

grown under aerobic condition in nitrogen-free medium was found to be 25 nm/hr/mg of protein.

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EVALUATION OF SOME ANTI-CHOLESTEROL AND ANTI-INFLAMMATORY DRUGS FOR MUTAGENICITY USING *BACILLUS SUBTILIS* HCR-9 MULTIGENE SPORULATION TEST

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NUMEROUS screening tests using a variety of genetic indicator organisms have been developed to identify

potential chemical mutagens. The multigene sporulation test¹ involving about 40–60 genes, detects forward mutations which are readily identified by their lack of brown pigment which normally accumulates in sporulating colonies^{2–5}.

In the present study the mutagenic effect of two anticholesterol drugs viz clofibrate (2,4-chlorophenoxy-2-methyl propanoic acid ethyl ester) and its calcium analog and four anti-inflammatory drugs viz tromaril (N-beta phenyl ethyl anthranilic acid), brufen (2,4-isobutyl phenyl propanoic acid), phenylbutazone (4-butyl-1,2-diphenyl-3,3-pyrazolidinedione) and indomethacin (1-para chlorobenzyl 5-methoxy 2-methyl 3-indolylacetic acid) were evaluated using *Bacillus subtilis* multigene sporulation test. MNNG (N-methyl N-Nitro N-nitroso guanidine) a known mutagen was used as the positive control.

Bacillus subtilis hcr-9 was inoculated in Arret and Kirshbaum medium⁷ and spore stocks of about 10¹⁰ spores/ml were prepared^{1,6}. Seventy µl were taken in Spizizen medium⁸ supplemented with DL tryptophan. Each test compound was evaluated by adding 10 µl of various concentrations (10–100 µl/ml) by employing standard test procedure¹¹ in the presence and absence of S₉ mix^{9,10}. Statistical analysis was done using Kastenbaum and Bowman¹² tables.

The results show that only clofibrate exhibited a marginal significant increase in the percentage of mutants i.e., 0.16 to 0.18% at higher concentrations of

Table 1 Mutagenicity of anti-cholesterol and anti-inflammatory drugs on sporulating genes of *B. subtilis* hcr-9

Durg	Concentration µg/ml	In absence of S ₉ mix			In presence of S ₉ mix		
		Total colonies scored	Total mutants observed	% of mutants	Total colonies scored	Total mutants observed	% of mutants
Control	0	8826	3	0.03	9004	3	0.03
1. Clofibrate	10–40	19523	11	0.06	19168	12	0.06
	60	9785	9	0.09	9286	11	0.12
	80	10026	13	0.13*	9482	12	0.16*
	100	9229	14	0.15*	9095	16	0.18*
2. Clofibrate (ca-salt)	10–100	59618	39	0.07	59329	44	0.08
3. Tromaril	10–100	61071	29	0.05	60343	37	0.06
4. Brufen	10–100	55639	24	0.04	55475	23	0.04
5. Phenylbutazone	10–100	54445	34	0.06	53412	41	0.08
6. Indomethacin	10–100	51628	22	0.04	51989	24	0.05

- Note:*
1. Dimethyl sulfoxide was used for controls as the durgs were dissolved in this solvent.
 2. Similar results for controls obtained in repeated experiments.
 3. *Significant increase in mutants.
 4. Total wild type colonies and mutant colonies obtained at the concentrations of 10, 20, 40, 60, 80 and 100 µg/ml were pooled and average percentage was calculated as the above drugs gave insignificant results except clofibrate which produced significant mutants at higher concentrations.

80 and 100 $\mu\text{l/ml}$ respectively (table 1). However, this drug was not effective in inducing mutations at concentrations of 10 to 60 $\mu\text{l/ml}$. Similar frequency of mutants was obtained in the presence as well as absence of S_0 mix. The mechanism of induction of mutations by clofibrate is not known, however, inhibition of DNA synthesis in *Tetrahymena pyriformis* by this drug is reported.¹³ In Salmonella/microsome assay, clofibrate did not show any mutagenic effect¹⁴ and this discrepancy could be attributed to false negatives in tests which are based on single specific locus^{1, 15-17}.

Calcium analog of clofibrate and the above four anti-inflammatory drugs (tromaril, brufen, phenylbutazone and indomethacin) did not induce any mutations at concentrations ranging from 10 to 100 $\mu\text{l/ml}$ either in the presence or in the absence of S_0 mix.

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A NEW SPECIES OF *PSEUDOCERCOSPORA* SPEG.

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DURING studies of fungi parasitizing phanerogamic flora of Gorakhpur region a parasitic fungus was collected on the leaves of *Casearia elliptica* Willd (Samydaceae). Microscopic examination revealed it to be an undescribed species of genus *Pseudocercospora* Speg which differed from the known species of *Pseudocercospora*¹⁻⁷ in major taxonomical characters. The fungus is characterized by the presence of well-developed stromata; short aseptate, unbranched conidiophores and mostly cylindrical, straight to curved conidia having obtuse to rounded apex (figure 1). There is no previous record of *Pseudocercospora* parasitizing the leaves of *Casearia elliptica*⁸ and therefore, the same is described and illustrated here as a new species.

P. samydacearum sp. nov.

Contagionis maculae, amphigenae, necroticae, irregulare, interdum plus minusve circulare, usque 1.5 cm in diam., albido vel griseae, atra brunnea margine; coloniae plerumque hypophyllae, atra brunneae, per paene totam maculae sparsae; mycelium ex hyphis immersis, fere hyalinis, 1-2 μm cr; stromata evoluta, atra brunnea, pseudoparenchymatica, usque 50 \times 35 μm ; conidiophora macronematica vel semi-macronematica, mononematica, parvea, olivaceo brunnea, fere aseptata, simplicia, haud ramosa, erecta vel flexuosa, leniter flaro ad basim, 8-10 μm longa et 3.5-4.6 μm in diam.; cellulae conidiogenae integratae, terminales, monoblasticae, interdum polyblasticae et sympodiales; cicatrices conidiales non incrassata,