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EFFECT OF SALINITY AND TEMPERATURE ON *COCCIDIOIDES IMMITIS* AND THREE ANTAGONISTIC SOIL SAPROPHYTES

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ABSTRACT

EGEBERG, ROGER O. (University of Southern California, Los Angeles), ANN F. ELCONIN, AND MARGARET C. EGEBERG. Effect of salinity and temperature on *Coccidioides immitis* and three antagonistic soil saprophytes. J. Bacteriol. **88**: 473-476. 1964.—Experiments exploring the factors which determine the ability of *Coccidioides immitis* to survive and reproduce in the soil *in situ* are described and discussed. Previous work indicated that soil temperatures and salinity influence the growth and distribution of *C. immitis*. It was thought that these factors might work through enhancing the growth of *C. immitis* or inhibiting the growth of its possible antagonists, or both. In searching for antagonists, samples of surface soil from an endemic area were screened by standard culture techniques. Three significant antagonists were isolated and were identified as two strains of *Bacillus subtilis*, and *Penicillium janthinellum*. Subcultures of each of these isolates were grown on Saboraud's media modified by sodium chloride and calcium chloride in parallel series. The salts used were the predominant salts, and the range of temperature was comparable to that found in the soil. The organisms were grown at 18 to 27 C and at 40 C. The latter temperature approximated the temperature of the top centimeter of soil for 4 to 6 hr a day in the spring when *C. immitis* is recovered. *P. janthinellum* was unable to survive 40 C. The growth of the other two, while unaffected by temperature change alone, was inhibited by the salts. Conversely, *C. immitis* was stimulated by the salts when grown at the higher temperature. These findings support the hypothesis that markedly increased salinity and seasonal high temperature of the surface soil inhibit or kill these antogonists while enhancing the growth of *C. immitis*.

We have recently reported (Elconin, Egeberg, and Egeberg, 1964) that the finding of *Coccidioides immitis* in abundance in the surface soil of an endemic area is always associated with a marked elevation of the soluble salts in the soil. It was suggested that this high level of soil salinity encouraged the growth of *C. immitis* in the soil and that this extreme environmental factor might be an important one in bringing about the spotty distribution pattern of *C. immitis* within an endemic area. We (Egeberg and Ely, 1956) earlier raised the question: could the high temperature of surface soil be an ally of *C. immitis* by giving it, for a brief period of time, a growth medium not thoroughly contaminated with its antagonists and competitors? It was postulated that both of these factors might work through enhancing the growth of *C. immitis* or inhibiting the growth of its possible antagonists and competitors, or both (Elconin et al., 1964).

The purpose of this paper is to describe the method by which several soil saprophytes antagonistic to *C. immitis* were found, and the effect of salts and temperature on the growth of *C. immitis* and the more significant of these antagonists.

In the search for antagonists, fresh samples of surface soil from an endemic area were screened by standard cultural techniques. All isolates recovered by this method were tested for antagonistic effect by placing a pinpoint inoculum of each subculture adjacent to a 24-hr-old streak of *C. immitis* on Saboraud's media. A zone of inhibition of *C. immitis* indicated antagonism.

To compare the effects of increasing salt concentrations and of higher ambient temperatures upon the growth of *C. immitis* and the antagonists thus found, each of these organisms was cultured on Saboraud's medium modified by soluble salts in various concentration at room temperature (18 to 27 C) and in an incubator at 40 C.

MATERIALS AND METHODS

Isolation of antagonists. Soil samples (288) were collected from an endemic area in the period just prior to and during which *C. immitis* was recovered from the soil. They were taken from the surface in sterile screw-top jars (4 oz); the jars themselves were used as scoops. Within 4 weeks of

collection, each sample was treated in the following manner in the laboratory. A 10-g portion of the soil sample was added to 90 ml of sterile distilled water, making a 1:10 suspension. A 10-ml portion of this suspension was further diluted 1:10 to make a suspension of 1:100. A 0.5-ml portion of each dilution was plated in duplicate on Saboraud's medium. Each of the 288 soil samples was processed in the above manner. After 1 week of growth at room temperature (18 to 27 C), subcultures were made of each grossly different type of colony. After 2 weeks of growth, a pinpoint inoculum from each subculture was planted at a distance of 1 cm from a 24-hr-old streak of *C. immitis* on Saboraud's media, and was allowed to grow at room temperature. If a zone of inhibition appeared, the isolate was classified as an antagonist to *C. immitis*, and the relative strength of each antagonist was grossly determined by the width of the zone it produced.

Cultures of C. immitis and its antagonists at various temperatures and concentrations of salts. Saboraud's medium was modified by the addition of sodium chloride and calcium chloride in amounts calculated to give the media 2, 4, 6, and 8% salt concentrations by weight. Aqueous sus-

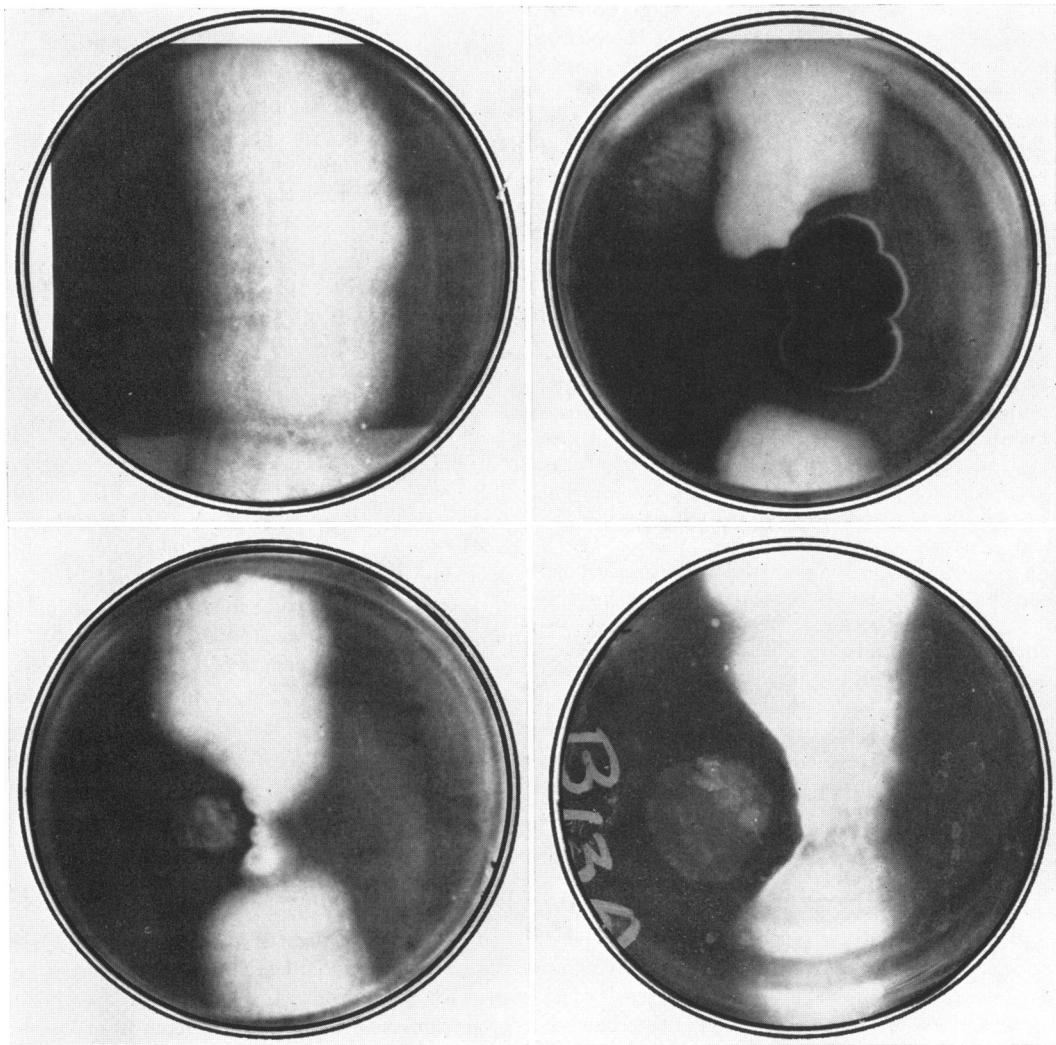

FIG. 1. *Coccidioides immitis and antagonists. Upper left, control; upper right, Penicillium janthinelum; lower left, isolate I of Bacillus subtilis; lower right, isolate II of Bacillus subtilis.*

pensions of *C. immitis* and of three major inhibitors (*Penicillium janthinellum* and two isolates of *Bacillus subtilis*, hereafter referred to as Pj[1], Bs[1], and Bs[2]) were streaked in duplicate series on the above-described culture media and on Saboraud's media without added salt as controls. One series was allowed to grow at room temperature. During the period of the experiment, the room temperature varied from 18 to 27 C. The other series was placed in an incubator at 40 C. All were observed and compared after 2 weeks of incubation at these temperatures.

RESULTS

Of the microorganisms isolated from the soil, three proved to be strong antagonists of *C. immitis*, showing clear, sharply defined zones of inhibition (0.5 to 0.7 cm) in the growth of *C. immitis* on Saboraud's media. These antagonists were identified as *P. janthinellum* and two strains of *B. subtilis* (Fig. 1).

The effects of the calcium chloride and sodium chloride in varying concentrations on the growth of *C. immitis* and the three antagonists grown at the ambient temperature range of 18 to 27 C is shown in Fig. 2. The growth area of all organisms at this range in plain Saboraud's media was designated as the control and, for the purpose of comparison, was given the value of $++++$.

At this ambient temperature, the growth of *C. immitis* was increasingly suppressed by the salts in higher concentrations, as was the growth of the two strains of *B. subtilis*. It is to be noted that *C. immitis* withstands the higher concentrations of the calcium chloride better than both strains of *B. subtilis*. *P. janthinellum* at this temperature range grew very well in both salts at all concentrations.

Figure 3 shows the growth of the same cultures at the same concentrations of both salts incubated at 40 C. Again, the growth area on plain Saboraud's media at 18 to 27 C was used as the control, with a value of $++++$. At this higher temperature, *C. immitis* was retarded by the heat in the absence of salt. Its area of growth, however, at 2% calcium chloride and 2 and 4% sodium chloride was greater than that of the control or when grown at this temperature without either salt. At this temperature (40 C) *P. janthinellum* failed to show any growth. Bs[1] was severely suppressed by both salts. Bs[2] was moderately suppressed by calcium chloride, but was not as adversely influenced by sodium chloride.

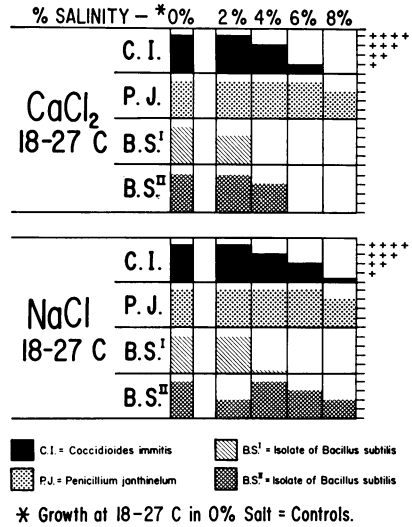

FIG. 2. *Coccidioides immitis and three antagonists grown at varying salt concentrations at 18 to 27 C.*

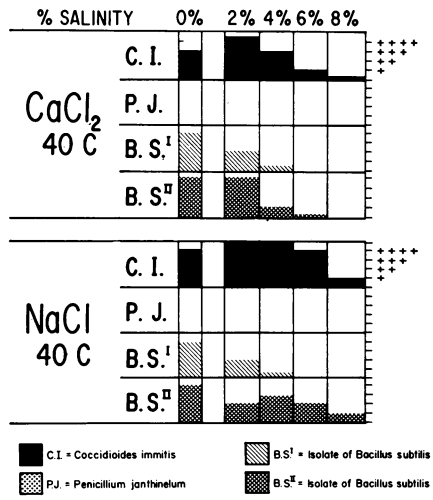

FIG. 3. *Coccidioides immitis and three antagonists grown at varying salt concentrations at 40 C.*

DISCUSSION

This laboratory study was planned to gain further information about the factors influencing the growth of *C. immitis* in the soil of an endemic area. During an 8-year period we have studied the soil of a 4- to 6-acre tract of land in an area where *C. immitis* is endemic. As a result of these studies, three facts became apparent concerning the growth pattern of the fungus within that area. (i) The fungus was recovered in abundance from the

soil only in the spring. (ii) Only surface soil yielded *C. immitis* in profusion. (iii) *C. immitis* appeared in the surface soil in the spring only during those years when the salinity of that soil was markedly elevated.

Three major antagonists prevalent in the soil were recovered. These antagonists are inhibited or killed by high temperature comparable to that of the surface soil of the endemic area in the spring or by the high concentrations of soluble salts existing in the soil at that time, or by both factors. Unlike the antagonists, *C. immitis* shows remarkably good growth at this higher temperature when these salts are present.

In this study the predominant salts found in the soil were used, and at concentrations found at the surface. The incubating temperature of 40 C corresponded to the temperature of the soil during the time of high recovery of *C. immitis*.

These findings further support the hypothesis that increased salinity and seasonal high temperature of the soil encourage the growth of *C. immitis* through inhibiting or killing its antagonists while stimulating its growth. This hypothesis suggests a changing milieu of living organisms responding to temperature changes and to varying concentrations of the chemical constituents of the soil. The latter, in turn, is governed by the washing and leaching of heavy rains and concentration by evaporation.

We would conclude, therefore, that *C. immitis*, a natural inhabitant of the soil, lives in a state of balance with many other organisms, and that the weather controls the organisms, among them *C. immitis* and its antagonists. A similar mechanism may explain changing prevalence and spotty distribution of other soil-carried pathogens.

ACKNOWLEDGMENTS

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