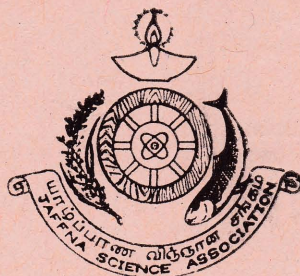


PROCEEDINGS  
OF  
JAFFNA SCIENCE ASSOCIATION

ABSTRACTS



*Third Annual Session*  
*27-29 April, 1994*  
**JAFFNA, SRI LANKA**

1994

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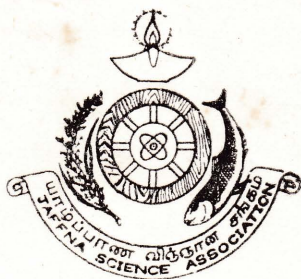
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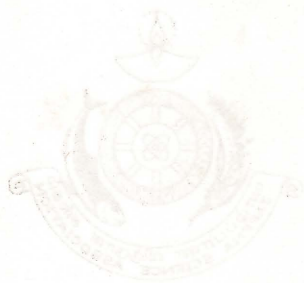
ABSTRACTS



*Third Annual Session*  
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1994

PROCEEDINGS  
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ABSTRACTS



Second Annual Session

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1994

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THE EFFECT OF NEEM SEED KERNEL EXTRACT ON THE  
LARVAE OF CABBAGE BORER, *Hellula undalis*.

Nithiyagowry Ratnasabapathy and V. K. Ganesalingam  
Department of Zoology, University of Jaffna.

This study was undertaken to determine the effect of neem seed kernel extract on weight loss, feeding rate and mortality of the larvae of *Hellula undalis*, the cabbage borer.

The extract was prepared by crushing the neem seed kernel with (a) petroleum ether (b) ethanol (absolute) (c) tap water (5gm / 20ml) and soaking the resulting mixture in the respective solvents for 24 hours. One ml of the extract was painted by a camel hair brush on both sides of the cabbage leaf discs of 5 cm in diameter. They were kept in a petridish which was ventilated by cheese cloth. A last instar larval stage of *H. undalis* was left in for 24hrs. After this period the larva was taken out and reared by giving them fresh mature leaves. Four replicates were carried out simultaneously. The rate of application was same for all treatments. The loss in weight, surface area of leaf consumed and percentage of mortality of larvae for four days after the treatment were determined.

It was found that the weight of the larvae on treated leaf discs was significantly lower than that of this control ( $P < 0.05$ ). However, the differences within the treatments were not significant ( $P > 0.05$ ).

The surface area of the leaf fed by the larvae on treated leaf discs was significantly lesser than that of the control ( $P < 0.05$ ).

Mortality of the larva was significantly greater in the extract treatment than that of the control ( $P < 0.05$ ), but there was no significant difference between the mortalities of larvae in the extracts prepared with petroleum ether, ethanol and water ( $P > 0.05$ ).

The neem kernel extract is effective in controlling the larvae of *H. undalis* and the effect of the extract is almost the same irrespective of the solvents used.

PREFERENCE OF *SITOPHILUS* sp.  
FOR SEVERAL VARIETIES OF RICE

Ratneswary Kanagaratnam and V. K. Ganesalingam  
Department of Zoology, University of Jaffna, Jaffna

In this study different varieties of rice grain were used to determine the most preferred variety of rice grain by *Sitophilus* sp.

Experiments were conducted using an "olfactometer", made up of a wooden box covered with glass, kept in a dark room. Forty normal specimens of *Sitophilus* sp., one day old, were introduced into the apparatus at its centre, keeping the samples of rice namely "Big young"; H-4, "White rice", "Black bold", 500-1, 34-6 and "Small young" at the inner margin of the "olfactometer" at 40 cm apart from one another and from the centre. At the end of 24 hrs. the number of specimens gathered around each grain sample was noted. Each experiment was replicated ten times. ANOVA test was used in the statistical analysis of the data.

Results showed that out of seven varieties of rice, *Sitophilus* sp. repelled only "White rice". All other varieties were preferred in the following order: H-4, "Black rice", "Big young", 500-1, 34-6 and "Small young". When bran was removed all rice varieties were equally preferred. When bran of all varieties were provided insects were equally repelled. When grains were washed with water, dried and provided, all the varieties were preferred.

It was concluded that the bran of rice varieties was not attractive, but kernel was responsible for preference by *Sitophilus* sp., presumably due to its chemical and physical nature.



THE EFFECT OF BROKEN RICE GRAINS OF DIFFERENT SIZES  
ON THE DEVELOPMENT OF RICE WEEVIL (*Sitophilus* sp.)

K Jeyasingham and V. K. Ganesalingam

Department of Zoology, University of Jaffna.

This study was undertaken to determine the size of the broken rice grain suitable for the development of rice weevil (*Sitophilus* sp.). Rice variety used was 500-1.

The rice grain was broken up using an electric blender and graded through a series of sieves to select broken particles of various sizes namely 6.25x3 mm (cracked), 5x2.5 mm, 3x2 mm, 2.5x2 mm and 2x2 mm. The uncracked 6.5x3 mm rice was used as control. Twenty grams of grain particles of each size were kept in separate containers. Five pairs (male and female) of the weevils were introduced into each treatment and left under the laboratory conditions until the emergence of  $F_1$  and  $F_2$  generations. The experiment was repeated three times.

It was observed that the number of adults emerged in  $F_1$  generation was the highest in the grains of 5x2.5 mm and least in grains of 2.5x2 mm and 2x2 mm. The number of weevils emerged from the grains of 6.5x3 mm (uncracked) and 6.25x3 mm were almost the same but less than that in 3x2 mm. In  $F_2$  generation too, the highest number of weevils were in grains of 5x2.5 mm and the least in 2.5x2 mm and 2x2 mm (cracked) than that from 6.5x3 mm (uncracked).

It is concluded that the rice grains of minimum size and uncracked condition of the rice grain is suitable for long term storage of rice against *Sitophilus* sp, since conditions are not suitable for egg laying and the development of larva within the grain (the loss due to attack by the weevil on the exposed surface area is taken to be negligible).

POTENTIAL USE OF SOME INDIGENOUS PLANT MATERIALS  
AGAINST RICE WEEVIL, *Sitophilus* sp. ON STORED RICE.

K. Jeyasingham and V. K. Ganesalingam  
Department of Zoology, University of Jaffna.

Investigation was carried out to determine the effects of some indigenous plant materials on *Sitophilus* sp. in causing mortality, inhibition of larval development and emergence of adults. The effects of ten plant species were tested.

Twenty grams of uninfected rice mixed with ten grams of dried and powdered leaves of each plant species were kept in different containers. Ten pairs of young male and female adult of *Sitophilus* sp. were introduced into each of the containers and kept under laboratory condition until the emergence of F<sub>1</sub> generation. Control experiments were conducted concurrently and the experiments were repeated four times.

When examined after two weeks of introduction it was found that percentage of mortality of introduced adults was the highest (87.5%) in *Glycosmis pentaphyla* (pannai (T)) when compared with the control (2.5%). There was no significant difference among the other plants used.

The development of larvae was found to be the least in *G. pentaphyla*.

The total number of weevils emerged in F<sub>1</sub> generation was the least in *G. pentaphyla*. *Azadirachta indica* was more effective than all the other plant species tested, but less effective than *G. pentaphyla*.

From this study it is considered that the *G. pentaphyla* forms an important part in pest management programme of *Sitophilus* sp. in stored rice.

\* SOME ASPECTS OF BIOLOGY OF *CHARYBDIS ANNULATA*  
(FABRICIUS) FROM THE JAFFNA LAGOON

K. Chitravadivelu

Department of Zoology, University of Jaffna.

The crab fishery in the Jaffna lagoon has been assuming commercial importance in recent years. *Charybdis annulata* (Fabricius) is one of the three species of economically important edible portunid crabs found in the lagoon.

Random samples of crabs caught by common fishing gear from four landing centres — Araly, Kakaitivu, Ariyalai and Columbuturai — were utilised in the investigation, carried out for two years from January 1991. Studies have been made on the morphometrics, length-weight relationship, rate of growth, sex composition and fecundity of *C. annulata*.

Relative growths (allometry) of the different parts of the body — carapace length (CL), Abdominal width (AW), Right chela height (RCH), left chela height (LCH) and gonopod length (GL) were determined applying the formula  $Y = ax^b$  to the morphometric data, using carapace-width (CW) as the reference dimension. The  $b$  values indicated positive allometry in AW, LCH and GL in males; negative allometry in CL in both sexes, RCH and LCH in females; while AW in females and RCH in males were very close to isometry.

The  $b$  values in length-weight relationship ( $Y = aW^b$ ) of 2.7336 in male and 2.2361 in female reflect their deviation from isometry.

Progression of monthly modal widths of unsexed *C. annulata* indicated an average rate of growth of 5.0 mm month<sup>-1</sup>.

The mean fecundity was found to be  $1.33 \times 10^5$  with a range of  $2.00 \times 10^4$  to  $2.25 \times 10^5$ .

The size of *C. annulata* caught in the Jaffna lagoon ranged from 41.0 mm to 85.0 mm in carapace width.

The monthly percentage variation of berried females shows that *C. annulata* to be a multispawner, breeding throughout the year except during June and July.

Analysis of the monthly sex composition shows that there is a noticeable preponderance of females in 1991 and 1992. The males have dominated during January, July, September and October in 1991 and only in January in 1992.

\* This research was funded by the University of Jaffna.

STATUS OF LOBSTER FISHERY AND SOME ASPECTS OF BIOLOGY  
OF *PANULIRUS ORNATUS* (FABRICIUS) IN THE COASTAL  
AREAS OF THE JAFFNA PENINSULA

K. Chitravadevelu and P. Shanmuganathan

Department of Zoology, University of Jaffna.

Lobster fishery is an important component of the subsistence fishery in the coastal areas of the Jaffna Peninsula. Five lobster Collecting Centres in the Jaffna Peninsula, had realised Rs. 18.8 million in 1983 by first sales of 75 metric tons of lobsters which were subsequently sent to Colombo mainly for export. The traditional lobster fishing grounds in the Jaffna Peninsula are near Thalayady, Pt. Pedro, Valvettiturai, Chulipuram, Mathagal, Karainagar and Kalmunai. This research commenced in January 1992 was to evaluate the state of the lobster fishery, identify the different species, assess the species composition and make a preliminary investigation on the biology of the predominant species. Data was obtained from observations at landing centres and lobster collecting centres, maintaining constant contact with fishermen.

Lobsters are caught mainly by trammel set nets and drift nets, specially designed for catching lobsters.

Five species of lobsters - *Panulirus ornatus* (Fabricius), *Panulirus versicolor* (Latreille), *Panulirus penicillatus* (Olivier), *Panulirus polyphagus* (Herbst) and *Panulirus homarus* (Berry) have been recorded in the area under investigation. The former three are the major species while the latter two have been recorded very rarely.

*P. ornatus* is the dominant species with a mean composition of 90.9% followed by *P. versicolor* and *P. penicillatus*, with 5.2% and 3.9% respectively.

The carapace-length weight relationships of males and females are  $W = 0.8399 Cl^{3.9559}$  and  $W = 0.6580 Cl^{3.809}$  respectively. Males of *P. ornatus* were found to be heavier than females of the same carapace length.

Fecundity of *P. ornatus* varied from  $1.56 \times 10^4$  to  $8.95 \times 10^4$  with mean of  $3.72 \times 10^4$ . The average rate of growth of *P. ornatus* was found to be 10 mm month<sup>-1</sup> irrespective of sex. 64.5% lobsters caught were below the carapace length of 80.0 mm, prohibited by the Fisheries Ordinance, indicating an urgent need for a realistic conservation programme.

## PRIMARY PRODUCTION OF TWO PONDS IN JAFFNA PENINSULA

Chandranthy Rajendram and N. Selvarajah

Department of Zoology, University of Jaffna

The quantum of net primary productivity in water samples gives information on the magnitude of organic production in ponds. The net primary productivity of two freshwater ponds were studied monthly from January, 1991 to December, 1992. The ponds selected were Neeraviaddy pond at longitude  $80^{\circ} 00' .50''$  and latitude  $9^{\circ} 40' 30''$  and Sivankovil pond at longitude  $80^{\circ} .01' .05''$  and latitude  $9^{\circ} .40' 55''$ . They were arbitrarily named as pond I and pond II respectively. The Light and Dark bottle method was followed to determine the productivity at the surface and bottom of the pond. The water temperature, pH, light penetration and salinity of the pond water were also determined at each survey. Nitrogen content was determined by Kjeldal's method.

The net production of pond I was found to be high in July 1991 ( $3.20 \times 10^{-4}$  g/1/hr). But for pond II the highest production was measured in October 1992 and it was  $2.10 \times 10^{-4}$  g/1/hr.

It was observed that light penetration has an Indirect relationship with the primary production and the effect was high in pond I. It was also found that there is a decrease in nitrogen content of water with the increase in net primary production. The value of net primary productivity obtained in the present investigation is low compared with those reported<sup>1,2</sup> by other workers from the same type of ponds in other countries.

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DACTYLOGYRUS VASTATOR, NYBELIN, 1924 (MONOGENEA;  
MONOPISTHOCOTYLEA), A GILL PARASITE OF EUROPEAN CARP,  
CYPRINUS CARPIO L.

P. Vinobaba and R. Wootten

Institute of Aquaculture, University of Stirling, Stirling FK9 4LA,  
Scotland, United Kingdom

Fish culture, whether in semi-natural or artificial pond conditions represents an important source of protein many countries of the world. With an increasing commercial interest in artificial rearing of fresh water fishes for food, there is a simultaneous need for veterinary practitioner to be aware of diseases and parasites of fishes.

*Dactylogyrus vastator* is an economically significant monogenean parasite of European carp, *Cyprinus carpio*. Scanning and transmission electron microscopic studies were conducted to show the detailed structure of the parasite and the pathology caused by it. *D. vastator* attaches to the gills of carp by means of an attachment organ namely opisthaptor, which carries two median hamuli and 14 peripheral marginal hooks. The parasite induces a severe hyperplastic tissue response in the gills which leads to heavy mortalities of young carp fry and fingerlings.

Experiments were conducted to study the effect of the temperature on *D. vastator* population at 12°C, 19°C and 20°C. The excised gills from samples of 20 fish taken at every two weeks, were examined and the number of adults and immature parasites in different parts of the gills were counted.

The results obtained at different temperatures indicate at the mean number per fish increased initially, reaching the maximum around fourth week of the experiment and then declined in all cases. 19°C appears to be the optimal for the parasitic development.

The distribution of *D. vastator* on the gill of carp is not random. The second gill arch has the most parasites followed by the first, third and fourth. There is no significant difference in numbers between dorsal and ventral hemibranchs in both left and right side gill arches. The dorsal part of the hemibranches have a lower number of parasites when compared to rest.

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## A STUDY ON AN ASSIGNMENT GAME

Z. T. Hoole and S. Srisatkunarajah

*Department of Mathematics and Statistics, University of Jaffna*

The assignment game is a model for a two sided market in which a product that comes in large indivisible units (eg. houses, cars etc.) is exchanged for money and in which each participant either supplies or demands exactly one unit. The units need not be alike and the same unit may have different values to different participants. It is shown here that the outcomes of such a game cannot be improved upon by any subset of players. The outcomes are arrived at by solving the corresponding linear programming problem. It is assumed that the assignment problem is balanced. The geometric structure of the feasible region shows that these solutions are insensitive to bargaining possibilities.

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SIMPLE COMPUTER PROGRAMS TO SHOW  
PICTORIALLY ELECTRON DENSITY DISTRIBUTION IN  
ATOMIC AND MOLECULAR ORBITALS

Rajeswary Mageswaran and Shanthithevi Saravanamuthu  
*Department of Chemistry, University of Jaffna.*

Two molecular fragments are isolobal if the number, symmetry properties, shapes and approximate energies of their frontier orbitals are the same. Isolobal molecular fragments will have chemical and/or structural similarities. An electron distribution diagram will help to compare the symmetry properties and shapes of different orbitals. The representations reported<sup>1</sup> earlier do not give the electron density distributions accurately. This paper discusses the development of simple computer programs to show pictorially the electron distribution in pure atomic, hybrid atomic and molecular orbitals. The psi-square value at a point  $(x, y, z)$  within a fixed volume element  $dv$  (where  $dv = dx \cdot dy \cdot dz$ ) was computed and points were chosen randomly within that volume  $dv$  so that the number of points chosen is proportional to the psi-square value. The dots were placed at orthographic projections of these points. With the programs developed it is possible to change the planes on which the orthographic projections are taken. Hydrogen atomic orbital wave functions<sup>2</sup> ( $\psi$ ) and Slater wave functions<sup>3</sup> ( $\psi$ ) were used separately to draw the pure atomic orbitals. The diagrams obtained for the 2s and 3s atomic orbitals using hydrogen atomic orbital wave functions showed the nodal planes while those obtained using Slater wave functions did not show any nodal plane as expected.

The hydrogen atomic orbital wave functions and Slater wave functions were used to draw the hybrid atomic orbitals of carbon. The shapes of  $sp$ ,  $sp^2$  and  $sp^3$  hybrid orbitals obtained using these two sets of wave functions were found to give the expected results. However the Slater wave functions gave slightly better diagrams. Also the 2s,  $2p_z$  hybrid orbitals obtained for BH and CH fragments had the same shapes and symmetry properties as the  $3d_{z^2}$ , 4s,  $4p_z$  hybrid orbital of Fe in  $Fe(CO)_3$  fragment showing that BH, CH and  $Fe(CO)_3$  fragments are isolobal. It is known<sup>4</sup> that these three fragments have chemical and structural similarities,

Slater wave functions were also used to draw the molecular orbitals. The diagrams obtained for the  $\sigma$  -molecular orbitals of  $H_2$ ,  $H_2O$  and  $NH_3$  and  $\pi$  -molecular orbitals of  $NO_2$ , allyl group and benzene are as predicted.

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## A USEFUL EXPRESSION FOR GREEN FUNCTION

S. Srisatkunarajah

Department of Mathematics &amp; Statistics

Let  $W(\alpha)$  be an infinite wedge of an angle  $\alpha$  and  $G(x, y; t)$  be the Green function associated with the heat equation:

$$\frac{\partial U(x, t)}{\partial t} = \Delta(x, t)$$

$$U(x, t) = 0 \text{ on } \partial W(\alpha), \text{ where}$$

$$\partial W(\alpha) = \text{Boundary of } W(\alpha).$$

The standard expression (1) known for  $G(x, y; t)$  was reported by Carslaw<sup>1</sup>

$$G(x, y; t) = \frac{1}{2\pi} \int_{-\pi/2}^{\pi/2} \sum_{n=1}^{\infty} e^{-(r^2 + \rho^2) / 4t} I_n(rp/2t) \sin n\phi \sin n\alpha \dots (1)$$

where  $x \equiv (\rho, \alpha)$ ,  $y \equiv (r, \phi)$ .

However this form is very difficult to handle in computations such as

$$\int_{D \leq W} G(x, y; t) dx dy, \quad \int_{D \leq W} G(x, x; t) dx$$

Kac in his famous work "Hearing the shape of a Drum" reported<sup>2</sup> an alternative expression (2) for  $G(x, y; t)$

$$G(x, y; t) = V(\alpha) - V(-\alpha), \text{ where}$$

$$V(\alpha) = \frac{1}{4\pi t} \sum_{\phi - \alpha - \pi < 2k\alpha < \phi - \alpha + \pi} \frac{\exp[-(r^2 - 2rp \cos(\phi - \alpha - 2k\alpha) + \rho^2)]}{4t} \frac{\sin(\pi^2 / \alpha) \exp[-(r^2 + \rho^2)]}{8\pi \alpha t} \int_{-\infty}^{\infty} \frac{\exp[-(rp \cosh y) / 2t] dy}{4t - \cosh[\pi y + i\pi(\phi - \alpha)] / \alpha + \cos(\pi / \alpha^2)} \dots (2)$$

However the proof for this expression (2) has not yet been reported. In this paper we are reporting the proof for expression (2) by using the Carslaw's representation of contour integral<sup>1</sup> for  $G(x, y; t)$  and performing difficult computations for calculating the contour integral.

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EFFECT OF SEED TUBER SIZE AND PLANTING SPACE ON GROWTH  
YIELD AND TUBER SIZE DISTRIBUTION OF POTATO (*Solanum tuberosum*)  
IN RED YELLOW LATOSOLS OF DRY ZONE UNDER IRRIGATION

S. Rajadurai

*Department of Agronomy, Faculty of Agriculture*

A field of experiment was conducted at the Faculty of Agriculture, Kilinochchi to investigate the effect of potato tuber size and planting space on growth and yield of potato. Experiment was laid out in a split plot design with tuber size in main plot and planting space in sub plot.

**Main plot treatment - tuber size**

15—30 mm diameter tuber (small) 27g / tuber

30—45 mm diameter tuber (medium) 38.5g / tuber

40—55 mm diameter tuber (large) 83.3g / tuber

**Sub plot treatment - Planting space**

60×20 cm

60×30 cm

60×50 cm

It was found that a number of main stem produced per tuber increase with increasing tuber size and planting space. Large size tubers produced more stems than small. Leaf area index (LAI) significantly influenced by tuber size. However it was found that LAI decreased with increasing planting space.

Number of tubers per plant increased with increasing tuber size and planting space. Number of tubers produced per plot significantly reduced by increasing planting space.

Final tuber yield increased with increasing tuber size. It was also found that the tuber size distribution was not significantly influenced by the different size tubers planted.

Based on the yield, cost of seed tubers, tuber size distribution and other agronomic characters, the spacing of 60×20 cm, 60×30 cm and 60×50 cm can be recommended to small, medium and large size tubers respectively.

**LOCALLY AVAILABLE LIME BASED CEMENTITIOUS MATERIALS**

S. Ruthiralingam & K. Raveendrakumaran

*"Vimalam" - Nallur Cross Road, Jaffna*

In the absence of cement for building works, local resources are being used to make cementitious materials. Hydraulic lime, pulverized quick lime, surki lime mix, lime-ash mix, are some of the lime based cementitious materials which have been used as binder in building construction.

Using the locally available varieties of lime, semi hydraulic lime, Surki-lime mix, made out of burnt clay such as tiles, bricks etc. lime-ash mix, are presently being used in the absence of cement.

No tests have been done so far on the locally available lime and lime based cementitious materials to determine the extent of their use.

In the absence of suitable laboratory facilities, field tests were carried out using proto-type destruction methods. Results obtained have been interpreted using design analysis wherever applicable.

Test results reveal that locally available lime based cementitious materials shall be used as alternative to Portland Cement with limitations.

**MORPHOLOGICAL AND HISTOLOGICAL STUDY OF BREAST LUMPS  
IN PATIENTS ADMITTED TO GENERAL HOSPITAL (TEACHING) JAFFNA**

**Sakunthaladevi Ambikaipakan**

*Department of Anatomy, University of Jaffna, Jaffna*

**V. S. Pathmanathan**

*Department of Pathology, General Hospital (Teaching), Jaffna*

**V. K. Ganeshalingam**

*Department of Zoology, University of Jaffna, Jaffna*

The above study was undertaken to determine the frequency of occurrence of benign and malignant lumps presenting as breast tumours clinically at General Hospital, Jaffna. Early detection of malignant tumours will help to prevent the spreading of malignant cells in those diagnosed as malignant by early treatment.

Examinations were made on sixty five female patients who were admitted at the Teaching Hospital, Jaffna, with breast lumps, by interviewing the patients with the help of questionnaires and by analysing the lumps morphologically and histologically.

It was found that the breast lumps were found to be very common in women between 17 to 45 years of age.

Of the lumps examined morphologically 83.6% were benign. Of these 95.4% had single lumps and 4.6% had multiple lumps.

With regard to their histological features, 45.5% were of fibroadenosis 34.5% were fibroadenoma and 3.6% were cystosarcoma phyllodes (giant fibroadenoma). The remaining 16.4% included 9 breast cancers, 2 lymph nodes and one fatty tissue.

It was observed that 5 patients examined had previous history of breast lumps. Among them, four had benign lumps.

A study of these patients shows that the benign single lumps are very common among the breast lumps.

## A COMPARISON OF AMYLOGUCOSIDASE PRODUCTION FROM ASPERGILLUS NIGER BY DIFFERENT FERMENTATION TECHNIQUES

Mangaleswary Ramadas

Department of Biochemistry, Faculty of Medicine, University of Jaffna

Olle Holst and Bo Mattiason

Department of Biotechnology, Chemical Center, Lund University,  
Box 124, S-221 00 Lund, Sweden

Amyloglucosidase (AMG) was produced from *Aspergillus niger* by solid state, submerged and aqueous two-phase fermentation systems. The solid state fermentation medium consisting wheat bran and water (1:1.5 W/W) was inoculated with a spore suspension ( $10^7$  spores/g medium). The cultivation was carried out at 30°C. The fermented bran was mixed with distilled water in the ratio of 1:9 W/V. The extraction was carried out at 30°C for 30 min in an agitated water bath. This method gave 7.5—8 ml extract from 1g moist fermented solids. The enzyme titres were calculated per ml of extract. In the fed batch process, fresh medium (wheat bran-water mixture) was added every 12h for 96h. The enzyme was extracted as in the batch process. Under solid state fermentation, the fed-batch mode of operation gave an yield of 64 U/ml compared to 44 U/ml by the batch mode. Submerged cultivation was carried out in a laboratory fermentor with a working volume of 2L. The fermentation medium consisted of ( $g\ l^{-1}$ ) soluble starch 50; yeast, extract, 10; KCl, 0.5;  $KH_2PO_4$ , 5;  $(NH_4)_2SO_4$ , 5;  $MgSO_4 \cdot 7H_2O$ , 0.5;  $FeSO_4 \cdot 7H_2O$ , 0.01;  $ZnSO_4 \cdot 7H_2O$ , 0.001 and  $CuSO_4 \cdot 5H_2O$ , 0.0001. The inoculum used was 100ml of 12h old culture grown in shake flask. Cultivation was carried out at 30°C and pH 6.0. The stirrer speed was increased from 300 to 600 rpm and the air flow was 2.6 L/min. Under fed-batch mode, the cultivation was started as above and fresh feed solution consisting of 10% W/V starch was added starting from 24h to 30h. However the feed rate was maintained at 110 ml/h from 24h to 27h and thereafter increased to 140 ml/h until 30h. Subsequently, the feed was stopped and the fermentation was continued until 66h. In submerged fermentation (SmF) also the fed-batch mode gave an yield of 102 U/ml compared to 66 U/ml in the batch mode. The yield was found to be higher for SmF than SSF, with an added advantage of shorter cultivation time (66h) compared to SSF (96h). Extractive AMG production was also studied in an aqueous two-phase system consisting of PEG (6000) (5% W/V) and salt (15% W/V  $KH_2PO_4$  and  $K_2HPO_4$  in the ratio 1:1). In the aqueous two-phase cultivation, the productivity and yield of AMG increased both by a factor of 2.0 compared to the reference fermentation.

MUTATION AND PROTOPLAST FUSION OF  
*CEPHAROSPORIUM EICHHORNIAE*

Vasanthi Arasaratnam

Department of Biochemistry, Faculty of Medicine,  
University of Jaffna Sri Lanka

Chuanpit Ek Namkul

Department of Biotechnology, Faculty of Science,  
Mahidol University, Bangkok Thailand

Strains of desired characters could be obtained by genetic engineering. Mutation and protoplast fusion are other techniques used in strain improvement. *Cepharosporium eichhorniae* an industrially important fungus was selected for the mutation and protoplast fusion studies. For protoplast fusion, two double auxotrophic mutants of *C. eichhorniae* were required. Hence *C. eichhorniae* 822, a single auxotrophic mutant for the amino acid methionine was mutated using nitrosoguanidine. The survival and mutation frequencies were 9.9% and 16.4% respectively. Out of the 81 mutants obtained, a double auxotrophic mutant for the amino acids methionine and proline was selected for protoplast fusion with *C. eichhorniae* 844, which is a double auxotrophic mutant for arginine and lysine. The two auxotrophic complement each other and the complementation selection mode was used in the identification of the fusants. The protoplasts of the two strains were prepared using Novozyme and fused in presence of 50% (w/v) polyethylene glycol. The fusants were regenerated in minimal medium. The fusion efficiency was 1.4%. The fusants showed 100% stability. To confirm the fusion, the DNA contents of the two double auxotrophic mutants and the fusant were estimated. DNA contents of *C. eichhorniae* 844, 822 mutant and the fusant were 0.71, 0.77 and 1.55  $\mu\text{g/g}$  cell respectively. These results show that the number of gene copies have been doubled in the fusant and their expression would yield higher primary and secondary metabolites.



## COMPARISON OF THE PERFORMANCE OF *LACTOBACILLUS DELBRECUKII* IN FED-BATCH AND REPEATED BATCH PROCESSES

A. Senthuran, Vasanthi Arasaratnam and K. Balasubramaniam

Department of Biochemistry, Faculty of Medicine,  
University of Jaffna, Shri Lanka

Lactic acid production by *L. delbrueckii*, was studied in fed-batch and repeated batch processes. In both cases the bacteria was grown in whey supplemented with glucose or glucose and nitrogen sources, at room temperature in static culture while maintaining the pH between 5.0 and 6.5. In fed-batch process, where the control had only whey containing  $30\text{g l}^{-1}$  total sugar, lactic acid production reached a maximum of  $15.5\text{g l}^{-1}$  at 48h. When whey medium was supplemented with yeast extract ( $20\text{g l}^{-1}$ ) or  $(\text{NH}_4)_2\text{SO}_4$  ( $10\text{g l}^{-1}$ ) lactic acid production reached a maximum of  $24.5\text{g l}^{-1}$  at 48h and  $23\text{g l}^{-1}$  at 60h respectively. When the above fermentation medium was supplemented with a total of  $40\text{g l}^{-1}$  glucose being added equally in two instalments at 36h and 96h of fermentation, the lactic acid production further increased to  $35\text{g l}^{-1}$  (120h) in  $(\text{NH}_4)_2\text{SO}_4$  containing medium and  $56\text{g l}^{-1}$  (168h) in yeast extract containing medium. In the above experiment instead of glucose supplementation at 36 and 96h, supplementation of glucose and nitrogen sources at 36, 84 and 144h further increased the lactic acid production to  $74\text{g l}^{-1}$  in  $(\text{NH}_4)_2\text{SO}_4$  containing medium and  $80\text{g l}^{-1}$  in yeast extract containing medium at 196h. In the repeated batch process whey medium contained  $30\text{g l}^{-1}$  total sugar and was supplemented with either  $(\text{NH}_4)_2\text{SO}_4$  ( $10\text{g l}^{-1}$ ) or a combination of yeast extract ( $5\text{g l}^{-1}$ ) and  $(\text{NH}_4)_2\text{SO}_4$  ( $7.5\text{g l}^{-1}$ ). As fermentation progressed and when the total sugar decreased to  $10\text{g l}^{-1}$ , 50% of the spent medium was removed and replaced with their respective media containing either total sugar ( $50\text{g l}^{-1}$ ) and  $(\text{NH}_4)_2\text{SO}_4$  ( $13.0\text{g l}^{-1}$ ) or total sugar ( $50\text{g l}^{-1}$ ) and a combination of yeast extract ( $6.6\text{g l}^{-1}$ ) and  $(\text{NH}_4)_2\text{SO}_4$  ( $9.8\text{g l}^{-1}$ ) to maintain total sugar at  $30\text{g l}^{-1}$  and elemental nitrogen level at 0.31% (w/v). The medium supplemented with  $(\text{NH}_4)_2\text{SO}_4$  was replaced thrice at 36, 96 and 144h and lactic acid content in this spent medium was 18, 23 and  $35\text{g l}^{-1}$ . Similar results were obtained in the whey medium supplemented with a combination of yeast extract and  $(\text{NH}_4)_2\text{SO}_4$ . The total lactic acid produced in fed-batch process at 196h was 14.8g in 200ml spent medium ( $74\text{g l}^{-1}$ ) and in the repeated batch process at 196h was 11.6g in 500ml spent medium ( $23.2\text{g l}^{-1}$ ). These results show that lactic acid production in fed-batch process was better than that of repeated batch process under these experimental conditions.

STIMULATION OF CITRIC ACID PRODUCTION BY METHANOL IN  
*ASPERGILLUS SP.*

P. Navaratnam, Sulojana Mahendran. Vasanthy Arasaratnam  
and K. Balasubramaniam

Department of Biochemistry, Faculty of Medicine  
University of Jaffna, Shri Lanka

A mutant strain of *Aspergillus sp* UV 1 developed in this laboratory was cultivated in a medium containing [gl<sup>-1</sup>] glucose, 50; NH<sub>4</sub> NO<sub>3</sub> 0.5; KH<sub>2</sub>PO<sub>4</sub>, 0.5; MgSO<sub>4</sub>, 0.1; peptone, 7.0; ZnSO<sub>4</sub>, 0.1 × 10<sup>-3</sup> ferrous ammonium sulphate 0.1 × 10<sup>-3</sup>; CuSO<sub>4</sub> .5H<sub>2</sub>O, 0.06 × 10<sup>-3</sup> for citric acid production in liquid surface culture at room temperature. To evaluate the effect of methanol on citric acid production different concentrations of methanol (20, 30, and 30 ml<sup>-1</sup>) were added to the test medium while to the controls glucose, equivalent to methanol, was added to equalise the carbon content. Maximum citric acid (11.54 gl<sup>-1</sup>) was produced in the medium containing 30 ml<sup>-1</sup> methanol at 88h. The stimulatory effect of methanol on citric acid production from glucose as carbon source was investigated by keeping the amount of total carbon (30.9 gl<sup>-1</sup>) constant. The control experiment having glucose (77.3 gl<sup>-1</sup>) with no methanol produced 10.6 gl<sup>-1</sup> citric acid in 188h. However when one-third (10. gl<sup>-1</sup>) of the carbon source from glucose was substituted with methanol (30 ml<sup>-1</sup>) citric acid production not only increased from 10.6 gl<sup>-1</sup> to 12.4 gl<sup>-1</sup> but the time required for maximum citric acid production also decreased from 188h to 88h.

Another mutant *Aspergillus sp* UV 2 was used to test the effect of poly-unsaturated fat (gingelly oil) on citric acid production. When different concentrations of gingelly oil (0 to 8 ml<sup>-1</sup>) were added to media containing glucose (50 gl<sup>-1</sup>) and methanol (30 ml<sup>-1</sup>), no marked difference in citric acid production was observed.

## AFFINITY PURIFICATION OF AMYLOGLucosIDASE ON CROSS LINKED STARCH

Mangaleswary Ramadas

*Department of Biochemistry, Faculty of Medicine,  
University of Jaffna, Shri Lanka*

Olle Holst and Bo Mattiason

*Department of Biotechnology, Chemical Center, Lund University,  
Lund, Sweden*

Substrate and substrate-analogues have been used in the affinity purification of enzymes. In this study, epichlorohydrin cross-linked starch was used as an affinity matrix for the purification of amyloglucosidase (AMG). Studies were carried out to find out the suitable pH for AMG adsorption on to cross linked starch. The enzyme solution was diluted in 0.1 M citrate-phosphate buffer of varying pH (3-7). The cross-linked starch beads were swollen in the corresponding buffers and transferred into the enzyme solution at respective pH. Contents in the test tubes were mixed on an end to end mixer at 22°C for 1h. The AMG adsorbed was calculated by mass balance and expressed as percentage adsorption. The enzyme adsorbed was maximal at pH 4.0 and was 38% of the total enzyme added. The effect of polyethylene glycol (PEG) and salt on the adsorption of AMG on cross-linked starch at pH 4.0 was investigated. Both PEG and salt increased the percentage of AMG adsorption from 38 to 81 and 55 respectively. As the adsorption of AMG was greater in the presence of PEG, adsorption of AMG in presence of PEG was adopted. After optimizing the adsorption of AMG to cross-linked starch, AMG was purified by affinity column chromatography. Crude AMG in 10% (W/W) PEG 6000 solution at pH 4.0 was applied to the column for adsorption of AMG. After washing the column with PEG solution at pH 4.0 enzyme was eluted with 1M maltose as a competitive inhibitor. About 85% of AMG activity were obtained in the elution fractions. The specific activity increased by a factor of 6. The purity of the isolated protein was checked by 12% separating and 4% stacking SDS-poly acrylamide gel electrophoresis. Gel was stained with coomassie blue solution (in 40% methanol and 10% acetic acid) and destained with 10% acetic acid. The preparation was homogeneous under the conditions used.

GLUCOSE CONCENTRATION AND INOCULUM SIZE ON  
CITRIC ACID PRODUCTION BY *Aspergillus* sp M 2

P. Navaratnam, Sulojana Mahendran, Vasanthi Arasaratnam  
and K. Balasubramaniam

Department of Biochemistry, Faculty of Medicine,  
University of Jaffna, Shri Lanka

Production of citric acid from glucose by a mutant strain of *Aspergillus* sp M 2 [developed in our laboratory] was investigated in surface culture using a liquid medium (100ml) containing two different concentrations (50 and 100g<sup>-1</sup>) of glucose with fixed amounts (g<sup>-1</sup>) of NH<sub>4</sub>NO<sub>3</sub>, 0.5; KH<sub>2</sub>PO<sub>4</sub>, 0.5; MgSO<sub>4</sub>, 0.1 peptone, 7.0; ZnSO<sub>4</sub>, 0.1 × 10<sup>-3</sup>; ferrous ammonium sulphate 0.1 × 10<sup>-3</sup>; CuSO<sub>4</sub>.5H<sub>2</sub>O 0.06 × 10<sup>-3</sup> methanol 30ml<sup>-1</sup> and squalidly oil, 2.0ml<sup>-1</sup>. In order to determine the total sugar required for citric acid production and to overcome the inhibitory effect of glucose on growth, the fermentation media containing initial concentrations of 50g<sup>-1</sup> and 100g<sup>-1</sup> glucose were supplemented with the same amounts of glucose as the glucose in the medium was used up. In both experiments, citric acid production reached the maximum of 27g<sup>-1</sup> and the total glucose utilized was 110g<sup>-1</sup>. Hence in future experiments, the initial concentration of glucose was kept at 140g<sup>-1</sup> even though only 110g<sup>-1</sup> glucose was utilized. Excess 30g<sup>-1</sup> glucose was added as a safety measure to ensure that the organism did not utilize citric acid as a carbon source. The effect of using spore inoculum and mycelial inoculum was investigated. The results show that the citric acid production with the mycelial inoculum reached a maximum on the 17th day and with spore inoculum on the 21st day. This apparently is not a significant advantage as the preparation of the mycelial inoculum takes about three days. When fermentation in 1 l scale was carried out with a glucose concentration of 140g<sup>-1</sup> and 65h old 10% (v/v) mycelium as inoculum, the citric acid production reached a maximum of 45.2g<sup>-1</sup> at 26th day. To produce the citric acid in large scale the inoculum should have sufficient amount of mycelium. Hence the growth pattern of *Aspergillus* sp M2 from spores was studied. The results showed that the mycelia reached mid log phase in 38h and 32h with the spore and mycelial inocula respectively. Maximum citric acid (28g<sup>-1</sup>) was produced from 140g<sup>-1</sup> glucose on 18th day when a mycelial inoculum (0.372g (dry wt)/ 100ml medium) at mid log phase was used.

PRELIMINARY STUDIES ON  $\alpha$ -AMYLASE PRODUCTION  
IN SOLID STATE FERMENTATION

W. S. Tambyrajah, Vasanthi Arasaratnam and K. Balasubramaniam

Department of Biochemistry, Faculty of Medicine,  
University of Jaffna, Shri Lanka

The inoculum preparation of *Bacillus licheniformis* 6346 showed a sigmoid curve with mid log phase at 20h. At this hour the optical density was 0.35 (620nm). This stage of culture (20%, v/v inoculum) was inoculated into fresh nutrient broth, incubated at room temperature (30°C) for 8h and used as the inoculum. Fermentation on rice bran medium having an initial reducing and total sugar contents of  $70\text{g l}^{-1}$  and  $210\text{g l}^{-1}$  respectively was compared with rice husk medium which contained no reducing sugar but had starch equivalent to  $150\text{g l}^{-1}$  total sugar while other nutrients in the bran medium and husk medium, and the fermentation conditions being the same, the production of enzyme in rich bran and rice husk media were 29 U/gDBM (Dry Bacterial Bran) and 250 U/gDBM respectively. This result indicates that the presence of reducing sugar repressed the  $\alpha$ -amylase production. The nutrient requirements of *Bacillus licheniformis* in solid state fermentation on rice husk medium was investigated keeping nitrogen, soluble starch and lipids (coconut oil:gingili oil = 1:1) at constant level but with combination of different nitrogen sources [ $(\text{NH}_4)_2\text{HPO}_4$ ;  $(\text{NH}_4)_2\text{SO}_4$  and soya flour] and carbon sources [wheat flour and unpolished red raw rice flour]. Maximum  $\alpha$ -amylase activity was observed with the combination of  $(\text{NH}_4)_2\text{HPO}_4$  and rice flour (433 U/gDBM). To find the significance of lipid sources, the oils were not added to medium containing  $(\text{NH}_4)_2\text{HPO}_4$  and wheat flour. The absence of oils decreased the enzyme production by 16%.

The extraction of enzyme from the rice husk medium was optimized under different conditions. It was observed that the mode of agitation and the contact time did not improve the recovery of the enzyme. The enzyme was extracted best when the DBM to buffer ratio was 1:10. The thermostability of the  $\alpha$ -amylase was studied in presence and absence of calcium ion. Presence of calcium ion doubled its stability at 85°C. In the absence of calcium ion at 85°C the activity decreased by 50% in 1h and at 95°C the activity decreased by 87.2% in 20 minutes.

## ISOLATION OF A YEAST STRAIN FROM TODDY FOR CELL RECYCLING

K. Sivaganeshan, Sulojana Mahendran, Vasanthi Arasaratnam and  
K. Balasubramaniam

*Department of Biochemistry, Faculty of Medicine,  
University of Jaffna, Sri Lanka*

Yeast strains were isolated in toddy samples obtained from four areas in Jaffna peninsula. The strains were isolated based on their morphological characteristics. The yeast strains were labelled as BT<sub>1</sub>, BT<sub>2</sub>, BT<sub>3</sub>, BT<sub>4</sub>, BT<sub>5</sub> based on the place from where the toddy sample was obtained. The best alcohol producing yeast strain from the five isolated strains was from Kokuvil area (BT<sub>1</sub>) and it produced 65.5g<sup>-1</sup> of alcohol from 150g<sup>-1</sup> of sucrose in 72h. The optimum pH for the ethanol production by BT<sub>1</sub> strain was 5.0 at room temperature. To reduce the fermentation time (to increase the productivity) the optimum initial cell number in the inoculum was determined which was 2.2x10<sup>8</sup>/ml and the ethanol produced was 67.3g<sup>-1</sup> at 24h instead of 72h. The productivity increased from 0.91g<sup>-1</sup>h<sup>-1</sup> to 2.8g<sup>-1</sup>h<sup>-1</sup>. To obtain a mutant yeast with improved ethanol producing ability BT<sub>1</sub> strain was exposed to UV radiation at 254nm for 20min at 6cm distance. The mutant (BT M) obtained showed 10% more efficiency than the parent strain. The yeast cells were recycled seven times for ethanol production. The productivity of ethanol reached the maximum at the second cycle (1.35g<sup>-1</sup>h<sup>-1</sup>) and then remained almost same in the next five cycles without any appreciable change in the efficiency of ethanol production. The average time per cycle to utilize the sugar completely was 48h. These results indicate that the BT<sub>1</sub> can be used in continuous cell recycle fermentation process for the production of ethanol without loss of productivity and efficiency.

## OPTIMIZATION OF CULTURE CONDITIONS FOR PRODUCTION OF RENNIN BY *ASPERGILLUS NIGER* BY SOLID SUBSTRATE FERMENTATION

Vasanthi Shanmuganathan, Vasanthi Arasaratnam and K. Balasubramaniam

*Department of Biochemistry, Faculty of Medicine,  
University of Jaffna, Sri Lanka*

Rennin production by solid state fermentation in rice bran medium was optimized. The fungus was cultivated in rice bran solid medium containing different moisture content. The maximum production of 12.9 U/g DMM (Dry Moldy Medium) of rennet was obtained at 50% moisture content at 40h. To increase the productivity of rennet, inoculum concentration on rennet production was studied. Maximum activity of 31 U/g DMM was obtained when the initial density of spores/g medium was  $1 \times 10^7$ . However for large scale rennet production, mycelial inoculum is more suitable than the spore inoculum. Hence, age of the mycelial inoculum in bran medium was optimized. It was observed that a solid inoculum of 10% (w/w) at about 60h of growth was the best and at this inoculum concentration, rennet production reached a maximum of 31.26 U/g DMM at 24h compared with the spore inoculum giving maximum rennet production at 40h. Rennin is a protease which hydrolyses caseinogen to casein. Hence the rennet production with casein as inducer was optimized. When different concentrations of casein ranging from 1-8% (w/w) were added to the medium, maximum rennet production (34.8 U/g DMM) was observed with 2% (w/w) casein. When casein (2%, w/w) was supplemented with soya flour (10%, w/w) rennet activity further increased by 50%.

## PRODUCTION OF ETHANOL USING STRACH IN CORN AND RICE FLOUR

Vasanthy Arasaratnam, N. Nithianantharajah, Ketheeswary  
Mylvaganam, K Sivaganeshen and K. Balasubramaniam

*Department of Biochemistry, Faculty of Medicine,  
University of Jaffna, Shri Lanka*

Starch (112g) in corn flour ( $160\text{g l}^{-1}$ ) was hydrolysed by the synergistic action of  $\alpha$ -amylase and glucoamylase at pH 5.0 and  $70^\circ\text{C}$ . The extract of the corn flour hydrolysate contained  $98\text{g l}^{-1}$  reducing sugar,  $116\text{g l}^{-1}$  total sugar and 76.6% dextrose equivalent (DE). When the sugar in the extract was fermented by a commercial yeast preparation (B. S. Fermipan),  $9.2\text{g l}^{-1}$  ethanol (yield 15.9%) was obtained. Since the yield of ethanol was low, a locally isolated yeast strain (BT<sup>1</sup>) was compared with Fermipan for ethanol production. The B. S. Fermipan, and BT<sup>1</sup> yeast produced  $9.2\text{g l}^{-1}$  (yield 15.9%) and  $20.7\text{g l}^{-1}$  (yield 35.7%) ethanol from an extract containing  $116\text{g l}^{-1}$  total sugar. From these results, it was decided to use the locally isolated strain in the subsequent experiments as it gave an ethanol yield of 35.7%. To increase the ethanol yield, the effect of initial yeast cell number in the medium was studied. When the cell number was  $10^6$ ,  $10^7$  and  $10^8$  / ml medium, the ethanol yield was 55.5, 60.0 and 73.6% respectively. From the results, it was decided to use an initial cell density of  $10^8$  / ml medium. Due to the present crisis corn is not freely available in the local market. Hence the carbon source was switched on from corn flour to rice flour. Hydrolysis of  $160\text{g l}^{-1}$  and  $320\text{g l}^{-1}$  of corn and rice flour was compared. The reducing sugar and the DE obtained were almost same for corn and rice at their respective concentrations. Therefore the fermentation experiments were continued with the substitution of corn flour extract with rice flour extract. Fermentation of  $120\text{g l}^{-1}$  of total sugar in rice flour extract at 48h produced  $31\text{g l}^{-1}$  ethanol. This is comparable to the ethanol obtained ( $27\text{g l}^{-1}$ ) with the fermentation of corn flour extract having a total sugar of  $116\text{g l}^{-1}$ . The effect of total sugar concentration on the efficiency of ethanol production was studied. When the sugar concentrations were 130, 200 and  $220\text{g l}^{-1}$ , the ethanol yield was 47.7%, 28% and 28% respectively. The results show that the ethanol yield was not improved by increasing the initial sugar concentration in the fermentation medium. Deficiency of nitrogen source in the medium could be a cause for the reduced ethanol production. When the medium containing  $130\text{g l}^{-1}$  sugar was supplemented with triammonium orthophosphate ( $1.0\text{g l}^{-1}$ ) the ethanol yield was reduced from 47.7% to 21.1%. Thus addition of inorganic nitrogen did not help to improve the ethanol yield. Further studies are in progress to improve the ethanol yield.



## GLUCOAMYLASE PRODUCTION FROM *ASPERGILLUS NIGER* BY SOLID STATE FERMENTATION

Vasanthi Arasaratnam, Ketheeswary Mylvaganam, K. Thayanathan  
and K. Balasubramaniam

*Department of Biochemistry, Faculty of Medicine,  
University of Jaffna, Sri Lanka*

The *Aspergillus niger* (CFTRI 1105) was cultivated in solid state medium for glucoamylase production. In the medium containing rice bran (100g), corn flour (2g), stock mineral solution (10ml) and tap water (90ml) the glucoamylase activity was 39.8 U/g DMM (Dry Moldy Medium). When the medium containing rice bran (100g) was substituted with rice husk (100g) or rice husk (70g) - rice bran (30g), the glucoamylase activity decreased from 39.8 U/g DMM to 28.7 U/g DMM and 16.0 U/g DMM respectively. To improve the glucoamylase production the corn flour (2g) in the medium was substituted with soya flour (2g) and this increased the glucoamylase production from 9.8 U/g DMM to 84 U/g DMM. When the effect of soya flour, urea or peptone at 3.2% elemental nitrogen level with corn as carbon source was investigated omitting rice bran and rice husk the glucoamylase activity obtained was 109.1, 5.0 and 70.6 U/g DMM respectively. From the results it can be concluded that soya flour is a better nitrogen source than urea or peptone. To enhance the enzyme production by increasing the intestinal air content, rice husk (460g) was added to the control medium containing corn flour (100g) soya flour (100g), stock mineral solution (20 ml) and tap water (180 ml). The glucoamylase activities of test and control were 243 and 109.1 U/g DMM respectively. These studies indicate that solid state fermentation is an appropriate technology for the production of glucoamylase in developing countries although glucoamylase is produced by submerged fermentation in the developed countries.

## PRODUCTION OF LACTIC ACID IN NATURAL AND SYNTHETIC MEDIA BY A *LACTOBACILLUS* SP. ISOLATED FROM SOURED MILK

A. Senthuran, Vasanthi Arasaratnam and K. Balasubramaniam

Department of Biochemistry, Faculty of Medicine,  
University of Jaffna, Shri Lanka

A strain of *Lactobacillus* sp. was isolated from soured milk in our laboratory for lactic acid production. The bacteria was isolated based on the morphological characteristics, gram positive staining and the specificity of lactic acid for LDH. All the experiments with *Lactobacillus* sp. were carried out at room temperature in static culture. The strain cultivated in milk containing  $60\text{g l}^{-1}$  total sugar produced  $23\text{g l}^{-1}$  lactic acid at 36h, with 80% substrate utilization. However when the pH was maintained by the initial addition of  $\text{CaCO}_3$ , the

the lactic acid production increased to  $34.5\text{g l}^{-1}$ , which corresponds to a 12% increase in the efficiency of lactic acid production. In the rest of the experiments pH was maintained by the initial addition of  $\text{CaCO}_3$  ( $60\text{g l}^{-1}$ ).

As the quantity of lactic acid produced from milk ( $34.5\text{g l}^{-1}$ ) was not sufficient for down-stream processing of lactic acid economically, it was decided to supplement milk with different sugars to a total concentration of  $150\text{g l}^{-1}$ . Among glucose, sucrose and lactose, the organism performed best in the lactose supplemented media in respect to lactic acid ( $43\text{g l}^{-1}$ ) production. This preference of lactose utilization may be due to the *Lactobacillus* sp. having [the enzymes for] lactose metabolism. As the proteins of milk are not used up in the fermentation, milk could be substituted with whey. Therefore further studies were carried out with whey as the fermentation medium. In whey the total sugar was  $30\text{g l}^{-1}$  and the organism fermented 86.7% of the total sugar and produced  $14.5\text{g l}^{-1}$  lactic acid at 24h. To compare lactic acid production in whey and milk, whey medium was supplemented with  $30\text{g l}^{-1}$  lactose to bring the total sugar level to  $60\text{g l}^{-1}$ . At 36h, in whey medium supplemented with lactose,  $29\text{g l}^{-1}$  lactic acid was produced with 86% substrate utilization. As a control, *Lactobacillus* sp. was cultivated in synthetic culture media containing different sugars. The organism preferred the synthetic medium containing lactose in preference to other sugars. A delay (12h) in attaining maximum lactic acid production in synthetic medium was observed when compared with milk and whey medium. Since the organism was isolated from soured milk and the inoculum was prepared in milk the organism might have been adapted to milk and whey. This could be one of the reasons for the delayed lactic acid production in the synthetic medium or else the medium may be deficient or lacking one or more of the essential nutrients needed for lactic acid production by the organism.

## ALCOHOLIC AND NON-ALCOHOLIC BEVERAGES FROM CORN MALT

Sulojana Mahendran, Ketheeswary Mylvaganam, Vasanthi Arasaratnam  
and K. Balasubramaniam

*Department of Biochemistry, Faculty of Medicine,  
University of Jaffna, Shri Lanka*

Malt powder was prepared by malting corn for 5 days, drying in the sun for two days and powdering in a commercial mill. Corn malt powder was roasted in an oven at 50°C for 4h to increase the flavour. Extract (3.5l) was prepared from 1kg corn malt powder by the action of endogenous and exogenous amylases. The malt extract contained 10g<sup>l</sup><sup>-1</sup> reducing sugar, 150g<sup>l</sup><sup>-1</sup> total sugar, 5g<sup>l</sup><sup>-1</sup> protein and .2g<sup>l</sup><sup>-1</sup> calcium. One litre of malt extract (DE 60) at pH 4.5 was inoculated with 5g<sup>l</sup><sup>-1</sup> bakers yeast and allowed to ferment at room temperature. After the fermentation (72h) a malty flavoured dark brown coloured clear alcoholic beverage was obtained. Alcohol strength, acidity and the amount of non-fermented carbohydrate of the alcoholic beverage were 30g<sup>l</sup><sup>-1</sup>, 4g<sup>l</sup><sup>-1</sup> and 137.5g<sup>l</sup><sup>-1</sup> respectively. The alcohol strength of the beverage increased from 30g<sup>l</sup><sup>-1</sup> to 78g<sup>l</sup><sup>-1</sup> and the nonfermented carbohydrate decreased from 137.8g<sup>l</sup><sup>-1</sup> to 36g<sup>l</sup><sup>-1</sup> when the extract was treated with glucoamylase and yeast simultaneously and the efficiency of ethanol produced was 89%.

In the preparation of non-alcoholic beverage (maltova) one liter of extract (DE, 60) was evaporated in a sand bath. The dry residue was mixed with milk powder, sucrose, cocoa and glucose, and was further dried and powdered. This preparation is comparable to Maltova in its organoleptic properties and could be readily reconstituted by mixing with milk, tea or coffee to provide a good non-alcoholic beverage.

## NITROGEN SUPPLEMENTATION OF WHEY IN LACTIC ACID PRODUCTION BY *LACTOPACILLUS DELBRUECKII*

A. Senthuran, Vasanthy Arasaratnam and K. Balasubramaniam

*Department of Biochemistry, Faculty of Medicine,  
University of Jaffna, Jaffna*

The objective of this study was to evaluate the supplementation of whey with different nitrogen sources on lactic acid production. *L. delbrueckii* was grown at room temperature in static culture. When whey containing  $30\text{g l}^{-1}$  total sugar was supplemented with 0.0, 10.0, 20.0 and  $30.0\text{g l}^{-1}$  of yeast extract, lactic acid produced at 48h was 12.0, 22.5, 23.0 and  $22.5\text{g l}^{-1}$  respectively and the sugar utilized was 55.0%, 86.6%, 90.0% and 90.0% respectively. The time of harvesting lactic acid in future experiments is based on the 90% utilization of the carbohydrate source. To increase the lactic acid production, glucose ( $20\text{g l}^{-1}$ ) was added at 36h to the whey medium which was supplemented with different concentrations of yeast extract (as in the above experiment) and the lactic acid produced at 84h was 27.5, 35.0, 40.0 and  $41.0\text{g l}^{-1}$  respectively and substrate utilization was 71.0%, 80%, 92% and 94.0% respectively. Based on these results,  $20\text{g l}^{-1}$  yeast extract was selected for supplementation and it contained 0.31% (w/v) elemental nitrogen. At the above elemental nitrogen concentration (0.31%, w/v), different nitrogen sources such as yeast extract, peptone, soya flour and  $(\text{NH}_4)_2\text{SO}_4$  were supplemented to whey and the amount of lactic acid produced at 48h was 24.5, 22.5, 23.0 and  $20.0\text{g l}^{-1}$  respectively and the substrate utilization was 92.0%, 85.7%, 90.0% and 83.0% respectively. The rate of lactic acid production at 36h was highest ( $0.57\text{g l}^{-1}\text{ h}^{-1}$ ) in whey supplemented with yeast extract. In the above experiment, containing different nitrogen sources, addition of glucose ( $20\text{g l}^{-1}$ ) at 36h further increased the amount of lactic acid production at 84h to 40.5, 35.0, 36.5 and  $30.5\text{g l}^{-1}$  substrate utilization was 92.0, 88.0, 90.0 and 80%. Since the best performance of *L. delbrueckii* in whey supplemented with yeast extract could be due to its vitamin B content, whey was supplemented with different nitrogen sources and vitamin B and no significant improvement in lactic acid production was observed. As yeast extract supplementation was not economically attractive, it was decided to use a combination of yeast extract and  $(\text{NH}_4)_2\text{SO}_4$ . When the elemental nitrogen ratio of yeast extract to  $(\text{NH}_4)_2\text{SO}_4$  was 1:3, the substrate utilization was 90.0% with 84.0% efficiency of lactic acid production which is same as in whey medium supplemented with  $20\text{g l}^{-1}$  yeast extract. These results show that the whey obtained as by product during cheese preparation from milk, can profitably be used for the production of lactic acid.

## THE POTSDHERD INSCRIPTIONS FROM POONAKARY A HISTORICAL PERSPECTIVE

S. K. Sittampalam and P. Pusparatnam

*Department of History, University of Jaffna, Shri Lnaka*

The aim of the present paper is to high light and to place in a historical perspective the recent discovery of Brahmi writings on Pottery discovered by the second author during the course of his explorations and which could be datable on Palaeographical grounds to the 3rd/2nd century B. C.. The noteworthy Brahmi characters are a, i, ka, ma, r, l, n, l and la. These along with potsherds bearing inscriptions have been found at Manithalai and Paraman Kirai. At Mannithalai two inscribed potsherds which have been diciphered as  $\bar{i}l\bar{a}/\bar{i}l\bar{a}$  and  $\bar{i}l\bar{a}$  respectively have been found. Similarly another two inscriptions which have been diciphered as  $\bar{l}o\bar{m}a$  and  $\bar{V}e\bar{l}\bar{a}n$  have been found at Paramankirai.

However, long before the discovery of these the evidence for the early use of Brahmi writing during the Pre-Christian era in the North has come from Kantarodai and Anaikoddai in the Jaffna Peninsula and Mahakaccatkodi, Eurupotana, Periyapuliyankulam and Vedikkinari malai in the Vavuniya district. However with the exception of the Bronze seal from Anaikoddai and the inscribed potsherds from Poonakary the evidence from the sites mentioned above clearly indicate their link with Buddhism. It is here only the Potsherds inscriptions from Poonakary assume new significance.

The assesment of these Potsherd inscriptions in the light of already found evidences in the North as well as similar evidences from the South by us shows that the Poonakary Potsherds present an earlier stratum of Brahmi script belonging to the Southern variety of Brahmi before it got submerged with the Northern Brahmi associate with Buddhism in the middle of the 3rd century B.C. in Sri Lanka. This further proves that Southern Tamil Nadu and Sri Lanka were in the same cultural zone before they succumbed to North Indian influences. Similar discovery of Potshed inscriptions from Anuradhapura in the Megalithic Cultural zone dated to 7th century B.C. is finally analyed to vindicate our point of view.

## THE DISTRIBUTION OF RAINFALL VARIABILITY IN BATTICALOA

C. Elankumaran

*Department of Economics*  
*University of Jaffna, Sri Lanka*

The mostly rainfall data recorded at the Batticaloa meteorological station from 1870 to 1985 was used to study the behaviour of the probability distribution of rainfall variability during the popular seasons of this region.

The distributions of rainfall variability during South-West monsoon and the Convictional season of October-November are found to be approximately normally distributed, while the distribution of annual rainfall variability is normal with little evidence. The rainfall variability during the popular North-East monsoon season for Agriculture in this region does not follow normal distribution, however it is found to be a symmetrical distribution. The distribution of the Convictional season of March-April was found to be asymmetrical. Rainfall probabilities have been calculated for the identified distributions. Further analysis on different periods indicate that the parameters of the above described distributions are not stable.

The statistical significance for high risk of floods during North-East monsoon season and the Convictional season of October - November and for high risk of drought during South-West monsoon season and the Convictional season of March-April in crop cultivation in the Batticaloa region have been confirmed.

*Key words* : North-East and South-West monsoons, Convictional seasons and normal probability distributions.

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## THEORY OF ANUMĀNA IN SAIVA SIDDHANTHA

S. Krishnarajah

*Department of Philosophy  
University of Jaffna, Sri Lanka*

In the Indian tradition, polemical exercises such as eristic debates, discussions and disputes play an important role in philosophical discourses. A system builder or a spokesman of a school of thought in Indian Philosophy is expected to argue out positively for the body of doctrines formulated to explain his philosophical position and at the same time he has to criticise and pinpoint the shortcoming of the rival schools of thoughts. In any case debates have to be conducted on the basis of certain rules. This process of debating is denoted by the term "Katha"

The term Katha defines "vakya-prabandha" (the sentence—discourse.) It is a treatise of a subject in which the entire subject is unified organically. The characteristic feature is that it concerns it-self with a "weighting" of the relative strength and weaknesses of the opposite system of thought, the champions of which enter the philosophical discourse as contestants. The view of the opponent is called Pūrvapaksa and one's own view is Uttarapaksa or Siddhanta. A careful analysis of the claims of the rival schools in the consideration of their strength and weaknesses are the essential features of "Katha".

The present study deals with the theory of Anumāna in Saiva-Siddhanta which is an integral part of "Katha". In order to articulate it and present its features, it is essential to take into account a number of well known Saiva Siddhanta texts like Pauskaragāma, Sivajnana Siddhiyar and their commentaries. Reference to other Saiva-Siddhanta texts wherever and desirable will not be overlooked. The commentaries of the above works play an important role in the theory of Anumāna and bring out the contours of the theory in sufficient details. The commentaries however do not exhibit uniformity in their explanations and seems to be misleading in certain points. This paper is attempt to present, the Anumāna theory immanent in the Saiva Siddhanta texts and its commentaries.







