Summary of completed/ongoing projects, funded by IERP, GBPIHED

BIOTRANSFORMATION OF LANTADENES, THE PENTACYCLIC TRITERPENOIDS FROM LANTANA *(LANTANA CAMARA)*

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Three bacterial isolates namely, *Pseudomonas pickettii, Alcaligenes odorans* and *Alcaligenes faecalis* were isolated at pH 7.0, 5.7 and 6.5, respectively, from soil in the presence of lantadene A (LA) as the sole carbon source. A fungal strain, *Cephalosporium* sp. was also isolated from soil in the presence of LA and glucose at pH 6.5. All these cultures were also investigated for their ability to degrade/transform LB. But none of the isolates could utilize LB, thus showing that these isolates were specific for LA. No fungal enrichment took place using LA as the sole carbon source at both the pH 5.7 and 6.5. The sets with LA and antibiotic at both the pH 5.7 and 6.5 were started with an attempt to obtained fungal enrichments and inhibit bacterial growth but these sets did not show the ability to utilize LA. There was no fungal enrichment in the presence of glucose and antibiotic at pH 5.7.

The bacterial strain *pseudomonas pickettii*, capable of degrading LA has been isolated from soil using LA as the sole carbon source at pH 7.0. The isolate is rod-shaped. Gram negative and motile. No metabolite was detected either by TLC or HPLC thus, implying that the organism is eliciting mineralization of LA. No enrichment for LA utilizing organism took place in the sets containing LA and glucose. The use of sucrose (20 mM) as cosubstrate did not enhance the utilization of LA. However, a metabolite M_5 with RRI, 0.84 (relative to LA) was detected in case of the sets containing sucrose as cosubstrate.

The isolate *Alcaligenes faecalis* brought about mineralization of LA when LA was used as the sole carbon source as well as in the presence of glucose (20 mM) as cosubstrate. Co metabolism studies using succinate (20 mM) caused transformation of LA to at least one metabolite M_4 having RRI of 0.57. At least two metabolities M_3 and M_4 could be detected when transformation studies were done in presence of sucrose as cosubstrate. The metabolite M_3 had the RRI of 0.92, which was the same as that of LB in the mobile phase, methanol-acetonitrile-water-acetic acid (71:20:9:0.01). However, it differed from the latter in channel ratio (A_{210}/A_{240}) which was higher in case of M_3. The other metabolite M_4 had the RRI of 0.57 and was produced in trace amount.

Alcaligenes odorans has been isolated at pH 5.7 from the soil under lantana bushes by enrichment technique. The isolate is Gram negative and motile. The isolate showed LA utilization of nearly 60% in 15 days. No metabolite was detected at 210 nm. However, at 240 nm, two metabolities M_1 and M_2 with RRI 0.239 and 0.478 were detected.

The biotransformation capacity of the mixed culture (pH 7.0) obtained by mixing of *A. faecalis* and *P. pickettii* was not better than the two pure cultures in the presence of LA as the sole carbon source. However, the utilization on LA was slightly higher in the presence sucrose (20 mM) as cosubstrate on incubation with this mixed culture.

A fungal strain, *Cephalosporium* sp. was isolated from soil in the presence of LA and glucose at pH 6.5. This strain brought about the transformation of LA to a metabolite M_6 having the same RRI as LB, a congener of LA. Two more metabolites M_7 and M_8 were also detected. However, the rate of transformation was very low. It utilized nearly 13% LA on incubation for 15 days.

Ten fungal cultures known to be potential degraders of complex organic molecules were used for their ability to degrade LA. LA was found to strongly adhere to the fungal mass. The protocol for the complete extraction of unutilized LA from the fermentation broth was developed. It involved filtration of fermentation broth through glass wool followed by extraction of unused LA from the mycelial mass by homogenisation of the same in the presence of acid-washed sand.

Strong abrasive action provided complete extraction of LA. *Merulius tremellosus* PRL 2845 and *Pleurotus sajor caju* did not utilize LA. No significant lass of LA was observed in case of *Phanerochaete chrysosporium* K3, *Phlebia radiata* 2 and *Sporotrichum pulverulentum*. *Aspergillus niger* van *Tieghem* MTCC 2425, *Trametes versicolor* MTCC 138, *Heterobasidion annosum* MTCC 146, *Pleurotus ostreatus* MTCC 142 and *Phellinus pini* RAB-83-19 were also not fast degrader of LA and caused 11-19.5% degradation of LA during an incubation period of 15 days.

Alcaligenes faecalis showed the ability to transform LA into various metabolities in the presence of LA and sucrose. The metabolite, M_3, was produced as the major metabolite. In case of other cultures showing transformation of LA to degradation products, the amount of metabolite produced was very less. The major metabolite M_3 appeared at the same retention time as LB during the HPLC analysis using the mobile phase, methanol-acetonitrile-water-acetic acid (71:20:9:0.01). LB in the sample at this stage was on account of minuscule impurity in the LA samples used for biodegradation. However, it had a much higher channel ratio (A_{210} /A_{240}) as compared to LB. Different mobile phase were tried for the resolution of these two compounds. The mobile phase, methanol-water-acetic acid (80:20:0.01) showed a better resolution of M_3 and LB. The mobile phase, acetonitrile-water-acetic acid (70:30:0.01) was found to be the most suitable for the complete resolution of M_3 and LB. The identity of M_3 was confirmed as lantadene X (22β-tigloyoxy-3-oxoolean-12-en-28-oic acid), a *trans* isomer of LA by spectroscopic analysis. Lantadene X is a novel compound not reported to be present in the leaves of lantana plant. The other metabolites could not be characterized due to paucity of materials.

Bioassays using guinea pigs as experimental animal were done for checking the biological effects of the extracts of fermentation broth after incubation of LA with *Alcaligenes faecalis*. The animals in group A were administered LA filled in gelatin capsules, those in group B were administered extract of the fermentation broth of *A. faecalis*. The animals in group C were given empty gelatin capsules and they served as the control. The animals in group A were found to be icteric in 24 h. The animals in group B were comparable to the controls. Their gall-bladder, liver and kidneys were normal. The plasma of group B and C was creamish while that of group A was pale. Bilirubin contents, both conjugated and unconjugated were within the normal range in case of animals in group B. There was a marked increase in the bilirubin content of the animals in group A. No clinical symptoms or biochemical lesions were observed in the animals administered extract of fermentation broth as was evident from the activities of various enzymes, which typify hepatotoxicity and cholestasis.

STUDY OF INDUCED SPAWNING AND MILT MORPHOLOGY OF SNOWTROUT SCHIZOTHORAX SPP. IN THE GARHWAL HIMALAYA

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The research project was under taken with the four major objectives: (i) Induced spawning of *Schizothorax plagiostomus* (ii) Study of the milt morphology (iii) Study of fertilization, hatching and survivability rate up to yolk absorption (iv) Physico-chemical analysis of natural and confined water where the breeding experiment was conducted.

1. Breeding experiment on snow trout *Schizothorax plagiostomus* were conducted during spawning season (September-October) by using pituitary gland extract (PGE), ovaprim and stripping method. Successful results have been obtained from the breeding experiments. Usual set of brooders, 1 male: 2 females or 2 males: 1 females were used in all the breeding experiments. The better result was obtained by inducing ovaprim and stripping method.
2. The rate of fertilization in *Schizothorax plagistomus* was ranged from 91-95% in different experimental sets. The percentage of fertilization was higher in case of stripping method instead of using hormone injections. The hatching percentage among fertilized eggs was ranged from 82 to 92 in natural ground while it was only 71 to 78% in laboratory.
3. The mortality percentage after hatching up to yolk absorption was 71.43% in natural ground during 1st year of experiment and 90% in laboratory, while after observing and providing natural habitat to hatched larvae the mortality rate was decreases up to 31.76% in natural ground and 38.88% in laboratory water system.
4. The physico-chemical parameter of both experimental sites was observed. The variation in water temperature was 1-2^{o}c and dissolve oxygen variation was 2-3.5 ppm in natural and laboratory water. The pH value was almost same in both the water bodies (7.0-8.2).
5. The growth of *S. plagiostomus* larvae was less in laboratory water system than the larvae growing in the natural water. Five months old *S. plagiostomus* larvae in natural water system got 77-78 mm length with 7.615 gm weight, while its growth in laboratory water system was 66-72 mm in length with 3.820 gm in weight. The difference in growth rate may be due to physico-chemical environment and availability, quality and proper supply of food.
6. The cyto-morphological study of spermatozoa of *Schizothorax plagiostomus* reveals that, the head of spermatozoa was oval shaped with long tail. The head was without acrosome. The nucleus was round and occupies most of the available space of the sperm head. The plasma membrane having very thin layer running towards the long tail forming a neck area. The tail was smooth having same thickness throughout the length without pointed end as in primitive spermatozoa of other aquatic animals.

Recommendations

Therefore, it is a great need to utilize the fishery potential of this region. This can be done not only by regulating fish landings, developing fish markets and providing selected fishing areas to the market. There is a great scope for reservoirs fishery in this region as a number of hydroelectric and irrigation projects are covering up.

So, development of hatcheries near reservoirs is very essential for propagation of fish. Certain parts of the rivers or reservoirs should be developed in to fish sanctuaries, which will be beneficial for the protection and propagation of important and endangered species. Such sanctuaries should be constructed at breeding sites/grounds of a particular species. The regulation of streams is the most important aspect in order to maintain as far as possible, the natural condition in river. The stream ahead of the dam often becomes a drain that can be breed a plethora of problems including destruction of the spawning grounds of the hill stream fishes in this region. The fishes and fish food with water quality near the barrage is highly affected. The minimum required discharge should be maintained in such parts of the river.

The fishes leave a very large number of eggs in this region in the shallow water on the side of the rivers, which ultimately develops in to fry, and fingerlings. But this fish seeds are

destroyed and damaged when the water recedes leaving small patches or pockets of water, which later on get dried up due to the fall in water level of river during winter. Among these seeds the most economically important fish (*Schizothorax* spp.) seed also get destroyed. Which cover up 90% of catchments out of the total fishing in this region. To protect and procure these seeds the efforts will have to be made by recent scientific methods (*i.e.* artificial breeding and propagation, collection of seeds from unprotected areas and transfer into hatcheries and safe ponds *etc*.).

ECOLOGICAL RECONNASSIANCE OF CHAIL WILDLIFE SANCTURY IN HIMACHAL PRADESH

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The Studies were undertaken to make an ecological reconnaissance of Chail Wildlife Sanctuary and to make appropriate recommendations for conservation of biodiversity and sustainable use of available resources. The main findings of the project are as follows:

Floral components of Chail Wildlife Sanctuary *viz.* trees, cultivated plants, shrubs, herbs and grasses have been identified and documented. In all 10 species of trees, 21 species of shrubs, 16 species of herbs and 6 species of grasses have been recorded from Chail Wildlife Sanctuary.

Seven transects were laid in the Blossom and Khariun areas of the sanctuary for systematic sampling of birds and mammals. Regular monitoring of these transects have confirmed the presence of thirteen species of large and medium sized mammals in the sanctuary. These include the endangered species like Panther *Panthera pardus* and Leopard Cat *Felis benghalensis*. Three species of ungulates *viz*. Goral *Nemorhaedus goral,* Barking Deer *Muntiacus muntjak* and Sambar *Cervas unicolor* are mostly confined to the Blossom and Khariun areas of the sanctuary. Other mammals recorded from the sanctuary are: Rhesus Macaque *Macaca mulatta,* Common Langur *Presbytis entellus,* Indian Porcupine *Hystrix indica,* Indian Hare *Lepus nigricollis*, Jackal *Canis aureus*, Himalayan Yellowthroated Marten *Martes flavigula*, Small Indian Civet *Viverricula indica* and Himalayan Palm Civet *Paguma larvata*. Census of Goral was carried out and its population was estimated to be around 44 individuals. Other aspects of ecology of Goral *i.e.* encounter rate, abundance, group size and habitat preference were studied. An exotic species European Red Deer *Cervus elaphus* introduced into the sanctuary about half a century ago couldn't be located and in all probability the species has become extinct from the area.

Observation and abundance of bird species has been carried out. In all 143 species of birds have been recorded. These belong to 35 families. Studies conducted on Cheer Pheasant reveals that transitional zone between grassland and oak forest is the most preferred habitat of these birds. The results of the study suggest that the population of Cheer Pheasant has remained static since the last estimate was made by Garson (1982-83). Group size of Cheer varied from 1-19. The overall encounter rate of Cheer Pheasant was 26.45 encounter per 100 hours. Sixty seven villages located inside the sanctuary were surveyed to get information of human population, number of livestock, crop predating wild animals, sources of fuel, fodder and timber extraction *etc*. to estimate their dependence or sanctuary's resources. Migratory shepherds along with their livestock pass through the sanctuary twice a year putting pressure on the food resources of the sanctuary. Chail being a tourist place attracts a large number of tourists every year. The increasing tourist pressure may affect the wildlife population in the long run.

NURSERY DEVELOPMENT AND PLANTATION OF CHYURA IN PITHORAGARH

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A total of 17000 saplings are planted in the target area. Apart from this area the Saplings were sent to Dehradun, Garhwal, to the VWSC's - a total of 7000 saplings.

From the 28000 saplings in the nursery the survival rate is 85.7%. As proposed the plantation was to take place in 25 hectares and the actual area covered in the proposed area is 27 hectares and another 11 hectares of plantation was covered by neighbouring villages. A total of 38 hectares is covered against 25 hectares as proposed. Some people have made their own nurseries and are supplying the sapling to other members of the community. DRDA has also shown interest considering in multidimensional use and are planning to use the species in their watershed programs.

Demonstration on the Cheura processing was done with the communities. The high participation of the community especially the women folk was appreciated. The plantation was done in the private land, which was lying as a wasteland. In case of VWSC's it was in the community owned land.

Major achievements

- Development of pesticide from Cheura Khali.
- Sensitization of the communities, of proposed area and also others towards multi dimensional usage.
- Communities interest in promotion of MPTS in this terrain.
- Understanding of the economic value of MPTS's which raises their economic status.
- Initiation of small nurseries by the villagers.
- DRDA's Consideration to use in their watershed programs.
- Village Churani regains its status again as the village of Cheura forest.