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

26 October 1984


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 test involving about 40–60 genes, detects forward mutations which are readily identified by their lack of brown pigment which normally accumulates in sporulating colonies.

In the present study the mutagenic effect of two anti-cholesterol 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-buty1-1,2-diphenyl-3,3-pyrazolidinedione) and indomethacin (1-para chlorobenzyl 5-methoxy-2-methyl 3-indolyliclastic acid) were evaluated using Bacillus subtilis multigene sporulation test. MNNN (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 medium and spore stocks of about 10¹⁰ spores/ml were prepared. Seventy μl were taken in Spizizen medium supplemented with DL tryptophan. Each test compound was evaluated by adding 10 μl of various concentrations (10–100 μl/ml) by employing standard test procedure in the presence and absence of S₉ mix. Statistical analysis was done using Kastenbaum and Bowman 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

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

Note: 1. Dimethyl sulfoxide was used for controls as the drugs 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 µl ml respectively (table 1). However, this drug was not effective in inducing mutations at concentrations of 10 to 60 µl.ml. Similar frequency of mutants was obtained in the presence as well as absence of S₉ 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. In Salmonella/microsome assay, clofibrate did not show any mutagenic effect and this discrepancy could be attributed to false negatives in tests which are based on single specific locus.

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 µl.ml either in the presence or in the absence of S₉ mix.

The authors are thankful to Dr Dillon of Scandia Laboratories of Albuquerque for providing bacterial strain, RRL, Hyderabad, Boots Company, Suhrid Geigy and Merck Sharp and Dohme Ltd. of Bombay for providing the pure forms of the drugs clofibrate and tromaril, brufen, phenylbutazone and indomethacin respectively.

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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* Wild (Samydaecae). Microscopic examination revealed it to be an undescribed species of genus *Pseudocercospora* Speg which differed from the known species of *Pseudocercospora* in major taxonomical characters. The fungus is characterized by the presence of well-developed stromata; short asceptate, 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* and therefore, the same is described and illustrated here as a new species.

*P. samydaecaeum* sp. nov.

Contagionis maculae, amphigenea, necroticae, irregularare, interdum plus minusve circulare, usque 1.5 cm in diam., albido vel griseae, atra brunnea margine; coloniae plerumque hypophyllae, atra brunnea, per paene totam maculae sparsae; mycelium ex hyphis immersis, fere hyalinis, 1–2 µm cr.; stromata evoluta, atra brunnea, pseudoparenchymatica, usque 50 × 35 µm; conidiophora macronematica vel semimacronematica, mononematica, parvae, olivacea brunnea, fere aseptata, simplicia, haud ramosa, erecta vel flexuosa, leniter flavo ad basim, 8–10 µm longa et 3.5–4.6 µm in diam.; cellulae conidiogenae integraeae, terminales, monoblasticae, interdum polyblasticae et sympodiales; cicatrices conidiales non incrassatae.