through deamination of phenylalanine by phenylalanine ammonia lyase and tyrosine by tyrosine deaminase. However the latter could not be traced out in the present study. The metabolic intermediaries of phenylalanine and tyrosine detected in the present study indicate that the metabolism of phenylalanine and tyrosine follows the same pathway in *Pythium aphanidermatum*.

**Division of Microbiology, R. BHASKARAN.**

Tamil Nadu Agricultural Univ.,


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**INHIBITORY ACTIVITY OF THE PARASPORAL CRYSTAL OF BACILLUS THURINGIENSIS VAR. THURINGIENSIS ON YOSHIDA ASCITES SARCOMA**

*Bacillus thuringiensis* produces two insecticidal components during its growth phase, the *β*-exotoxin and the *δ*-endotoxin or the proteinaceous crystal. The *β*-exotoxin was isolated, characterised and found to be toxic for mammals by inhibiting the ribosomal RNA synthesis. The *δ*-endotoxin was reported to have a high insecticidal activity without any chronic or acute toxicity for warm blooded animals and fishes. In the insect system it changes the membrane permeability and inhibits the active transport mechanism of the cell.

During the process of screening for anti-tumour agents of bacterial origin, we found that the proteinaceous crystal of *B. thuringiensis* has anti-Yoshida ascites sarcoma activity, which is being reported herein.

**MATERIALS AND METHODS**

*Tumour*—Yoshida ascites sarcoma (YAS), a rapidly developing, chemically induced tumour was maintained in isogenic Wistar rats (A/ISONC) by serial intraperitoneal (i.p.) transfer of $2 \times 10^7$ tumour cells, once in 4 days.

**Purification of the crystal of B. thuringiensis**—The strain of the test organism *B. thuringiensis* var. *thuringiensis* serotype I was kindly supplied by Dr. De Barjac of Institut Pasteur, Paris.

The toxins of *B. thuringiensis* were produced by growing the organism as described by Delafield *et al.* The crude spore crystal complex and pure crystal preparations were obtained by following the procedures of Dulmage *et al.* and Pendelton and Morrison.

The crystal suspensions were prepared freshly by mixing the freeze-dried materials in sterile saline such that the required concentrations were contained in 0.2 ml of the suspension. Toxicity and evaluation of anti-tumour activities of the crystal preparations were carried out in healthy isogenic Wistar rats of 100–120 gm weight and in Swiss mice of 20–25 gm weight. These animals were provided with 'pellet' diet supplied by Hindustan Lever Ltd., Bombay, and water ad libitum. Experimental rats were injected intraperitoneally with 20 million actively dividing YAS cells. Treatment consisted of a single i.p. dose of the crystal preparation given 24 hours after tumour transplantation. Regular observations were made on weights and general behaviour of all the animals.

**RESULTS AND DISCUSSION**

The acute toxicity data with crude and pure crystal preparations on the experimental animals (Table I) indicate that in contradiction to earlier reports, high doses of the crude-spore-crystal complex were lethal to mice and rats.

**TABLE I**

**Toxicity of crystal preparations of B. thuringiensis in experimental animals**

<table>
<thead>
<tr>
<th>Drug</th>
<th>Animal Route</th>
<th>Maximum Lethal dose tolerated ng/kg</th>
</tr>
</thead>
<tbody>
<tr>
<td>Spore-crystal complex</td>
<td>Mice, i.p.</td>
<td>200</td>
</tr>
<tr>
<td>Rat, i.p.</td>
<td>120</td>
<td></td>
</tr>
<tr>
<td>Pure crystal</td>
<td>Mice, i.p.</td>
<td>60</td>
</tr>
<tr>
<td>Rat, i.p.</td>
<td>120</td>
<td></td>
</tr>
</tbody>
</table>

An interesting observation is that with the purification of the crystal preparation, the toxicity was reduced as seen in the increase of the maximum tolerated dose.
The anti-YAS activity of the spore-crystal complex is shown in Table II. The minimum protective dose was found to be 5 mg/kg.

**TABLE II**

*Anti-YAS activity of spore-crystal complex of B. thuringiensis*

<table>
<thead>
<tr>
<th>Dose*</th>
<th>Route</th>
<th>No. of injections</th>
<th>Survival period (days)</th>
<th>Survivors**</th>
<th>No. of survivals after 6 months</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>i.p.</td>
<td>1</td>
<td>10.5</td>
<td>0/10</td>
<td>..</td>
</tr>
<tr>
<td>30</td>
<td>i.p.</td>
<td>1</td>
<td>All surviving</td>
<td>10/10</td>
<td>10</td>
</tr>
<tr>
<td>20</td>
<td>i.p.</td>
<td>1</td>
<td>,,</td>
<td>10/10</td>
<td>10</td>
</tr>
<tr>
<td>10</td>
<td>i.p.</td>
<td>1</td>
<td>,,</td>
<td>10/10</td>
<td>10</td>
</tr>
<tr>
<td>5</td>
<td>i.p.</td>
<td>1</td>
<td>,,</td>
<td>10/10</td>
<td>10</td>
</tr>
<tr>
<td>2.5</td>
<td>i.p.</td>
<td>1</td>
<td>†</td>
<td>5/5</td>
<td>2</td>
</tr>
<tr>
<td>1</td>
<td>i.p.</td>
<td>1</td>
<td>†</td>
<td>5/5</td>
<td>2</td>
</tr>
<tr>
<td>0.5</td>
<td>i.p.</td>
<td>1</td>
<td>12</td>
<td>2/5</td>
<td>..</td>
</tr>
</tbody>
</table>

*: mg/kg given 24 hrs after transplantation. Controls injected with saline.
**: Survivors at the time of the death of the controls.
†: Out of 5 animals, 3 of them died with an average survival period of 29 days.
‡: Out of 5 animals, 3 of them died with an average survival period of 24 days.

**TABLE III**

*Anti-YAS activity of pure crystal of B. thuringiensis*

<table>
<thead>
<tr>
<th>Dose*</th>
<th>Route</th>
<th>No. of injections</th>
<th>Survival period (days)</th>
<th>Survivors†</th>
<th>No. of survivals after 1 month</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>i.p.</td>
<td>1</td>
<td>14</td>
<td>0/5</td>
<td>..</td>
</tr>
<tr>
<td>10</td>
<td>i.p.</td>
<td>1</td>
<td>All surviving</td>
<td>5/5</td>
<td>5</td>
</tr>
<tr>
<td>5</td>
<td>i.p.</td>
<td>1</td>
<td>,,</td>
<td>5/5</td>
<td>5</td>
</tr>
<tr>
<td>2.5</td>
<td>i.p.</td>
<td>1</td>
<td>,,</td>
<td>5/5</td>
<td>5</td>
</tr>
<tr>
<td>1</td>
<td>i.p.</td>
<td>1</td>
<td>,,</td>
<td>5/5</td>
<td>5</td>
</tr>
</tbody>
</table>

*: mg/kg given on the day after transplantation. Controls injected with saline.
†: survivors at the time of the death of the controls.

The surviving animals were challenged with a lethal dose of tumour cells at monthly intervals for 4 months. Every time the animals had rejected the tumour, indicating the development of immunity in these animals.

The anti-YAS activity of the purified crystal preparation is shown in Table III. The minimum protective dose was 1 mg/kg, which is hundred times less than the maximum tolerated dose.

It is observed that the parasporal crystal body formed by *B. thuringiensis* is a potent anti-YAS agent with high therapeutic index. The purification of the active protein or polypeptide from the crystal and its mode of action on tumour cells is in progress.

The authors thank Professor M. Sirsi for his advice and helpful discussion. One of us (S.S. V. P.) is grateful to the University Grants Commission for the award of a Junior Research Fellowship.


**FISSION TRACK AGES OF SOME BUNDELKHAND GRANITES AROUND JHANSI**

**ABSTRACT**

Nine granite samples from different localities around Jhansi have been dated by fission track method. The ages support the view that the region is affected by later granitisation episodes in Aravalli and Delhi times.

The recent development of the fission track technique\(^1\)\(^{-3}\) offers a new and inexpensive tool for dating of an igneous or metamorphic event over a time span not generally accessible by other methods. The method depends on the fact that most minerals contain small quantities of uranium which undergo spontaneous fission, thereby creating damage tracks which are easily revealed by suitable chemical etching. Some of the minerals present in the common igneous and metamorphic rocks can be exploited for the fission track dating. Suitable minerals include apatite, biotite, muscovite, glasses, sphene, epidote, zircon\(^{4,9}\), etc. The purpose of this paper is to present apatite fission track ages of some granites found near Jhansi.

**Experiment.**—The experimental technique has been explained in details elsewhere\(^10\)\(^{-11}\), but for the sake of completion, we mention briefly the main steps involved in the procedure. Transparent sections of thickness 200-300\(\mu\) are prepared from the rocks on a glass piece with a cross marked at its back. The sections are finally polished and then etched in 5\% HNO\(_3\) at 27\(^\circ\)C for 20-30 seconds to reveal fission tracks in apatite nodule.

The etched section is put on the microscope slide with a cross marked on it and the two crosses are made to coincide with each other. Then apatite crystals in the sample are located under the microscope with magnification of 1,500\(\times\), their co-ordinates are noted and fossil densities (\(r_\phi\)) are determined with the help of 5\times5 grid graticule in the eyepiece. These samples are then sent for irradiation to a known dose of thermal neutrons at CIRUS atomic reactor at B.A.R.C., Bombay, for uranium content determination. The integrated thermal neutron dose (\(\phi\)) is measured with the help of standard glass of known uranium concentration\(^12\) irradiated simultaneously.

The process of grinding, polishing and etching is repeated for the irradiated samples. With the help of noted co-ordinates the apatite crystals are relocated and induced track densities (\(r_\phi\)) are measured. The age \(T\) in years and uranium concentration \(U\) are calculated from the following relations\(^5\):

\[
T = 6.57 \times 10^7 \log_e \left(1 + 9.25 \times 10^{-18} \frac{r_\phi}{r_\phi} \phi \right) \text{yrs.}
\]

\[
U = \frac{r_\phi}{r_\phi} (3.6 \times 10^5) \text{ atom/atom.}
\]

The results are shown in Table I. The errors indicated are only statistical errors (\(\sqrt{n}\)) in track density. Overall spread of ages are shown by mean standard deviation. The uranium concentration is found to vary from grain to grain and from sample to sample ranging from 0.2 to 16 ppm.

**Discussion.**—The samples used in the present study have not been directly dated by any other radiometric methods, moreover the age data of the region is very much lacking in the literature. Only some whole rock age determinations by Rb-Sr method are available\(^13\)\(^{-15}\) for the Bundelkhand granites indicating an age of \(\sim 2,500\) m.y. almost equivalent to banded gneissic complex which is very much older than our measured fission track ages.

This difference of the ages can be understood as follows:

1. The measurements are being made on apatite mineral which is thermally very sensitive as far as track stability is concerned\(^16\).
2. The fission track ages have generally been found to be correlated to the last orogenetic-metamorphic cycles in the region.

Therefore the present data indicate the closing phase of last metamorphic activity in the region which must have occurred \(\geq 639 \pm 108\) m.y. ago, and it supports the views of Naha et al.\(^14\) that the