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Preliminary studies of Bacillus cereus distribution near a gold vein and a disseminated gold deposit

By

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CONTENTS

ILLUSTRATION

TABLE

ABSTRACT

The distribution of Bacillus cereus, a penicillin-resistant microorganism, was studied in O1 and A1 soil horizons overlying a vein and a disseminated gold deposit and adjacent unmineralized areas. Based on microbial culture techniques, the number of viable B. cereus spores in soil samples adjacent to mineralization (background) was much lower than in soils directly over mineralization. The number of B. cereus spores in soil samples collected from an aspen and pine forest, overlying a vein-type gold deposit, was much higher than in soil samples collected from a semi-arid desert environment overlying a disseminated gold deposit. However, the relative difference in counts between background and mineralized-area samples was similar both in desert and forest environments. The apparent association of this organism with subsurface gold mineralization in glaciated or pediment-covered areas suggests that geomicrobial surveys may be useful for prospecting when conventional geochemical methods are inadequate. The response of B. cereus to gold mineralization at depth in two diverse environments further suggests its use as an exploration technique in a variety of climates. However, further studies of the distribution of B. cereus in natural environments are necessary.

INTRODUCTION

Microorganisms have been shown to develop both genetic resistance and population demographic responses to environments with soils and waters enriched in heavy metals (Letunova and Koval'sky, 1978; Marques and others, 1979; Timoney and others, 1978; Austin and others, 1977; Nelson and Colwell, 1975; Kendrick, 1962; Updegraff, in press; Olson and Thornton, 1981; and Watterson and others, 1983; 1984a, b). Data compiled by Watterson and others (1983a, b) indicates that B. cereus, a penicillin-resistant soil microorganism, is found in above-background abundance in soils and stream sediments associated with mineral deposits of Au, Ag, Mo, Bi, As, and Cu. This organism, therefore, may be useful in conducting reconnaissance geochemical surveys for locating mineral deposits.

In order to measure the association between B. cereus content in surface soils and mineralization at depth, two areas were chosen that differ in type of gold deposit and environment. Near Empire, Colorado, veins containing gold, pyrite, chalcopyrite, and quartz, within Precambrian Boulder Creek granite, are overlain by 15-20 feet of glacial overburden and covered with aspen and pine forest vegetation. Near Elko, Nevada, an undeveloped disseminated epithermal gold deposit within silty limestone, is overlain by an unknown amount of transported overburden and covered with cold-desert, sagebrush vegetation.

MATERIALS AND METHODS

Straight-line traverses, with samples collected every 20 feet, were used at both the vein and disseminated gold deposit study sites. Near Empire, Colorado, the traverse was perpendicular to the gold-vein orientation and crossed two known veins. Near Elko, five samples were collected from areas known to be underlain by mineralization (disseminated) and three samples were collected from areas known to overlie unmineralized rock of the same type. At each sample site, the upper two inches of soil were removed and discarded and the two-inch soil zone immediately below was collected in new plastic bags. In the laboratory, all samples were air dried and sieved to minus 30 mesh.

For each soil sample, B. cereus was isolated using a minimal agar containing: 1.0g K_2HPO_4; 0.2g $MgSO_4$ $7H_2O$; 0.01g $FeSO_4$ $7H_2O$; 0.01g $CaCl_2$; 1.0g glucose; 1.0g NH_4Cl; and 15g agar per liter deionized water. Sodium citrate (0.5%) and fresh egg yolk (2.5%) (both volume/final volume) were added to liquid agar after cooling to 47°C. One gram of sample was aseptically transferred into sterile screw-cap culture tubes containing 9 ml sterile deionized water, shaken for 10 minutes in a mechanical shaker, and centrifuged for 3 minutes at 50 x g. Culture tubes were heat treated for 60 seconds at 90°C and then two 1/10 serial dilutions were made. This was done so that dilutions containing between 30 and 300 colonies could be selected for counting. Pour plates were made with 1.0 ml aliquots, and samples were incubated at 30°C for 14 hours. Colonies surrounded by typical egg-yolk positive opaque zones were enumerated as B. cereus. For a more detailed account of this procedure, see Watterson (1984b).

RESULTS AND DISCUSSION

Preliminary B. cereus culture counts of ten A1 horizon and ten O1 horizon soil samples from the gold-vein study show B. cereus counts to be an order of magnitude higher over mapped gold veins than in adjacent unmineralized areas. The counts were also higher by several thousands of colonies per gram in the O1 horizon soils compared to the A1 horizon (fig. 1). However, the B. cereus halo in A1 horizons is more tightly centered upon the veins than the halo for O1 horizons. Chaffee (1972) and Curtin and others (1971) showed that geochemical anomalies associated with gold mineralization in the Empire area occur chiefly in the mull material (O1 horizon). The geochemistry of surface soils (A1), in contrast to the mull material, poorly reflects the known distribution of gold veins in bedrock beneath the cover of glacial drift (Curtin and others, 1971). Whereas these surface soils have little value as a sampling medium for conventional geochemical analysis, they evidently have great diagnostic value for geomicrobial exploration using the B. cereus assay.

The disseminated gold deposit in Nevada shows contrast in B. cereus counts directly over, and adjacent to, gold mineralization similar to that found in the gold-vein study (table 1). However, higher counts were found in soil samples collected in the forested area than in soils collected in the desert environment (table 1). The difference between background and mineralized area B. cereus counts is an order of magnitude in both areas, suggesting that the method is applicable for gold prospecting in both types of environments. The drop in B. cereus counts observed in samples 1-4 (Nevada) may possibly be related to the direction the ore deposit is dipping. However, our information on the depth and attitude of the ore body is limited. Studies concerned with the relationship between depth of the deposit and B. cereus counts need to be carried out.

Although B. cereus counts appear to correspond to areas of gold mineralization in two different environments, the data presented here are preliminary. Studies concerned with the mechanism of population increase, the seasonal variation of B. cereus over gold deposits, the organisms' response to mineralization in different environments, and whether this organism indicates

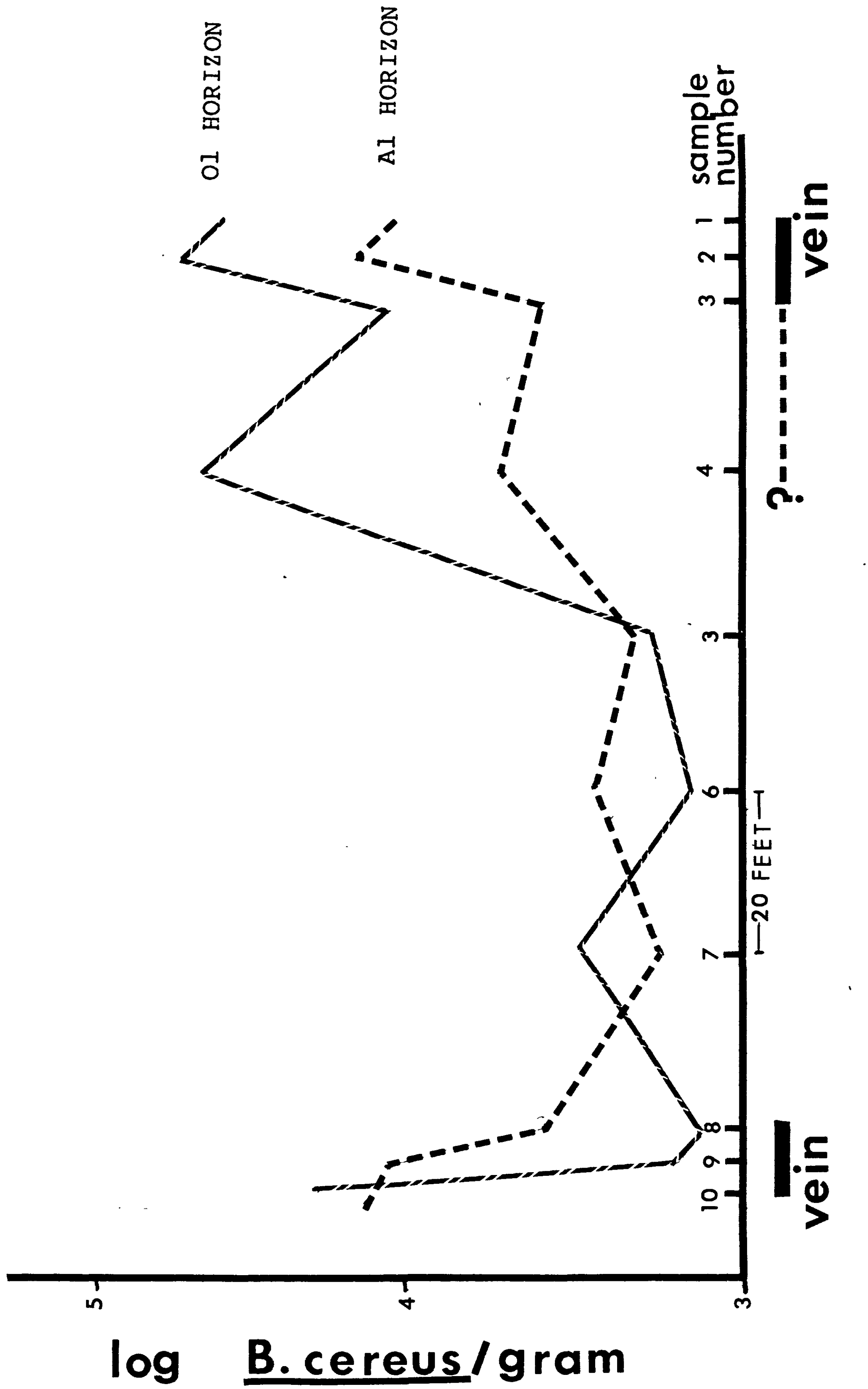

FIGURE 1.--Relationship between B. cereus counts in forest litter (O1 horizon) and in surface soils (A1 horizon) over vein-type gold mineralization.

the presence of other types of ore deposits need to be undertaken in order to establish the limits and practicality of using this apparent "indicator" organism for prospecting.

CONCLUSIONS

Comparison of a published geochemical study (Curtin and others, 1971) and preliminary B. cereus assays of mull and soil samples near Empire, Colorado, indicates that the latter method may be a more effective way of locating gold veins concealed beneath glacial till. Geochemical methods are rendered ineffective presumably because initial overlying soils have been removed or buried by glaciation. B. cereus soil assays may therefore be especially useful for prospecting in glacial or pediment-covered terrains where the effectiveness of conventional geochemical methods is limited.

The population increase of B. cereus in soils over a gold vein deposit and a disseminated gold deposit suggests that further work on the distribution of this organism in the vicinity of other known gold deposits should be conducted. Anomalous values of B. cereus associated with subsurface deposits of gold and the relative ease of soil sampling and enumeration of this organism suggest that microbial prospecting may become a competitive prospecting technique in the future.

TABLE 1.--**Bacillus cereus counts per gram for vein-type and disseminated gold deposits**

Colorado Forested, Vein-type			Nevada Desert, Disseminated	
		Soil Horizon		
	A1	01		A1
		Mineralized		
Sample no.			Sample no.	
1	11,370	41,000	1	1,100
2	15,450	54,300	2	1,000
3	4,300	12,100	3	320
4	5,880	45,700	4	380
Unmineralized			5	125
5	2,230	2,000	Unmineralized	
6	2,980	1,470	6	0
7	1,830	3,290	7	10
8	4,060	1,430	8	20
Mineralized				
9	12,490	1,700		
10	15,000	21,000		

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