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Ethanol precipitates of Cephalobus sp. (Nematoda: Cephalobidae) inhibiting the growth of Staphylococcus pyogenes var. aureus

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Ethanol precipitates of *Cephalobus* sp. (*Nematoda: Cephalobidae*) inhibiting the growth of *Staphylococcus pyogenes* var. *aureus*[1]

By

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Abstract. Dried ethanol precipitates prepared from nematodes incubated for 7, 8, 15, 21 and 22 days produced significant inhibition of *Staphylococcus aureus*. The 7 and 15 day precipitates showed a linear increase in inhibition. Microscopic observation indicated that plates incubated for 8, 15, and 20 days had the greatest fraction of molting nematodes Molting was correlated with inhibition.

Previous studies (1, 2, 3, 4, 12) and others have cited the possible relationship of nematodes to animal cancer. Relatively few studies have been published on organic substances released by nematodes in plant or animal tissues which very likely contribute to symptoms of the disease (5, 6, 7). Other studies (9, 10, 11) have shown that the metabolism of free-living nematodes differs substantially from that of an expected animal species. The object of this study was to determine if the free-living nematode *Cephalobus* contains or releases a substance which induces changes in the growth pattern of the bacterium *Staphylococcus aureus*.

METHODS

The nematode was grown in petri plates on moistened dehydrated potatoes and collected by washing from the lids. After harvesting and concentrating, the nematodes were washed with 2 liters of sterile distilled water in an aspirated Buchner funnel with filter paper. The nematodes were suspended in 100 ml of pH 7.5 phosphate buffer and the volume adjusted to a optical density of .770 mμ with a colorimeter. The suspension was homongenized with 177-250 μ glass beads in a Braun MSK homogenizer at 4000 rpm for five minutes at 10°C.

Partial fractionation of the lipids was achieved by subjecting 10 ml of the homogenate to solvent extractions with acetone and ether. Proteins were precipitated by repeating the lipid extraction and then heating the protein-carbohydrate mixture for 10 minutes at 90°C. The denatured portion was removed by centrifugation at 4000 rpm for 20 minutes. Warmed (50°C) 95% ethanol was added to the carbohydrate supernatant to precipitate medium to low molecular weight polysaccharides. The precipitate was washed twice

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in 10 ml of 95% ethanol and reconstituted in 5 ml of sterile distilled water. The precipitates were dried for 18 hours at 52°C and were used immediately or stored at -15°C.

The growth pattern of *S. aureus* was followed by using a sterile 300 ml nephelo-culture flask with a 19- by 130 mm sidearm cuvette. Concentrations of the dried precipitates, 150 ml of sterile nutrient broth (Difco) and 0.1 ml of an 18 hour culture of *S. aureus* were placed in the flask and mixed. Control flasks with 0.1 ml of *S. aureus* were maintained for each extract tested. Culture turbidity was determined by a regulated Spectronic 20 colorimeter with a light transmittancy of 100 per cent at 600 mμ. Bacteria populations were determined with a Coulter Counter model B. Particles counted were 1 μ or less in diameter and a 30 μ orifice was used with a aperture current setting of 4. Background noise was kept below 100 for all counts.

Results and Discussion

In the method of extraction it was necessary to eliminate first the possibility that lipids or phospholipids caused the inhibition. This was done by extracting the lipid portion with acetone and ether. Secondly, enzyme activity was eliminated by heat denaturation. Warm ethanol caused the medium molecular weight polysaccharides, amino acids, nucleic acids and their derivatives, creatine, mucoproteins, glycoproteins, and other protoplasmic constiutuents to precipitate out. The ethanol precipitate may have contained other cellular compounds in addition to the inhibitor.

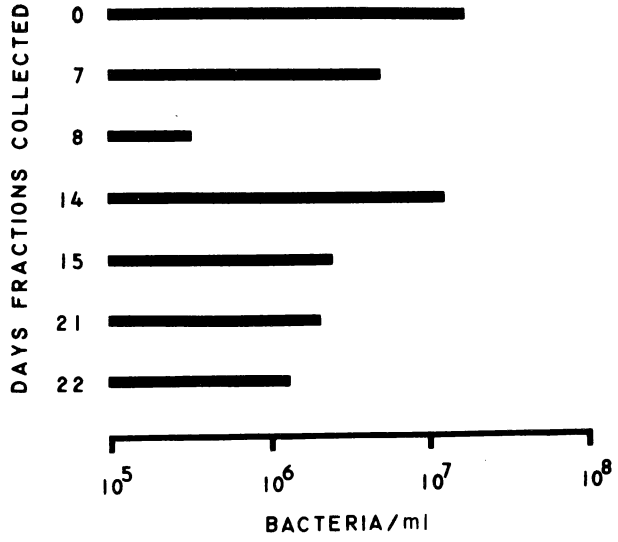

Fig. 1. Terminal growth readings of *Staphylococcus aureus* in the presence of ethanol precipitates.

Terminal growth readings of *S. aureus* versus .01 g of ethanol precipitates collected from plates incubated for 7, 8, 14, 15, 21, and 22 days are summarized in Fig. 1. All readings, except those from the 14 day plates, were significantly different from the control at the .01% level of confidence.

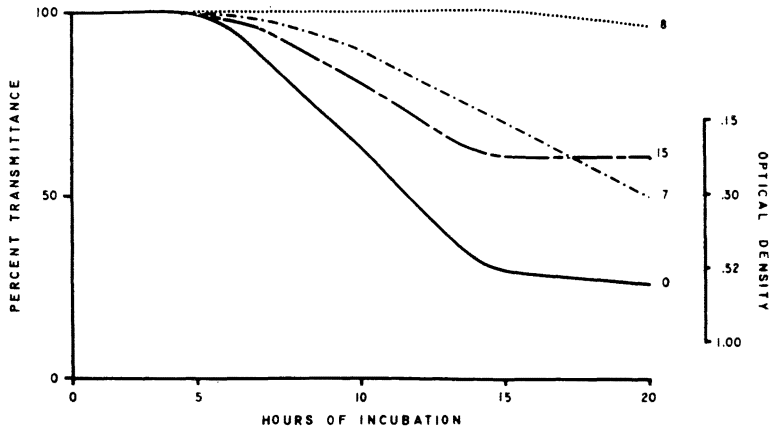

Fig. 2. Growth curves of *Staphylococcus aureus* treated with 0, 7, 8, and 15 day ethanol extracts.

Certain of the ethanol precipitates changed the growth pattern of *S. aureus,* Fig. 2. The patterns established with the 21 and 22 day old precipitates were comparable to the control whereas the 14 day precipitate is comparable to the 7 day precipitate. *S. aureus* containing the 7 day ethanol precipitate shows a smiliar latent and lag phase to that of the control. The next phase is more representative of a linear phase. The 8 day precipitate inhibits cell fission until the 16th hour when an extended lag phase was observed.

In the presence of the 15 day precipitate *S. aureus* exhibited a shorter linear phase of growth than the 7 day precipitate. Fission probably took place until *S. aureus* became susceptible to the inhibitor causing the organism to go into the negative growth acceleration phase.

Concentration correlations were established with 7 and 15 day nematode ethanol extracts (Fig. 3). Both curves show a linear relationship between concentration and inhibition and are very similar in percent inhibition over the range of concentrations. Two possible explanations for the greater inhibition caused by the 15 day precipitate at the higher concentration are that the precipitate may actually contain a more active form of the substance causing the inhibition, or if two or more substances caused the inhibition, they may be in better proportion.

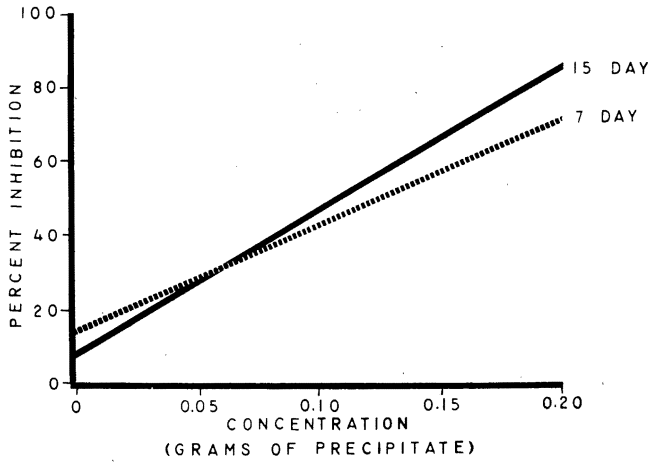

Fig. 3. Percent inhibition of *Staphylococcus aureus* caused by various concentrations of 7 and 15 day nematode ethanol extracts.

Microscopic observations were made on nematode plates from the 5th through 24th days of growth of the nematode. The results show definite periods when the fraction of nematodes molting is greater when compared to the total number of nematodes counted (Fig. 4). The increased molting on the 8, 15 and 22 days shows a

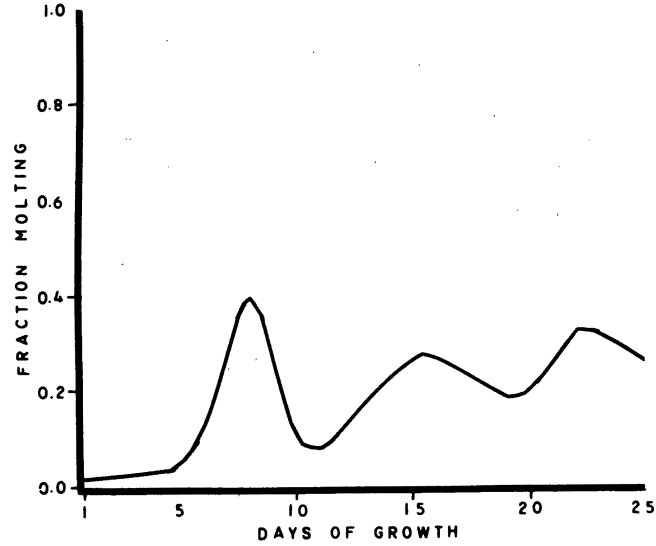

Fig. 4. Fraction of nematodes molting of the total counted from the 5th to 24th days of growth.

direct correlation to the greatest inhibition of *S. aureus* by the ethanol precipitate.

Fluids taken from exsheathing larvae bring about exsheathment of unstimulated larvae which then produce a sequence resembling a chain reaction (13). Most larvae require different types of stimuli for exsheathment. The stimulants may range from moisture content in the medium to the concentration of carbon dioxide respired. The whole process of exsheathment takes about three hours but the action of the stimulus is completed after 15 minutes (8). The entire mechanism which initiates exsheathment and causes the accumulation and subsequent release of exsheating fluid originates between the base of the pharynx and the region of the nerve ring of the excretory pore. In free-living nematodes it is possible that neurosecretory cells release hormones which activate the tissue and bring about changes in structure and activity and stimulate the production of molting fluid. Molting was detected by a decrease in physical activity which probably avoids bacterial contamination and helps in completing the formation of the new cuticle.

Summary

An extraction procedure was developed to obtain a precipitate from *Cephalobus* which at various days of incubation caused a change in the growth pattern of *Staphylococcus aureus*. Significant changes were observed for 7, 8 and 15 day precipitates. The growth in the presence of the 7- and 15-day's precipitates appeared similar when the percent inhibition was correlated to concentrations of precipitates. A linear relationship was observed when concentration of precipitate was compared with percent inhibition. Molting increases were noted at 8, 15 and 22 days of nematode growth. The amount of molting showed a direct correlation with the changes in the growth pattern of *S. aureus*.

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