female rat may have arisen from a non-disjunction event in oogenesis or spermatogenesis in the parents or non-disjunction at a post-zygotic stage (figure 2).

Survival of a monosomic individual poses questions as genetic imbalance is not favoured in the developing organism. However, the fact that autosomal monosomies do exist, and that trisomy, the countertype of a given monosomy, is always detectable, appear to suggest that autosomal monosomy per se is not necessarily lethal. If this is true, it can be assumed in the present case that the remaining homologue of chromosome 9 is devoid of any recessive lethal gene.

Why was monosomy of only chromosome 9 detected? It is possible that chromosome loss is not a random process. Earlier workers have shown that chromosomes 1, 9 and 13 in the rat are most vulnerable to structural rearrangements. In the present case it seems chromosome 9 is a sensitive element in the chromosome complement, but its loss exerts no effect on the phenotype or survival of the animal.

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A CASE OF FURUNCULOSIS IN LABORATORY-REARED LATES CALCARIFER

S. C. MUKHERJEE, M. PEER MOHAMED and S. V. ALAVANDI

Physiology, Nutrition and Pathology Division, *Fisheries Environmental Management Division, Central Marine Fisheries Research Institute, Cochin 682 031, India.

Of the bacterial diseases of fishes, furunculosis has received the most attention. The pathogen responsible for this disease, Aeromonas salmonicida, commonly causes systemic disease in salmonids and in several freshwater forms. Sometimes the skin may also be affected in these species of fishes. As the disease is infectious, it is very important to take precautionary measures to prevent the infection from spreading. This paper deals with the skin form of furunculosis in Lates calcarifer and its recovery through a successful treatment.

Fifteen L. calcarifer measuring about 8–10 cm in length were reared in the laboratory in a 2001 capacity perspex tank. The fishes were maintained under optimum oxygen level and 30 ppt salinity. During the period of observation (25 days), one fish developed marked dullness and skin lesions, and was removed from the group and kept separately in another tank. Water in both the tanks was changed and the fishes observed closely for these symptoms in the rest of the group.

Clinical materials, namely swabs from lesions and exfoliated tissue, were inoculated on nutrient agar,
Zobel's marine agar 2216 and TCBS agar plates and incubated at room temperature for 48 h. The isolates were identified according to the methods described earlier\(^1\sim3\).

**Treatment:** (i) Affected skin was swabbed with a solution prepared by dissolving 10 mg of acriflavine in 100 ml of distilled water. A second application was made after 24 h.

(ii) Three mg of oxytetracycline was dissolved in 31 of seawater (30 ppt) and the animal was given a bath for 24 h every two days in the medicated water from the 5th day onwards until recovery.

The fish showed greyish-yellow areas of erosion with haemorrhagic border on the skin of the back, proximal third of the lateral aspects of the body, and near the head. The animal became extremely sluggish in movement and markedly anorexic. On the second day the affected areas extended around the eyes and the muscle tissue showed blister-like necrotic areas and oedema (figure 1). There was continuous shedding of the cuticle and epidermis with widening of the abraded surface of the skin.

Bacterial smears from abraded lesions and exfoliated cells from the epidermis revealed gram-negative rods that were identified both morphologically and biochemically as *Aeromonas salmonicida* subsp. *salmonicida*. The bacteria were nonmotive and sensitive to oxytetracycline and streptomycin. *A. hydrophila* subsp. *proteolytica* was also isolated, the cells were motile and resistant to oxytetracycline but sensitive to streptomycin.

Twenty-four hours after the application of acriflavine there was excessive shedding of dead tissue from the skin surface. On the second day noticeable improvement was observed, with reduction in tissue shedding. The condition improved considerably from the sixth day onwards after treatment with oxytetracycline, given after antibiotic sensitivity tests. The abraded skin lesions started healing and were soon replaced by healthy tissue. By the twelfth day the animal had recovered completely and no skin lesions were seen (figure 2).

Furunculosis of fish usually erupts in a fishery or farm on introduction of a carrier fish. Roberts\(^4\) opined that the disease is usually associated with high temperature, low oxygen levels and overcrowding in the tank. In the present case the affected animal could have been a carrier, exhibiting the disease resulting from stress associated with overcrowding and, possibly low oxygen level. Duijn\(^5\) opined that the infection first occurs in the gills and alimentary tract, and may be present latent for a long time. The disease breaks out when such fishes are weakened by adverse conditions. According to Sindermann\(^6\) furunculosis is a highly infectious disease in freshwater fish. However, the pathogen is sufficiently salt-tolerant.

Perusal of the available literature has not revealed reports of the occurrence of furunculosis in *L. calcarifer* in India. This is therefore the first report in India in laboratory-reared species. The isolation of *A. hydrophila* subsp. *proteolytica*, which is known to cause secondary infections like fin rot and haemorrhagic septicemia, suggests that this organism might have acted in association with *A. salmonicida*, aggravating the lesion.

Nitrofurans, sulphonamides, oxytetracycline and chloramphenicois in the feed for 14 days and sulphamethazine, terramycin and nitrofurazone in varied doses in the diet for several days have been advocated earlier for furunculosis\(^4\sim6\). Our trial with aqueous acriflavine solution and subsequently with oxytetracycline has proved effective in treating the disease.

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**Figure 1.** Furunculosis in *Lates calcarifer*. Note the areas of erosion on the skin of the back, above the eye, and on lateral aspects.

**Figure 2.** Furunculosis-affected *L. calcarifer* on twelfth day after treatment. Note the healthy skin in areas that showed abrasion.
ENZYMATIC DIFFERENTIATION OF AZOTOBACTER AND AZOMONAS

S. C. JANA, P. K. CHAKRABARTTY and A. K. MISHRA

Department of Microbiology, Bose Institute, 93/1 APC Roud. Calcutta 700 009, India.

According to the ninth edition of Bergéy’s manual of systematic bacteriology, the family Azotobacteraceae consists of two genera, Azotobacter and Azomonas. Members of the two genera show strong immunological cross-reaction and high rRNA citron homology. Cyst formation by Azotobacter and DNA base composition differentiate members of the two genera. The grouping is further supported by bacteriophage typing. It is, however, not known if there is any enzymatic basis for this separation. Previous investigations reported the operation of the Entner-Dudoroff (ED) pathway for glucose oxidation in Azotobacter. Enzymes of the Embden-Meyerhof-Parnas (EMP) pathway and pentose phosphate (PP) pathway were also demonstrated in Azotobacter vinelandii. The present investigation attempts to make a comparative study of the key enzymes involved in the different pathways of carbohydrate metabolism.

Azotobacter chroococcum B11, Azotobacter chroococcum B2, Azotobacter vinelandii Ka were isolated in our laboratory from soil and identified according to the Bergey’s manual. Azotobacter vinelandii 2821, Azomonas agilis 2819 and Azomonas macrocytogenes 2454 were procured from the National Collection of Industrial Microorganism (NCIM), Pune, India. The organisms were maintained on slants of Burk’s nitrogen-free medium between experiments. The cells were grown in nutrient broth (Hi-Media, Bombay) supplemented with 0.5% sucrose as carbon source for 16 h, when the cells reached late log phase of growth. The cells were harvested by centrifugation at 10,000 g in a Sorvall centrifuge. Cell-free extracts were prepared by sonication of cells for a total period of 3 min and the extracts were clarified by centrifugation at 30,000 g for 15 min. The supernatant was used for the enzyme assays. 6-Phosphogluconate dehydrogenase (6PGD), ED enzymes (combined activities of 6-phosphogluconate dehydratase and 2-keto-3-deoxy-6-phosphogluconate aldolase), 6-phosphofructokinase (PFK) and fructose diphosphate aldolase (FDA), glyceraldehyde-3-phosphate dehydrogenase (GL3PD) and isocitrate dehydrogenase (ISDH) were assayed according to published procedures. Protein was determined in cell-free extracts according to Lowry et al.

From a survey of the enzymes of the ED, EMP, PP and tricarboxylic acid (TCA) cycle pathways (Table 1), an operational ED pathway was detected in all the strains, in confirmation of the previous findings. However, the specific activities of the ED enzymes were several-fold higher in the cell-free extracts of the strains of Azomonas than in those of the strains of Azotobacter under identical conditions of assay. The reason for the higher specific activities of ED enzymes in strains of Azomonas is not clear. Small differences in the specific activities of carbon metabolism enzymes have been noted in Azospirillum brasilense wild-type strain and its mutants, while significant differences in the activities of ED enzymes were noted in strains of Rhizobium and Bradyrhizobium. Considerable differences in specific activities of ED enzymes in the strains of Azotobacter and Azomonas would, as such, lend further support to this separation at the generic level. The presence of high levels of PFK, a key enzyme of the EMP pathway, in many strains (Table 1) suggests an operational EMP pathway in them, though the activity of FDA, another key enzyme of the EMP pathway, is comparatively low in all the strains of Azotobacter studied. This would imply that EMP pathway operates at a lower efficiency in Azotobacter than in strains of Azomonas. The activity of GL3PD, an enzyme involved in the EMP, ID and PP pathways, was high in all the strains. The high activity of ISDH, a TCA cycle enzyme, in all the