

# BIOCERIN: AN ANTIBIOTIC PRODUCED BY *BACILLUS CEREUS*<sup>1</sup>

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A strain of *Bacillus cereus* isolated from milk was reported to exhibit marked antagonism to both gram-positive and gram-negative organisms (Goodlow, Johnson, and Shafer, 1947). This antagonism was most marked against the genera *Salmonella* and *Shigella*. The antibiotic substances reported in this study were produced by an organism isolated from the soil and identified by following a key to the identification of aerobic sporeforming organisms (Smith, Gordon, and Clark, 1946) as *Bacillus cereus*.<sup>2</sup> It is suggested that the substance be called "biocerin." When *B. cereus* was streaked on the surface of proteose peptone agar plates previously seeded with various gram-positive and gram-negative organisms, zones of inhibition ranging from 5 to 24 mm in diameter were observed after an incubation period of 24 hours at 37 C.

Biocerin was produced by growing *B. cereus* in a medium of the composition given in table 1 for 48 hours at 37 C. The medium containing the organism was then extracted with ether. A yellow color was observed in the ether after extraction. Spontaneous evaporation of the ether extract resulted in a brownish-yellow, oily residue. This material was insoluble in water but soluble in 95 per cent ethanol, acetone, and chloroform.

The inhibition of various bacterial species was tested by means of proteose peptone agar plates containing varying amounts of crude biocerin per ml of agar. The plates were streaked with a 2-mm loopful of a saline suspension containing approximately 300 million cells of the organism per ml and incubated for 24 hours at 37 C. Inhibition was recorded as complete suppression of all visible growth of the bacteria. All bacterial species tested were inhibited by a concentration of 1.0 mg of crude biocerin per ml of agar. *Salmonella paratyphi* A, *Salmonella paratyphi* B, and *Sarcina lutea* were the only species not inhibited by a concentration of 0.5 mg of crude biocerin per ml of agar. The lower concentrations of 0.1 mg and 0.05 mg of biocerin per ml of agar did not inhibit any of the species tested except *Staphylococcus aureus* and *Staphylococcus albus*. None of the species tested were inhibited by a concentration of 0.025 mg per ml of agar. These results are presented in table 2.

The effect of rabbit serum on the activity of biocerin was determined by adding various concentrations of serum to proteose peptone agar containing 1.0 mg and 0.5 mg of crude biocerin per ml of agar. A concentration of 10 per cent serum did

<sup>1</sup> The organism was originally isolated by Miss Laura Brilliatine, Department of Bacteriology, The University of Southern California, Los Angeles, California.

<sup>2</sup> Identification confirmed by Dr. Ruth Gordon, American Type Culture Collection, Washington, D. C.

TABLE 1  
*Medium for the production of biocerin*

Glucose.....	2.0 g
NH <sub>4</sub> H <sub>2</sub> PO <sub>4</sub> .....	1.0 g
NaCl.....	2.0 g
MgSO <sub>4</sub> .....	1.0 g
K <sub>2</sub> HPO <sub>4</sub> .....	1.0 g
Methionine.....	0.005 g
Agar.....	5.0 g
Distilled H <sub>2</sub> O.....	1,000 ml
Final pH = 7.0	

TABLE 2  
*Bacterial growth in the presence of various concentrations of crude biocerin*

ORGANISM	MG OF CRUDE BIOCERIN PER ML OF AGAR				
	1.0	0.5	0.1	0.05	0.025
<i>Staphylococcus aureus</i> .....	-	-	-	-	+
<i>Salmonella typhosa</i> .....	-	-	+	+	+
<i>Salmonella paratyphi</i> A.....	-	+	+	+	+
<i>Salmonella paratyphi</i> B.....	-	+	+	+	+
<i>Corynebacterium diphtheriae</i> .....	-	-	+	+	+
<i>Bacillus anthracis</i> .....	-	-	+	+	+
<i>Bacillus subtilis</i> .....	-	-	+	+	+
<i>Sarcina lutea</i> .....	-	+	+	+	+
<i>Staphylococcus albus</i> .....	-	-	-	-	+
<i>Escherichia coli</i> .....	-	-	+	+	+
<i>Brucella suis</i> .....	-	-	+	+	+
<i>Aerobacter aerogenes</i> .....	-	-	+	+	+
<i>Neisseria catarrhalis</i> .....	-	-	+	+	+

TABLE 3  
*Effect of rabbit serum on the growth of bacteria in the presence of various concentrations of crude biocerin per ml of agar*

ORGANISM	10 PER CENT SERUM		20 PER CENT SERUM	
	1.0 mg	0.5 mg	1.0 mg	0.5 mg
<i>Staphylococcus aureus</i> .....	-	-	-	+
<i>Sarcina lutea</i> .....	-	-	-	+
<i>Escherichia coli</i> .....	-	-	-	+
<i>Salmonella typhosa</i> .....	-	-	-	+
<i>Brucella suis</i> .....	-	-	-	+
<i>Bacillus subtilis</i> .....	-	-	-	+
<i>Bacillus anthracis</i> .....	-	-	-	+
<i>Corynebacterium diphtheriae</i> .....	-	-	-	+

not decrease the inhibitory effect of 1.0 mg and 0.5 mg of biocerin against any bacteria tested during a 24-hour period of incubation at 37 C. A concentration of 20 per cent serum did decrease the inhibitory effect of 0.5 mg of biocerin per

ml of agar during a 24-hour period of incubation at 37 C. A decrease in the inhibitory effect of both concentrations of biocerin in the presence of serum was observed after 48 hours in all instances, except when 1 mg of biocerin per ml of agar was tested in the presence of 10 per cent serum. The results are presented in table 3.

The toxicity of crude biocerin was determined by suspending the material in sterile mineral oil and injecting it intraperitoneally into white mice weighing approximately 18 to 20 grams. A single injection of 20 mg of the crude substance produced no fatalities in a group of 10 mice at the end of a 5-day test period. Toxic symptoms were not apparent during the course of the experiment.

These experiments have, by necessity, been preliminary in nature. The authors feel that further experimentation is desirable in view of the antibacterial activity obtained from varying amounts of crude biocerin against various gram-negative and gram-positive bacteria.

#### REFERENCES

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