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Cytologia **58**: 9–19, 1993

Mutagenic Potential of the Non-nodulous Nitrogen-fixing Bacterium, *Xanthobacter flavus* in Treated Male Parent Mice and Verified to Their F_1 Progeny

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Accepted October 12, 1992

The genotoxic potential of log culture and other samples of the bacterium, *X. flavus* in treated male and female mice was reported positive earlier (Sadhukhan and Manna 1986, 1992). Even the leucocyte cultures of normal human when contaminated with different samples of *X. flavus*, the results were also positive (Manna and Sadhukhan 1992). The present study has been aimed at to verify further the mutagenic potential of *X. flavus* in treated male parent mice after mating for 7 weeks with virgin untreated females and to their F_1 progeny. That 'Microbes as Living Mutagens' advocated by Manna (1973) has been evidenced uptill now in one or more mutagenicity tests deployed on various mammalian models infected and/or treated with samples of some 40 species of animal viruses, 31 species of bacteria, 10 species of lower fungi, 4 species of parasitic protozoans (Manna 1992a, b) and of late 3 species of algae viz., unicellular *Euglena* sp (Manna and Mohanty 1992) and 2 prokaryotic cyanophytans, *Plectonema baryanum* and *Anacystis nidulans* (Manna and Mohanty unpublished). Thus all groups of microbes (Cruickshank 1970) have been covered in showing mutagenic potential in mammals, specially in mice system. The paper presented here has a special importance because the mutagenic effect of microbes has rarely been followed up in successive generation.

Materials and methods

Laboratory bred Swiss albino mice, *Mus musculus* were used as experimental model. The log culture (20×10^7 cells per ml) of the non-nodulous nitrogen-fixing gram-negative aerobic bacterium, *Xanthobacter flavus*, a member of the alpha subclass within the Proteobacteria (Malik and Claus 1979), was intraperitoneally injected @ 1ml per 100 g b. w. into 10 male parent mice and parallely as control another 10 male mice were injected at the same rate with sterile Burk's modified N_2-free medium for N_2-fixer. The treated and control male parent mice were mated separately to different sets of 30 virgin untreated normal females per week for 7 consecutive weeks to cover the cycle of mouse spermatogenesis (Adler 1982). Cytogenetic assays of 8 treated and 5 control male parents after 7 weeks mating programme, and in F_1 generation (I) the lethal test in vivisected pregnant females at 15 day of gestation and at 1 month after parturition and (II) the cytogenetic assays of some of the living embryos and adults were conducted. Since the methods of cytogenetic assays of male parents and F_1 progeny were the same, both the data of each test have been presented in the same table to facilitate direct comparison.

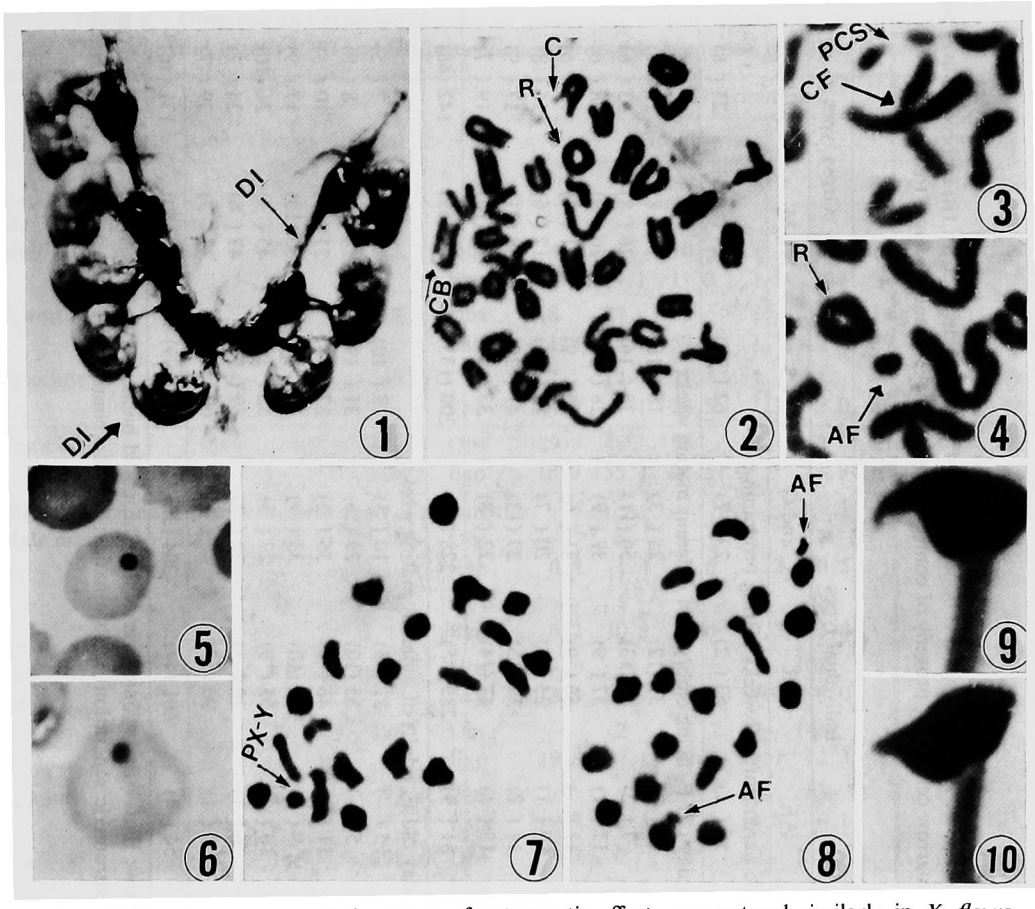
The methods described by Manna (1985) were followed here. (I) The lethal test in F_1 progeny before and after parturition for mating of control and *X. flavus* treated male parents with separate sets of virgin normal females and (II) the cytogenetic assays of male parents after mating programme and some of the F_1 living progeny of control and treated series were conducted. The standard colchicine-sodium citrate-acetic alcohol-flame dry-Giemsa stained

Table 1. Data of lethal test in F_1 generation assessed on 15 day of gestation and 30 day after parturition in pregnant mice for mating of normal virgin females with separate sets of control and *X. flavus* treated males

Mat week	Series	15day gestation				After parturition				Combined data				Per mother			%	
		Mo	Imp	LI	DI	Mo	Imp	LI	DI	Mo	Imp	LI	DI	Imp	LI	DI	MI	IL
1wk	Co	2	15	15	—	3	17	17	—	5	32	32	—	6.40	6.40	—	—	—
	Tr	3	17	17	—	3	12	12	—	6	29	29	—	4.83	4.83	—	—	—
2wk	Co	3	21	21	—	2	13	13	—	5	34	34	—	6.80	6.80	—	—	11.00
	Tr	5	34	32	2	4	22	18	4	9	56	50	6	6.22	5.55	0.66	10.71	—
3wk	Co	3	25	25	—	3	16	16	—	6	41	41	—	6.83	6.83	—	—	16.00
	Tr	6	37	31	6	4	14	12	2	10	51	43	8	5.10	4.30	0.80	15.68	
4wk	Co	2	16	15	1	4	21	21	—	6	37	36	1	6.16	6.00	0.16	2.70	2.06
	Tr	4	27	25	2	3	16	16	—	7	43	41	2	6.14	5.85	0.28	4.65	
5wk	Co	3	19	19	—	3	14	14	—	6	33	33	—	5.50	5.50	—	—	—
	Tr	7	41	41	—	5	21	21	—	12	62	62	—	5.16	5.16	—	—	
6wk	Co	3	23	23	—	4	23	23	—	7	46	46	—	6.57	6.57	—	—	24.00
	Tr	9	67	52	15	6	27	20	7	15	94	72	22	6.26	4.80	1.46	23.40	
7wk	Co	3	22	22	2	3	19	18	1	6	41	38	3	6.83	6.33	0.50	7.31	13.04
	Tr	5	31	24	7	4	21	18	3	9	52	42	10	5.77	4.66	1.11	19.23	
Total	Co	19	141	138	3	22	123	122	1	41	264	260	4	6.43	6.34	0.09	1.53	11.22
	Tr	39	254	222	32	29	133	117	16	68	387	339	48	5.69	4.98	0.70	12.40	

Abbreviations: Mat=Mating; Co=Control; Tr=Treated; Mo=Mother; Imp=Implant; LI=Living implant; DI=Dead implant; MI=Mutation index; IL=Induced lethal.

preparations for somatic chromosome aberrations (SCA) of adult bone marrow (BM) and embryonic liver (EmL) cells, May-Gruenwald-Giemsa stained smear preparations for micronucleus (MN) test (T) of polychromatic (P) and normochromatic (N) erythrocytes (E) in BM and embryonic peripheral blood (EmPB); seminiferous tubules fixed in acetic alcohol, squashed and stained in Giemsa for meiotic chromosome aberration (MCA) and sperm suspension of individual epididymis made in 8 ml distilled water smeared on slides and stained with Giemsa for sperm head abnormality (SHA) were carried out separately for control and treated series specimens.



Figs. 1–10, Some representative types of cytogenetic effects encountered similarly in *X. flavus* culture treated male parent mice and to their F_1 progeny. 1, vivisected uteri of a treated series pregnant female showing DI by a gap and another with the impaired umbilical cord. 2–4, metaphases of BM and EmL cells showing CB, AF, R, CF, C and PCS types chromosome aberrations. 5–6, MNE in EmPB and BM respectively. 7–8, metaphase I showing PX-Y and AF respectively. 9–10, each showing a SHA.

Results

(I) Lethal test

Out of the total 210 normal virgin females put for mating separately with 10 control and 10 *X. flavus* treated male parents for 7 consecutive weeks, 121 (57.6%) females in control and 93 (44.2%) females in treated series became pregnant, indicating a fall of 13.4% fertility in *X. flavus* treated series against controls. Among 121 in control and 93 in treated series pregnant females, 19 and 39 at 15 day of gestation and 22 and 29 at 1 month after parturition

Table 2. Somatic chromosome aberrations in (A) bone marrow of male parents, and in (B) embryonic liver and (C) adult bone marrow cells of F_1 progeny of control (data in brackets) and *X. flavus* treated series

Mat time	Total metaphases	Aberrations								
		Individual types					Gross types		Total	Net % incr
		CB	AF	TR	C and G	PCS	NUM	MISC		
(A)	BM chromosome aberrations in male parents after 7 weeks mating programme									
50d	800 (500)	25 (1)	16 (1)	53 (2)	23 (2)	62 (8)	24 (6)	23 (8)	266 (28)	22.65
(B)	EmL chromosome aberrations in 15 day old F_1 living embryos of different mating weeks									
1wk	1000 (800)	18 (1)	12 (2)	37 (12)	25 (3)	18 (18)	19 (8)	10 (6)	139 (50)	7.65
2wk	1900 (1000)	37 (1)	7 (2)	46 (13)	56 (11)	38 (16)	26 (8)	33 (6)	243 (57)	7.08
3wk	1500 (1200)	21 (—)	17 (1)	37 (9)	37 (9)	28 (23)	26 (9)	28 (6)	194 (57)	8.18
4wk	1900 (700)	21 (1)	11 (—)	56 (9)	41 (6)	23 (9)	41 (4)	19 (6)	212 (35)	6.15
5wk	1000 (1000)	3 (1)	10 (1)	23 (13)	20 (7)	19 (26)	17 (6)	16 (8)	108 (62)	4.60
6wk	1300 (1400)	18 (2)	11 (2)	39 (15)	27 (13)	41 (20)	38 (21)	17 (9)	191 (82)	8.80
7wk	1400 (1000)	6 (2)	13 (1)	47 (14)	22 (8)	33 (21)	17 (13)	19 (7)	157 (66)	4.61
Tot	10000 (7100)	124 (8)	81 (9)	285 (85)	228 (57)	200 (133)	184 (69)	142 (48)	1244 (409)	6.68
(C)	BM chromosome aberrations in 90 day old F_1 adults of different mating weeks									
1wk	700 (700)	2 (1)	5 (—)	21 (3)	10 (3)	18 (10)	26 (9)	7 (5)	89 (31)	8.29
2wk	900 (900)	6 (1)	5 (—)	55 (10)	29 (6)	31 (19)	24 (5)	8 (8)	158 (49)	12.11
3wk	1000 (800)	13 (1)	11 (—)	46 (4)	35 (2)	27 (18)	21 (3)	10 (5)	163 (33)	12.18
4wk	1300 (1200)	7 (1)	9 (1)	64 (10)	33 (9)	35 (14)	50 (13)	17 (9)	215 (57)	11.78
5wk	1200 (1000)	4 (1)	10 (1)	55 (9)	27 (5)	52 (16)	45 (7)	26 (10)	219 (49)	13.35
6wk	1400 (800)	10 (—)	6 (1)	70 (3)	22 (2)	59 (14)	47 (6)	24 (2)	238 (28)	13.50
7wk	1200 (700)	11 (—)	8 (1)	90 (5)	48 (5)	69 (8)	23 (7)	39 (7)	288 (33)	19.29
Tot	7700 (6100)	53 (5)	54 (4)	401 (44)	204 (32)	291 (99)	236 (50)	131 (46)	1376 (280)	13.20

Abbreviations: BM = Bone marrow; EmL = Embryonic liver; CB = Chromatid break; AF = Acentric fragment; TR = Translocation; C and G = Constriction and Gap; PCS = Precocious centromeric separation of chromatids; NUM = Numerical changes; MISC = Miscellaneous types.

respectively were assayed. Thus, of total pregnant females 33.8% in control and 73.2% in treated series were sacrificed using variable numbers at different mating weeks (Table 1). The uteri of treated series mothers contained relatively more dead implants (DI) of various forms as a gap for its complete absorption between other developing foetuses (Fig. 1), a small scar, some without umbilical blood supply, heart-beat stopped, discoloured blackish large sac around DI for accumulation of fluid and so on. The number of DIs and the frequency of mutation index (MI) were relatively high in treated series than those of controls, rendering the elevated frequencies of induced lethals (IL) in 2nd, 3rd, 4th, 6th and 7th weeks, while there was no sign

Table 3. Observed and expected number of group-wise and/or region-wise distribution of (A) chromatid breaks (CB) and (B) precocious centromeric separation (PCS) of chromatids

Sample	Tissue	Series	Total		Distribution							
					Group-wise					Region-wise		
					I	II	III	IV	V	Prox	Mid	Dist
(A) Chromatid breaks												
Male parents	BM	Tr	22	Obs	4	4	10	3	1	2	10	10
				Exp	2.2	4.1	11.0	3.3	1.3	7.3	7.3	7.3
F_1 embryos	Liv	Tr	118	Obs	18	27	65	7	1	14	46	58
				Exp	11.8	22.1	59.0	17.7	7.3	39.3	39.3	39.3
F_1 adults	BM	Tr	49	Obs	7	17	21	3	1	5	20	24
				Exp	4.9	9.1	24.5	7.3	3.0	16.3	16.3	16.3
Combined			189	Obs	29	48	96	13	3	21	76	92
				Exp	18.9	35.4	94.5	28.3	11.8	63.0	63.0	63.0
(B) Precocious centromeric separation of chromatids												
Male parents	BM	Co	8	Obs	—	—	—	1	7			
				Exp	0.8	1.4	4.0	1.2	0.5			
〃	〃	Tr	62	Obs	—	1	—	13	48			
				Exp	6.2	10.8	31.0	9.3	3.8			
F_1 embryos	Liv	Co	133	Obs	—	—	2	23	108			
				Exp	13.3	24.9	66.5	19.9	8.0			
〃	〃	Tr	198	Obs	—	2	3	47	146			
				Exp	19.8	37.1	99.0	29.7	12.3			
F_1 adults	BM	Co	99	Obs	—	—	—	16	83			
				Exp	9.9	18.5	49.5	14.8	6.1			
〃	〃	Tr	291	Obs	—	2	5	46	238			
				Exp	29.1	54.5	145.5	43.6	18.1			
Combined		Co	240	Obs	—	—	2	40	198			
				Exp	24.0	45.0	120.0	36.0	15.0			
〃		Tr	551	Obs	—	5	8	106	432			
				Exp	55.1	103.2	275.5	82.6	34 4			

of lethality for mating in 1st and 5th weeks (Table 1). In the combined data of 7 weeks, the number of DIs was significantly high in treated series than that of control (Tou-test, $P<0.001$), and the frequency of MI was 12.40% in treated against 1.53% in control rendering 11.2% as IL (Table 1).

(II) Cytogenetic assays

The *X. flavus* treated male parents and the F_1 living progeny of treated series revealed more or less the same types of qualitative effect, some representatives of which have been presented (Figs. 2–10), but their frequencies considerably differed (Tables 2–6).

(a) Somatic chromosome aberration (SCA)

The SCAs found in cells of F_1 EmL and BM of adults and of male parents were commonly categorized as individual types comprising chromatid break (CB, Fig. 2), acentric fragment (AF, Fig. 4), translocation (TR) in the form of ring (R, Figs. 2, 4) and centric fusion (CF, Fig. 3), constriction (C, Fig. 2), gap (G) and precocious centromeric separation of chromatids (PCS, Fig. 3) and gross types comprising numerical change (NUM) in the form of polyploidy and

Table 4. Micronucleus (MN) test (T) in poly- and normo-chromatic erythrocytes of (A) BM of male parents, and (B) EmPB and (C) BM of F_1 progeny of control and treated series

Mat time	Series	Erythrocytes									
		Polychromatic			Normochromatic			Combined			
		Total	MN	%	Total	MN	%	Total	MN	%	% incr
(A) MNT in BM of male parents after 7 weeks mating programme											
50d	Co	12500	9	0.07	12500	14	0.11	25000	23	0.09	0.44
	Tr	20000	113	0.56	20000	99	0.49	40000	212	0.53	
(B) MNT in peripheral blood of 15 day old F_1 living embryos of 7 mating weeks											
1wk	Co	20000	7	0.03	20000	13	0.06	40000	20	0.05	0.19
	Tr	25000	50	0.20	25000	72	0.28	50000	122	0.24	
2wk	Co	20000	11	0.05	20000	25	0.12	40000	36	0.09	0.40
	Tr	30000	147	0.49	30000	152	0.50	60000	299	0.49	
3wk	Co	30000	21	0.07	30000	33	0.11	60000	54	0.09	0.59
	Tr	30000	193	0.64	30000	217	0.72	60000	410	0.68	
4wk	Co	20000	17	0.08	20000	23	0.11	40000	40	0.10	0.35
	Tr	30000	159	0.53	30000	117	0.37	60000	272	0.45	
5wk	Co	25000	29	0.11	25000	26	0.10	50000	55	0.11	0.75
	Tr	40000	376	0.94	40000	315	0.78	80000	691	0.86	
6wk	Co	30000	34	0.11	30000	40	0.13	60000	74	0.12	0.99
	Tr	35000	398	1.13	35000	381	1.08	70000	779	1.11	
7wk	Co	20000	27	0.13	20000	19	0.09	40000	46	0.11	0.67
	Tr	20000	173	0.86	20000	141	0.70	40000	314	0.78	
Tot	Co	165000	146	0.08	165000	179	0.10	330000	325	0.09	0.59
	Tr	210000	1496	0.71	210000	1391	0.66	420000	2887	0.68	
(C) MNT in BM of 90 day old F_1 adults of 7 mating weeks											
1wk	Co	5000	—	—	5000	3	0.06	10000	3	0.03	0.21
	Tr	10000	29	0.29	10000	20	0.20	20000	49	0.24	
2wk	Co	5000	7	0.14	5000	5	0.10	10000	12	0.12	0.30
	Tr	5000	23	0.46	5000	19	0.38	10000	42	0.42	
3wk	Co	5000	10	0.20	5000	—	—	10000	10	0.10	0.45
	Tr	10000	47	0.47	10000	64	0.64	20000	111	0.55	
4wk	Co	5000	8	0.16	5000	13	0.26	10000	21	0.21	0.32
	Tr	10000	59	0.59	10000	48	0.48	20000	107	0.53	
5wk	Co	10000	11	0.11	10000	10	0.10	20000	21	0.10	0.54
	Tr	10000	73	0.73	10000	56	0.56	20000	129	0.64	
6wk	Co	10000	19	0.19	10000	26	0.26	20000	45	0.22	0.79
	Tr	10000	112	1.12	10000	91	0.91	20000	203	1.01	
7wk	Co	10000	11	0.11	10000	17	0.17	20000	28	0.14	0.80
	Tr	10000	99	0.99	10000	89	0.89	20000	188	0.94	
Tot	Co	50000	66	0.13	50000	74	0.14	100000	140	0.14	0.49
	Tr	65000	442	0.68	65000	387	0.59	130000	829	0.63	

Table 5. Male meiotic chromosome aberration data in (A) parents and (B) F_1 progeny of control and terated series mice

Mat time	Sr	Spermatogonial meta					Diakinesis					Metaphase I					Metaphase II				
		Total plate	Aberration				Total plate	Aberration				Total plate	Aberration				Total plate	Aberration			
			Ind	Gr	Tot	Net %		Ind	Gr	Tot	Net %		Ind	Gr	Tot	Net %		Ind	Gr	Tot	Net %
(A)	Male meiotic chromosome aberrations in partents																				
50d	Co	176	4	6	10	15.73	112	8	1	9	22.03	300	8	4	12	23.33	87	1	3	4	14.10
	Tr	258	25	38	63		163	43	6	49		300	66	16	82		123	13	10	23	
(B)	Meiotic chromosome aberrations in F_1 males of different weeks of mating																				
2wk	Co	67	3	1	4	11.88	105	7	2	9	10.09	200	5	1	6	8.50	81	1	1	2	12.40
	Tr	56	4	6	10		150	21	7	28		200	17	6	23		74	8	3	11	
3wk	Co	84	3	3	6	13.91	140	3	—	3	6.08	200	5	4	9	9.50	73	3	1	4	18.82
	Tr	95	12	8	20		165	17	6	23		200	22	6	28		66	11	5	16	
4wk	Co	53	1	2	3	13.57	112	7	1	8	16.66	200	7	4	11	15.50	95	1	2	3	13.51
	Tr	78	12	3	15		126	27	3	30		200	30	12	42		120	16	4	20	
5wk	Co	83	2	2	4	14.75	200	9	1	10	13.94	200	6	—	6	10.00	56	3	—	3	8.62
	Tr	138	10	17	27		190	27	9	36		200	23	3	26		93	11	2	13	
6wk	Co	79	2	2	4	15.94	160	8	—	8	20.16	200	8	—	8	15.50	74	5	1	6	13.39
	Tr	119	14	11	25		155	36	3	39		200	34	5	39		107	20	3	23	
Comb	Co	366	11	10	21	14.22	717	34	4	38	14.55	1000	31	9	40	11.80	379	13	5	18	13.33
	Tr	486	52	45	97		786	128	28	156		1000	126	32	158		460	66	17	83	

aneuploidy and miscellaneous ones (MISC) like stickiness, pycnoses *etc.* In treated male parents even after 7 weeks mating programme, the frequency of each type of SCAs was strikingly high than that of controls, and in total the difference was statistically highly significant ($P<0.001$). The net increase was 22.65% (Table 2 A). Similarly, in F_1 living progeny of treated series, the frequency of each type of SCA both in EmL and BM cells at each of 7 weeks mating was always higher than respective controls. In the combined data, the difference was significantly high ($P<0.001$). The net increase in frequency of SCA ranged between 4.60% and 8.80% with an average of 6.68% in EmL, and between 8.29% and 19.29% with an average of 13.20% in BM (Table 2 B, C). Further, in treated series the frequency of SCAs was relatively high in BM than that of EmL cells of each week and in their total (Table 2 B, C).

Among individual types of SCAs, the data of CB (Table 3 A) and PCS (Table 3 B) were tested for their non-random distribution, if any, following the method of Manna (1986). It revealed that CB in chromsomes belonging to groups I and II for group-wise analysis, and the middle and distal regions for region-wise analysis were relatively more vulnerable when the observed numbers were compared with the expected ones (Table 3 A). The data of PCS showed that chromosomes belonging to group V were highly vulnerable than that of group IV,

Table 6. Data of sperm head abnormality in (A) parents and (B) F_1 progeny of control (data in brackets) and treated series male mice

Mat time	No. of sperm	Sperm with abnormal head		
		No.	%	Net % incr.
(A) parents				
50d	8000 (5000)	396 (62)	4.95 (1.24)	3.71
(B) F_1 progeny				
1wk	2000 (2000)	51 (19)	2.55 (0.95)	1.60
2wk	2000 (2000)	58 (13)	2.90 (0.65)	2.25
3wk	2000 (2000)	61 (17)	3.05 (0.85)	2.20
4wk	2000 (2000)	72 (31)	3.60 (1.55)	2.05
5wk	3000 (3000)	96 (12)	3.20 (0.40)	2.80
6wk	3000 (3000)	129 (39)	4.30 (1.30)	3.00
7wk	3000 (3000)	77 (31)	2.56 (1.03)	1.53
Comb	17000 (17000)	544 (162)	3.20 (0.95)	2.25

while those of groups I, II and III were strongly resistant when observed and expected numbers were compared (Table 3 B).

(b) Micronucleus test (MNT)

The MNT in BM of male parents and in F_1 EmPB (Fig. 5) and BM (Fig. 6) of each week mating revealed that in each case the frequency of MN-PE and MN-NE and the total MNE was strikingly high in treated series over controls. The average net increase was 0.44% in male parent, and 0.5% in EmPB and 0.49% in BM of F_1 progeny (Table 4 A, B, C). In total, the difference in frequencies of MNE between control and treated series was highly significant ($P<0.001$) in both EmPB and BM cells of F_1 progeny (Table 4 B, C) and in BM of male parents (Table 4 A). On the whole, the trend of MNT data (Table 4 A, B, C) was complementary to that of SCA (Table 2 A, B, C).

(c) Male meiotic chromosome aberrations (MCA)

As in somatic cells, the types of MCA in different stages (Figs. 7, 8) were basically the same, but the bivalents in first division added some new types like precocious desynapsis of X and Y chromosomes (PX-Y, Fig. 7) and autosomes of some bivalents, multivalent formation *etc.* The occurrence of all other meiotic stages was relatively lower than metaphase I to en-

counter 100 plates (Table 5 A, B). The frequency of individual and gross type MCAs in each stage was always strikingly high in treated series than in controls found in male parents and in F_1 male progeny of each week. In F_1 progeny, though the net increase in frequency of MCA of different stages varied but in combined data of 7 weeks, the average increases were somewhat close to one another except that of metaphase I (Table 5 B). In total, the difference of MCA frequencies between treated and control male parents and that of F_1 males was statistically significant ($P<0.001$), indicating the same trend as found for studies of SCA and MNT.

(d) Sperm Head Abnormality (SHA)

The smear of sperm suspension of male parents and 90 day old F_1 males yielded the same types of SHA (Figs. 9, 10), but the frequencies were significantly high ($P<0.001$) in each treated series than that of controls (Table 6 A, B). In F_1 males of each week mating, the net increase of SHA in treated series over control ranged between 1.53% and 3.00% with an average of 2.26% in the combined data, whereas in male parents it was 3.71% (Table 6 A, B). Thus, the SHA data also showed the same trend of effect as found in all other tests.

Discussion

The log culture of *X. flavus* appeared to have reduced the fertility of the treated male parent mice, because the average number of implants per mother was 5.69% in treated series against 6.43% in controls. Besides that, the strikingly high frequencies of IL in lethal test and effect in various cytogenetic assays of F_1 living progeny of treated series over parallel controls implied that the mutation was induced to the *X. flavus* treated male parent mice. The higher frequencies of effect assessed by various cytogenetic tests in treated male parents after 7 weeks consecutive mating also implied that the effect was present during mating period. If the week-wise lethal test data were correlated with the time-table of mouse spermatogenesis (Adler 1982), it would indicate that the treatment of *X. flavus* had differential meiotic stage sensitivity, because the highest frequency of DIs at 6th week represented the stage of differentiated spermatogonia, the relatively lower frequency in 7th week the stem-spermatogonia and that also in 2nd and 3rd weeks the period for metamorphosis of spermatids, while the lowest frequency in 4th week and no effect in 5th week covered the stages of spermatocytes and that in 1st week represented the stage of already mature spermatozoa in epididymes. However, we refrained from making a definite claim for the differential stage sensitivity for the lethal effect, because the data were not so extensive. But, even if the stage sensitivity was disregarded, the combined data of 7 weeks would leave no doubt about the lethality for mating of *X. flavus* treated male parent mice. The week-wise data of cytogenetic assays of the F_1 embryos and adults, however, did not reflect any differential sensitivity except for in most tests the effect was lowest in 1st among 7 weeks, but the reason was not known.

The types of DIs encountered in vivisected females at the 15th day of gestation during lethal test as well as in embryotoxic effect (Manna and Sadhukhan 1991) were of similar nature, but the frequencies differed. The average number of DIs per mother was 1.10 in treated against 0.10 in control series for embryotoxic effect conducted in normal pregnant mice injected with *X. flavus* at their different days of gestation, while it was 0.70 in treated against 0.09 in control series for lethal test. The difference could be attributed due to some sort of direct effect as the treatment of bacterium to pregnant mice might reach the foetuses through maternal circulation while the effect was indirect being mediated by mutated gene inherited from treated male parent to F_1 progeny through spermatozoa as vehicle in lethal test. The same trend of results was also recorded for the treatment of spores of the fungus, *Aspergillus niger* to mice, bacause in lethal test the number of DIs per mother was 0.75 in treated against 0.07 in control series (Manna and Kundu 1991) while for embryotoxic effect it was 1.67 in treated

against 0.07 in controls (Manna and Kundu 1992 b).

The cytogenetic assays made by deploying SCA, MNT, MCA and SHA between treated male parents and F_1 progeny of treated series revealed the same trend in results. The data of lethal test and cytogenetic assays could be explained by a common hypothesis. Unlike heritable translocation (Cattanach 1982), the treatment of *X. flavus* to male parent mice possibly induced mutation at some genetic locus/loci. The lethal and various cytogenetic effects in F_1 progeny could have been inflicted for the transmission of above-mentioned mutated gene from treated male parent. As advocated elsewhere (Manna 1986, 1989 a) the structural integrity of chromosomes in normal state was controlled by some gene(s) which when mutated under the influence of some mutagen, as in the present case it was associated with *X. flavus*, would loose the control. As a result various types of cytogenetic effects in successive cell divisions might occur in treated male parents, and to the F_1 progeny if they inherited the mutated gene from treated male parents. In F_1 it might cause lethality to some developing embryos due to severe or else vital chromosomal damage which was not possible to verify in dead embryos while its activity in living progeny was followed by different cytogenetic assays. The effect of the gene seemed to be pleiotropic in nature. The results were compatible with similar studies conducted in male parent mice treated with bacterium, *Mycobacterium tuberculosis* and followed in F_1 generation (Manna and Pal 1992), spores of fungus, *Aspergillus niger* (Manna and Kundu 1992 a) and others (Manna 1989 a).

The occurrence of non-random distribution of CB and PCS in parents and progeny could be explained on the basis of the presence of inherent weaker regions in mouse genome (Manna 1989 b). The manifold increase in frequency of PCS in treated male parent and F_1 progeny of treated series when compared with those of controls would render indirect support to the mutagenic potential of *X. flavus*.

Summary

The mutagenic potential of log culture (20×10^7 cells per ml) of the non-nodulous nitrogen-fixing free living bacterium, *Xanthobacter flavus* in treated male parent mice after mating with different sets of virgin untreated normal females for 7 consecutive weeks and in their F_1 progeny verified by lethal test in vivisected mothers and in living ones by various cytogenetic assays was found positive in each test as compared to parallel controls. In male parents as well as in both sexes of F_1 embryos and adults, as the cases might be, the frequencies of chromosome aberration and micronucleated erythrocytes in somatic cells, male meiotic chromosome aberration and sperm head abnormality were strikingly high in treated series than in respective controls, indicating the same trends of cytogenetical effects in parent and F_1 progeny of treated series. The results have been explained with the hypothesis that the treatment of log culture of *X. flavus* to male parent mice induced mutation to some genetic locus/loci which in normal state maintained the structural integrity of chromosome, but when mutated lost the control. This might have led to various cytogenetic effects in F_1 progeny, in some cases caused lethality while in others (living ones) produced visible cytogenetic anomalies of various forms assessed by different testing protocols.

Acknowledgements

Grateful acknowledgements are made to Prof. S. P. Sen, Department of Botany, Kalyani University for supply of the bacterial culture, University Grants Commission and Indian National Science Academy, New Delhi for financial assistance and Vidyasagar College, Calcutta for cooperation to the work.

References

Adler, I. D. 1982. Male germ cell cytogenetics. In: Cytogenetic Assays of Environmental Mutagenesis. Ed. T. C. Hsu, Allanheld, (Eds.), Osmun & Co. Publ., Totowa, N. J. p. 249–276.

Cattanach, B, M. 1982. The heritable translocation test in mice. In: Cytogenetic Assays of Environmental Mutagenesis. T. C. Hsu, Allanheld, (Eds.) Osmun & Co Publ., Totowa, N. J. p. 289–323.

Cruickshank, B. 1970. Ed. Medical Microbiology. A Guide to the Laboratory Diagnosis and Control of Infection. 11th edn., The ELBS and E & S, Livingstone Publ. p. 1–1070.

Malik, K. A. and Claus, D. 1979. *Xanthobacter flavus*, a new species of nitrogen-fixing hydrogen bacteria. Int. J. Syst. Bacteriol. **29**: 283–287.

Manna, G. K. 1973. Chromosome Aberrations Induced by Living Mutagens. Presidential Address. Sec. Biol. Sci., 43rd Ann. Sess., Nat. Acad. Sci., India, Jodhpur, pp. 1–15.

— 1985. The living mutagens. In: Laboratory Mannual of Instructional Workshops on Perspectives in Chromosome Research. S. C. Roy (Ed.), Dept. of Botany, Calcutta University, pp. 55–72.

— 1986. Mouse bone marrow as a means of testing clastogenic agents. Nucleus **29**: 141–168.

— 1989a. Microbes as living mutagens and the possibility of genic control of cytogenic effects in treated parents and their progeny. Perspectives in Cytology and Genetics **6**: 47–58.

— 1989b. Chromosome structure, inherent weaker region and fragile site. Presidential address. Proc. Zool. Soc. (Calcutta) **42**: 1–12.

— 1992a. Microbes as environmental mutagens. In: Environmental Mutagenesis. R. C. Sobti (Ed.), Narosa Publ. House, New Delhi, (*in press*).

— 1992b. Microbes—are they potential mutagenic danger to man? Perespectives in Cytology and Genetics **7**: 53–70.

— and Kundu, B. 1991. Lethal test in F_1 and F_2 generations for mating of male parent mice treated with *Aspergillus niger* spore with virgin normal female mice. Proc. Nat. Acad Sci. India, Sec. B Pt II **61**: 169–176.

— and — 1992a. Cytogenetic assays of F_1 progeny produced after mating of *Aspergillus niger* spores treated male parents with virgin normal female mice. Proc. Nat. Acad. Sci. India, Sec. B Pt. II **62**: (*in press*).

— and — 1992b. Assays of embryonic deaths in normal pregnant mice injected at their different days of gestation with spores of two species of fungi. Cytologia **57**: (in press).

— and Mohanty, K. C. 1992. Mutagenic potential of the unicellular alga, *Euglena* sp tested in experimental mice. CIS **52**: 9–11.

— and Pal, S. 1992. Mutagenic induction by the tubercule bacilli to the treated male parent mice verified in F_1 and F_2 living progeny by different cytogenetic assays. Cytologia **57**: 127–142.

— and Sadhukhan, G. C. 1991. Lethality induced to embryos by the bacterium, *Xanthobacter autotrophicus* to normally pregnant female mice at various days of their gestation. CIS, **51**: 10–12.

— and — 1992. Bacterium, *Xanthobacter flavus* induced chromosome aberrations in treated human leucocyte culture. Nucleus **35**: 70–78.

Sadhukhan, G. C. and Manna, G. K. 1986. Clastogenic effects of nitrogen-fixing *Xanthobacter* culture on treated mice. Perspectives in Cytology and Genetics **5**: 943–948.

— and — 1992. Genotoxic potential of different samples of the bacterium, *Xanthobacter autotrophicus* in experimental mice. Perspectives in Cytology and Genetics **7**: 671–680.