeruginosa* treated *A. testudineus* at 24 hr and the data were compared with that of kidney cells scored at 24 hr (Table 4). Qualitatively both the tissues had the same types of aberrations comprising CB (Fig. 30), ICG (Fig. 29), AF (Figs. 27, 28), TR like R (Figs. 28, 31, 32), CF (Figs. 33, 34), C, G as individual types and polyploidy (Fig. 26), stickiness etc. as gross types but the frequencies differed indicating kidney cells were relatively more vulnerable than that of gill epithelia. However, the genotoxic potential of *P. aeruginosa* was found in both the tissues and if the differential response to the two tissues was due to the differential tissue sensitivity could not be definite because the data were limited.




Figs. 26–40. Some cytogenetic effects in *A. testudineus* treated with *P. aeruginosa*. 26, a polyploid metaphase. 27, an AF. 28–34, more magnified part of metaphases showing in 28 a R and an AF. 29, an ICG. 30, a CB. 31, a R, 32, a R and 33, 34, each with a CF. 35–37, MN indicated by arrows in 35, blood, 36, gill and 37, kidney cell. 38–40, MN in blood cells of *L. rohita*, *C. catla* and *C. mrigala* respectively marked by arrows.

Table 5. MNT data at various intervals in blood, gill and kidney cells of *A. testudineus* treated with *P. aeruginosa* (Since equal number of cells in control had no MN, data are omitted in table)

| Fixa time | Cells per tissue exam. | Blood | | Kidney | | Gill | |
|---|---|---|---|---|---|---|---|
| | | MN | % | MN | % | MN | % |
| 1 hr | 6000 | 2 | 0.03 | 0 | 0 | 0 | 0 |
| 8 hr | 6000 | 1 | 0.01 | 0 | 0 | 0 | 0 |
| 18 hr | 6000 | 8 | 0.13 | 3 | 0.05 | 3 | 0.05 |
| 24 hr | 6000 | 10 | 0.16 | 5 | 0.08 | 4 | 0.06 |
| 30 hr | 6000 | 6 | 0.10 | 2 | 0.03 | 1 | 0.01 |
| 48 hr | 6000 | 4 | 0.06 | 0 | 0 | 0 | 0 |
| Combi. | 36000 | 31 | 0.08 | 10 | 0.02 | 8 | 0.02 |

(b) *MNT*: The MNT revealed that MN was found almost exclusively in cells of blood (Fig. 35), gill (Fig. 36) and kidney (Fig. 37) of *P. aeruginosa* treated *A. testudineus* while none in peptone controls assessed in 6000 cells of each tissue at 1 hr, 8 hr, 18 hr, 24 hr, 30 hr and 48 hr. In treated specimens the frequencies varied at different intervals with the peak at 24 hr

(Table 5). The MNT data (Table 5) in *A. testudineus*, like that of *O. mossambicus* showed the same trends with little less effect for the treatment of *P. aeruginosa* as in the combined data the frequency of MN erythrocytes and that of gill and kidney was significantly high ($p < 0.001$) but not between gill and kidney; MN could be seen at different time but it was more palpable around peak at 24 hr and so on. Anyhow, MNT and somatic chromosome aberration study revealed the mutagenic potential of *P. aeruginosa*.

3. Three species of Indian major carps, *L. rohita*, *C. catla* and *C. mrigala*, 2n=50 (Fam: Cyprinidae)

*MNT*: The data presented before (Manna and Biswas 1986) have been extended more, which revealed no difference in the number and morphology of MN (Figs. 38–40) in the three species and out of 12000 cells of peripheral erythrocytes assessed in each species not a single MN was found in peptone control, while in *P. aeruginosa* treated specimens 53 in *L. rohita*, 51 in *C. catla* and 22 in *C. mrigala*, indicating thereby the genotoxic potential of *P. aeruginosa* in all of them but it was almost equal in *L. rohita* and *C. catla* and more than double of *C. mrigala*.

That the mutagenic potential of *P. aeruginosa* is very far and wide because the results were positive whenever one or more tests were conducted in experimental mouse or several species of fish. However, the genotoxic active principle of *P. aeruginosa* remained unknown as was the case for nearly 2 dozens of bacterial species tested by us on mice system. In general the log culture and isolated bacteria in saline suspension were higher or equally genotoxic, followed by culture filtrate while the heat killed sample or specific toxin as PPD in tubercular bacilli, vaccine (BCG) etc. were less genotoxic; sometimes no difference was found from control. The sonicated bacterial cells yielded more chromosome aberration than cell wall fraction in treated mice. Therefore, it would take time to know precisely the active genotoxic principle associated with bacterial species.

## Summary

Various species of the bacteria including *Pseudomonas aeruginosa* have been recognized as pathogens of fish. The mutagenic potential of *P. aeruginosa* log culture ($17 \times 10^7$ cells/ml) has been assessed after intraperitoneal injection @ 1 ml per 100 g b. w. to cichlid mouth brooding fresh water tilapia, *O. mossambicus* by chromosome aberrations in gill epithelia and first spermatocyte metaphase, micronucleus test (MNT) in peripheral blood, gill epithelia and kidney cells, and lethal test for unfertilized eggs and dead embryos during development after mating treated male parents with normal virgin females. Moreover, dose dependent effect of 1/2 ml, 1 ml and 2 ml doses for chromosome aberrations in gill epithelia and MNT in cells of blood, gill and kidney were found to be highly positive against respective sterile peptone medium injected controls. The sperm head abnormality test yielded no positive result. Further, the intraperitoneal injection of log culture of *P. aeruginosa* of 1 ml dose to Indian climbing perch, *A. testudineus* yielded positive results in chromosome aberrations studied from kidney and gill epithelia and MNT in cells of blood, gill and kidney. The three species of Indian major carps, *L. rohita*, *C. catla* and *C. mrigala* also yielded positive genotoxic potential of *P. aeruginosa* by MNT. There was intraspecific tissue sensitivity among blood, gill and kidney cells found in MNT of *O. mossambicus* and interspecific sensitivity shown by MNT of blood of three species of Indian major carps. The mutagenic potential of *P. aeruginosa* evaluated by a battery of tests in directly treated and in $F_1$ and $F_2$ generations of experimental mouse recorded before revealed that *P. aeruginosa* can act as a potential mutagen to fish in aquatic media and to mice in terrestrial one. Thus 'Microbes as living Mutagens' advocated by Manna (1980, 1989) has been supported from the present result on fish.

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