

A PRELIMINARY NOTE ON *BACILLUS BREVIS* STRAIN DEMONSTRATING MODERATE VIRULENCE AGAINST *BIOMPHALARIA* *PFEIFFERI* AND *BULINUS TRUNCATUS*, SNAIL HOSTS OF SCHISTOSOMES

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Abstract

A *Bacillus brevis* strain isolated from soil was found to produce 100 per cent mortality on 40 *Biomphalaria pfeifferi* and *Bulinus truncatus* after 6 h and 24 h exposure periods, respectively, at a spore concentration of $7 \times 10^7 \text{ ml}^{-1}$. The isolate also produced moderate activity on *Anopheles gambiae*, *Aedes aegypti*, *Culex decens* and *Cx. quinquefasciatus* larvae in that order of susceptibility. More potent strains of the species could be isolated and used to control both snail and mosquito vectors which tend to co-exist.

Introduction

Research into the biological control of disease vectors is gaining considerable momentum. This growing interest is due to the development of resistance in target organisms to chemicals used to control them. Other reasons are the relative safety of biological agents, their pollution-free nature and specificity which make them safe, for field application.

Much of the research on biological control has, however, focused on insect vectors and little attention has been given to snail vectors. This little work that has been done on the biological control of snail vectors has been on the use of competitor snails (Yousif, El-Eman & Roushdy, 1993) or fish predators (Graber & Euzeby, 1974.). Microbial pathogens seem to have received the least attention.

Résumé

OFORI, J.: Note préliminaire sur la souche de *Bacillus brevis* démontrant une virulence modérée contre *Biomphalaria pfeifferi* et *Bulinus truncatus*, hôtes escargots de schistosomes. Une souche de *Bacillus brevis* isolée du sol était découverte de produire 100% de mortalité sur 40 *Biomphalaria pfeifferi* et *Bulinus truncatus* respectivement après 6 heures et 24 heures de périodes d'exposition, à une concentration de spore de $7 \times 10^7 \text{ ml}^{-1}$. L'isolée produisait également une activité modérée sur les larves d'*Anopheles gambiae*, *Aedes aegypti*, *Culex decens* et *Cx. quinquefasciatus* dans cet ordre de susceptibilité. Plus de souches puissantes des espèces pourraient être isolé et utilisé pour le contrôle de vecteurs d'escargot et des moustiques qui ont la tendance de coexister.

The present study was undertaken to search for potential pathogens to control the snail intermediate hosts of schistosomes.

Experimental

Soil water and snail samples were taken from a wide range of snail breeding habitats as possible sources of bacterial pathogens.

To isolate pathogens from soils, samples were collected with sterile disposable spoons and placed in plastic cups. In the laboratory, one gram samples were weighed out and dispersed by shaking in 10 ml sterile distilled water and pasteurised at 80 °C for 12 min to eliminate non-spore formers. From each pasteurised sample 0.1 ml aliquots were plated on nutrient agar fortified with 0.1 per cent yeast extract and incubated at 37 °C. After 48 h

incubation the plates were examined for bacteria colonies. Selected colonies were subcultured for purity.

Loopfuls of the pure cultures were inoculated into flasks containing nutrient broth plus 0.1 per cent yeast extract. The flasks were incubated at 35 °C in an orbital shaker orbiting at 240 rpm for 48 h. The suspensions were serially diluted in sterile distilled water. Each dilution was tested against 10 *Bulinus truncatus* snails in glass jars containing 500 ml of the suspension. Each dilution was replicated four times. The snails were fed with a few pieces of sterile lettuce leaves to eliminate mortalities due to hunger. Jars containing only distilled water and snails served as controls. Viable and spore counts were carried out alongside the bioassays. Another bioassay was performed on *B. pfeifferi*.

B. truncatus and *B. pfeifferi* are responsible for the transmission of urinary and intestinal schistosomiasis, respectively, in Ghana.

To isolate pathogens from water, 10 ml portions of the water sample were aseptically pipetted into sterile test tubes, pasteurised and treated as before.

For the isolation of pathogens from snails those which showed signs of disease were selected and crushed in sterile mortars. The flesh was aseptically transferred to similar mortars containing 0.1 ml portions of distilled water. The suspensions were transferred into sterile test tubes containing 10 ml distilled water, pasteurised and treated as above.

Results and discussion

Three pathogens were isolated from the soil samples but only one demonstrated an appreciable level of activity against the above snails. This strain was designated IAB 395. It was observed within 5 min of exposure to the preparation that the snails had retracted into their shells. They remained in this state until total mortality was

achieved, i.e. until they failed to respond to mechanical probing.

The activity of the strain seemed to be stronger on *B. pfeifferi* causing all 10 snails in each jar to succumb within 6 h of exposure at 1.6×10^8 cells/ml (97.1×10^7 spores/ml). With regard to *B. truncatus* a minimum of 24 h exposure was necessary to produce 100 per cent mortality at the same concentration. At a 10^7 cell/ml concentration (10^6 spore/ml), 20 per cent of *B. pfeifferi* died within 72 h.

An interesting feature about the strain was that it also possessed moderate activity against *Anopheles gambiae*, *Aedes aegypti*, *Culex decens* and *Cx. Quiquesfasciatus* in that order of susceptibility, causing 48, 42, 22 and 19 per cent mortalities, respectively, at 107 cells/ml. One hundred larvae were tested per species.

Unlike pathogenic strains of *B. sphaericus* and *B. thuringiensis*, this bacillus strain did not possess any discernible crystals. The isolate was identified as a strain of *B. brevis* (H. de Barjac, Institute Pasteur, Paris, personal communication).

Schistosomiasis is a disease that afflicts thousands of Ghanaians and millions worldwide. Its control has been focused on the use of drugs which have invariably caused side effects. Additional effective approach to controlling the disease is to control the snail hosts. The purpose of this study is to generate interest in the search for more active strains of this agent to control both snail and mosquito vectors, which tend to occur in the same, habitat.

References

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