SPORES OF THE FUNGUS *ALTERNARIA BRASSICICOLA* AS A CLASTOGEN IN TREATED MICE

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ABSTRACT

The cultured spore suspension (6×10^6/ml) of a fungus, *A. brassicicola* intraperitoneally injected into mice yielded the frequency of affected metaphases in the bone marrow cells with chromosome aberrations like chromatid breaks, acentric fragments, translocation, polyploidy, aneuploidy, precocious centromeric separation etc. The frequency was strikingly high as compared to control and the average net increase over control was 14.6%. The data of clastogenic potentiality of spores of *A. brassicicola* in mice system added another evidence to the genotoxic potentiality of microbes as living mutagens in the environment.

INTRODUCTION

The chromosome-breaking effect in bone marrow cells of mice after treatment with cultured spore suspension of a fungus, *A. brassicicola* has been investigated with a view to extending the data of microbes as living mutagens[1–3]. The genotoxic potentiality of another two species of fungi imperfecti, *Aspergillus fumigatus* and *A. niger* has been shown by more than one mutagenicity test[3–6]. Some mycotoxins specially aflatoxin B_1 was found mutagenic to different testing models by various workers[7–9].

MATERIALS AND METHODS

Alternaria brassicicola belonging to fungi imperfecti is a plant pathogen which also causes respiratory trouble in man on infection. Their spores were cultured in Czapek-Dox glucose-agar slant. On the 7th day after incubation, the spores were isolated in distilled water. The isolated spore suspension (sample of 6×10^6 spores/ml) was intraperitoneally injected into male and female mice at the rate of 1 ml/100 g b.w. A different set of mice injected with culture medium washed distilled water at the same rate served as controls. The chromosome preparations of bone marrow cells of both the control and the treated mice were made following the standard colchicine-sodium citrate-acetic alcohol-flame drying- Giemsa staining schedule to assess the aberration frequency at 8 intervals between 1 hr and 35 day (table 1); 75–100 plates were examined per individual.

RESULTS AND DISCUSSIONS

The chromosome aberrations underwent numerical changes due to aneuploidy and polyploidy (figure 1) and diffuse ones as stickiness, pycnosis etc comprised the gross types (table 1) while constriction (C), gap (G), subchromatid break (SC), chromatid break (CB), acentric fragment (AF), centric fusion (CF) and ring (R) as translocation (T), precocious centromeric separation (PCS) etc involving structural changes as individual types (figures 2–6). Since there were plates with one or more individual type aberrations (figures 2–6), the frequencies were determined for total aberrations as well as the number of affected metaphases irrespective of the number and type of aberrations (table 1). Among the individual types, the frequency of precocious centromeric separation in chromosomes belonging to group V (figure 6) was highest and the next was in group IV[1] but the genetic significance of this type of aberration is debated[10]. The frequency of aneuploid plates was a little more than that of polyploid ones but in one treated female examined on day 7, it was abruptly very high (58%) while in others it was generally 1 or 2%. If the frequency of the total aberrations was considered, including and excluding cases of precocious centromeric separation, it was 14% and 5% in the control series and 22.6% and 14.6% at 1 hr, 32% and 15.3% at 24 hr, 46% and 15% at 48 hr, 38% and 22% at 72 hr, 32% and 27.6% on 7d, 23% and 11.5% on 14d, 38% and 8% on 21d, 41% and 25% on 35d and 32.8% and 18.4% in all the respective intervals of the treated series (table 1). Therefore, the frequency of chromosome aberration with and without cases of PCS was strikingly high as compared to that of control. Similarly if the frequency of the affected metaphases with and without the cases of PCS was considered (table 1), it was 14% and 5% in the control and 22.6% and 14.6% at 1 hr, 26% and 14.6% at 24 hr. 41% and 15% at 48 hr, 36% and 20% at 72 hr, 31% and 28.3% on 7d, 19% and 9.5%

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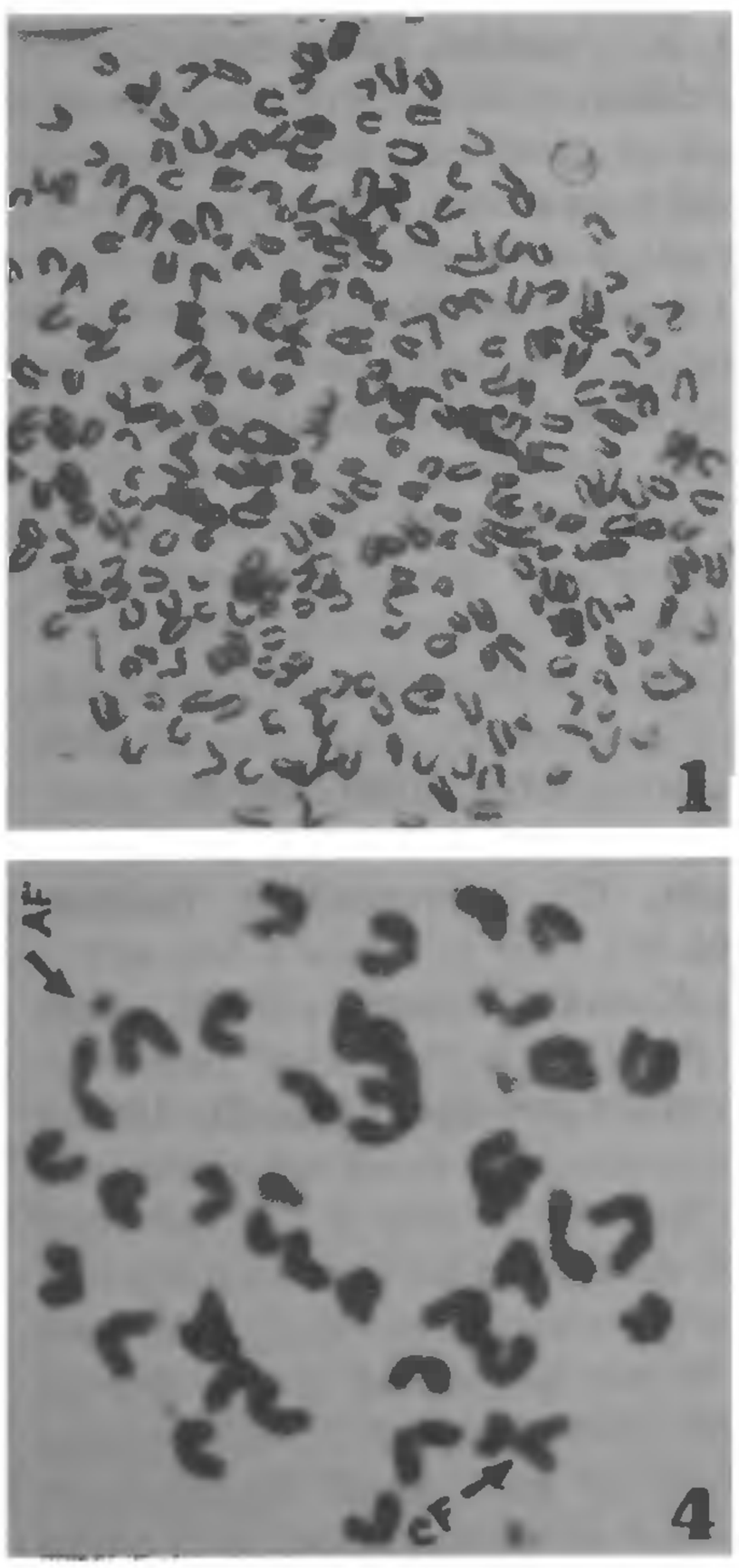
Table 1 *Frequency of chromosome aberrations and affected metaphases in bone marrow of control and A. brassicicola treated mice*

Series	Individual types: Break SC	CB	AF	T	Others C&G	PCS	Gross type: Numerical	Diffuse	Total aberr.	No. of metaphases: Exam	Affected with PCS	Affected without PCS	Net increase %
Control	–	1	–	5	2	18	2	–	28	200	28	10	–
Treated													
1 hr	–	4	–	8	8	12	2	–	34	150	34	22	8.6
24 hr	–	6	1	6	5	25	4	1	48	150	39	22	12.0
48 hr	–	4	1	1	3	31	3	3	46	100	41	15	27.0
72 hr	2	1	–	9	9	16	1	–	38	100	36	20	22.0
7 d	1	11	–	2	8	13	60	1	96	300	93	85	17.0
14 d	–	6	2	6	5	23	2	2	46	200	38	19	5.0
21 d	–	2	1	1	3	30	1	–	38	100	25	8	11.0
35 d	1	6	3	4	9	16	2	–	41	100	38	22	24.0
Total	4	40	8	37	50	166	75	7	387	1200	344	213	14.6



Figures 1–6. Several types of chromosome aberrations in bone marrow cells of mice injected with spores of *A. brassicicola.*

on 14d, 25% and 8% on 21d, 38% and 22% on 35d and 28.6% and 17.7% in the average respectively in the treated series also revealing higher frequency at different intervals than in the control. The net increase in the frequency of affected metaphases with PCS in the treated series over control in the above intervals was 8.6%, 12%, 27%, 22%, 17%, 5%, 11%, 24% and 14.6% in the average (table 1). The data further revealed that the frequency of individual type aberrations except on 7d was much higher than that of gross types and in the treated series, the chromatid break, translocation, constriction and gap, precocious centromeric separation and aneuploidy/polyploidy were found in all fixation intervals showing no significant difference with the lapse of time after treatment of spores of *A. brassicicola* (table 1). Though the frequency at different individuals fluctuated as the increase in percentage data varied erratically, it was still apparent that the genotoxic effect of the treatment of spores continued even on day 35. The occurrence of more or less the same type of aberrations at all fixation intervals might indicate that the affected chromosome or cell did not survive and fresh batch of cells had aberrations as they were entering the division.

The trequency of chromosome aberrations in control specimens was relatively high as compared to that of only distilled water injected control (0.7% average) indicating the presence of some genotoxic agent in Czapeak-Dox medium or else it was due to the metabolic product the nature of which has not yet been resolved. However, in treated series the frequency of aberration or the affected metaphases was strikingly high clearly indicating high genotoxic potentiality of the spores of *A. brassicicola* in the mice system. The agent responsible tor the induction of higher frequency of chromosome aberrations in bone marrow cells of treated mice is not known. It was suspected that spores of *A. brassicicola* after the intraperitoneal injection into mice might have released some genotoxic metabolic products into the system of mice. The specific toxin liberated by *A. brassicicola* is not known while the alternaric acid, a phytotoxin, is liberated by the *Alternaria*. Therefore, further studies are in progress. However, the present study revealed that spores of *A. brassicicola* like that of *A. fumigatus* and *A. niger* belonging to fungi imperfecti migh act as environmental mutagens like some other groups of microbes as reported earlier[3,11].

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