Bacteriocinogeny in *Lactobacillus fermenti*

BACTERIOCINS are antibiotics produced by bacteria and which act on strains of the same or closely related species. They are protein in nature. De Klerk and Coetzee[1] reported bacteriocin production by homofermentative and heterofermentative species of *Lactobacillus*. Bacteriocin was detected in the supernatants of 10 day old broth cultures of eleven out of fifty-nine strains of *Lactobacillus acidophilus* and one out of forty-two strains of *L. fermenti*. The production of bacteriocins often depends on growth conditions[2], and strains which produce bacteriocin on agar may show little or no activity in broth[3]. Because the methods previously used may not have been optimal[2] it was decided to re-investigate the incidence of bacteriocinogeny in *L. fermenti* strains. One hundred and twenty-one strains of *L. fermenti* isolated locally from as many different sources of human saliva were tested. Many of the strains showed different lytic patterns when tested with a series of lactobacillus bacteriophages (unpublished observations). The media used were MRS broth and agar[4] and cultures were incubated at 37° C in an atmosphere of carbon dioxide. Bacteriocin production was investigated by stabbing single colonies of the 121 strains into fresh plates. After overnight incubation the plates were sterilized with chloroform vapour. They were then layered with soft agar seeded with an indicator strain and re-incubated overnight. All 121 strains were also used as indicators of bacteriocinogeny in different experiments. Twenty-five of the strains showed clear rings of inhibition of indicator organisms 2–3 mm wide. All twenty-five strains inhibited the same forty-four *L. fermenti* indicators. Sixty-four homofermentative strains of lactobacillus were then also tested for susceptibility. Five strains of *L. acidophilus* were inhibited by the same twenty-five *L. fermenti* strains. Subcultures of agar fragments from clear areas of inhibition never showed growth and the inhibitory activity could not be serially transmitted to fresh lawns of the original indicator organisms. Subsequently inhibitory activity in undiluted supernatants of overnight broth cultures of the producer strains was demonstrated by techniques previously used[2]. This activity was variable and could frequently be discerned only as a hazy spot of inhibition in the indicator lawn. The inhibitory activity of supernatants could be concentrated by precipitation with saturated ammonium sulphate and the agents diffused more than 1 cm from a central hole in agar. This was demonstrated by filling the hole with a concentrated solution of the agent and allowing diffusion to occur overnight. The plates were then covered with soft agar containing the indicator organism. These properties label the inhibitory agents as bacteriocins[5]. No resistant mutants of indicator strains were obtained and this precluded the use of cross-resistance tests[6] to classify these bacteriocins.

The use of solid media has increased the incidence of bacteriocinogeny in *L. fermenti* from the previously reported (ref. 2) 2·4 per cent to 15·5 per cent. This work was done in an attempt to subdivide strains of *L. fermenti*, but the identity of the spectra of activity of the bacteriocins discovered limits their use in this respect.

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PATHOLOGY

Complete Development of the Human Hookworm, *Necator americanus*, in Golden Hamsters, *Mesocricetus auratus*

STOLL[1] estimated that more than a fifth of the world's population carries hookworm infection involving *Ancylostoma duodenale* or *Necator americanus* or both. In view of their widespread distribution in both tropical and subtropical regions and severe pathogenicity, the importance of developing a laboratory-adapted human hookworm strain for experimental investigation in controlled conditions has long been recognized. The information in the literature suggests that dogs and cats may be experimentally infected with *N. americanus* in controlled conditions[2], but these large animals are unsuitable for various laboratory investigations. Partial development of this parasite in guinea-pigs and hamsters has been reported by Schwartz and Alicata[3] and Nagahana *et al.*[4]. According to them, after oral or percutaneous infections the infective larvae of *N. americanus* developed to fourth stage larvae in the small intestines but failed to grow to maturity. They had all been expelled by 3–4 weeks after infection.

The object of our investigation was to determine whether or not infective larvae of *N. americanus* could reach maturity in suckling hamsters less than 1 week old. This communication gives the results of a series of experiments and presents evidence that baby hamsters are suitable hosts for human hookworm infections.

The infective larvae of *N. americanus* were obtained from a 14–15 day old faecal culture prepared from a human patient according to the techniques described by Sen *et al.*[5] and with a Baermann apparatus. A dose of 0·02–0·05 ml. of suspension containing approximately 500–1,000 infective larvae was placed on the abdominal skin or in the mouth of 3–5 day old suckling hamsters under ether anaesthesia. Hamsters thus infected were kept under control for 30 min. After infection the animals were replaced in plastic cages provided with cotton pads with their respective mothers until they were weaned, and necropsies were made at various intervals. The parasites were collected, counted and fixed in hot A.F.A. for measurements.

The worms recovered from the small intestines 14–18 days after infection were in the fourth stage, while after 21–27 days they were immature adults which had completed the fourth ecdysis. After 35 days the average length of the males was found to be 6·56 mm, while that of females was 7·87 mm. Results of two experiments are summarized in Table 1.

The recovery of adult worms at necropsy demonstrates that baby hamsters are easily infected with larvae of *N. americanus*; the result is a satisfactory host–parasite

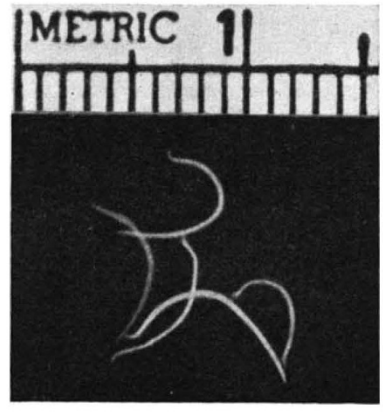

Fig. 1. Adult worms recovered from a 3-day-old hamster after 35 days of infection.