INFECTION AND IMMUNITY, Apr. 1978, p. 310-311
0019-9567/78/0020-0310$02.00/0

Vol. 20, No. 1
Printed in U.S.A.

Siderophore Production by *Vibrio cholerae*

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Received for publication 23 November 1977

Vibrio cholerae produces a phenolate-type siderophore that stimulates growth of the organism in low-iron medium. This compound is similar, but not identical, to enterochelin, the siderophore produced by *Salmonella* and *Escherichia coli*.

Iron is essential for microbial growth, but the acquisition of this element is complicated by the extreme insolubility of the ferric ion (12, 16). Microorganisms produce high-affinity iron-binding compounds which bind and solubilize iron and transport it across the cell membrane (10). These iron-binding compounds, collectively referred to as siderophores (10), have been isolated from a number of bacterial species including *Escherichia coli* (2), *Salmonella typhimurium* (13, 19), *Bacillus megaterium* (6, 11), and *Mycobacteria* (15).

This report describes the detection and partial characterization of a similar compound in culture supernatants of *Vibrio cholerae*.

V. cholerae 569B Inaba was grown in a simple, chemically defined, synthetic medium (herein called Synbase, as opposed to Syncase medium, which has added Casamino Acids [4, 5]) without added iron or manganese. The medium was deferrated by magnesium carbonate precipitation (8) followed by conalbumin treatment. A solution of iron-free conalbumin (Sigma, type 1, 10 mg/ml, in 5 mM sodium bicarbonate) was added to a final concentration of 1 mg of conalbumin per ml and stirred for 30 min, and the iron-conalbumin complexes were removed by ultrafiltration through a PM-10 membrane (Amicon Corp.). The final concentration of iron was less than 0.5 μM as determined by the method of Stookey (17) using ferrozine (U.S. Biochemicals).

The medium was inoculated with *V. cholerae* (10^7 colony-forming units per ml) and, after overnight growth, diluted into fresh medium (10^6 colony-forming units per ml) and incubated for an additional 24 h at 30°C on a rotary water bath shaker. Viable counts (colony-forming units per milliliter) were determined by dilution plating on meat extract agar. The cells were removed by centrifugation, and the supernatant was extracted with ethyl acetate (Mallinckrodt) as described by Rogers (14). This extract was assayed for its ability to stimulate growth of *V. cholerae* (Fig. 1). The extract was equally as effective as iron in enhancing growth of *V. cholerae* in the deferrated medium. No activity was detected in extracts of cultures grown in the same medium with added $FeCl_3$ (10 μg of iron/ml).

The ethyl acetate extract appeared to contain a catechol- or phenolic acid-type siderophore as determined by the Arnow test (1). Since two other enteric pathogens, *E. coli* and *S. typhimurium*, produce a catechol-type iron chelator, enterochelin (enterobactin), a sample of the *V. cholerae* extract was analyzed by paper chromatography (14) in comparison with a sample of enterochelin provided by J. B. Neilands. Chromatography was carried out using Whatman no. 1 paper and 5% ammonium formate plus 0.5% formic acid as the solvent. Both the *V. cholerae* extract and the enterochelin separated into two fractions, which were detectable by ultraviolet light absorption or by spraying the paper with ferric chloride. However, the catechols produced by *V. cholerae* did not co-migrate with enterochelin, and a mixture of the two samples yielded four distinct spots after chromatography. R_f values for the *V. cholerae* extract were 0.09 and 0.6, as compared to 0.16 and 0.52 for the enterochelin sample. Some growth-stimulating activity remained in the culture filtrate after extraction with ethyl acetate, but the nature of this activity has not been determined.

It is concluded that *V. cholerae* produces a phenolate-type siderophore when grown in low-iron medium. This factor is distinct from enterochelin, the siderophore composed of 2,3-dihydroxybenzoylserine that is produced by *Salmonella* and *E. coli*, perhaps differing in the amino acid component, as do the siderophores produced by *Klebsiella* (9), *Azotobacter vinlandii* (3), *Micrococcus denitrificans* (18), and *Bacillus subtilis* (7). Interestingly, the *V. cholerae* factor stimulates growth of a variety of gram-negative bacteria in low-iron medium, including *Neisseria gonorrhoeae* and *N. meningitidis*,

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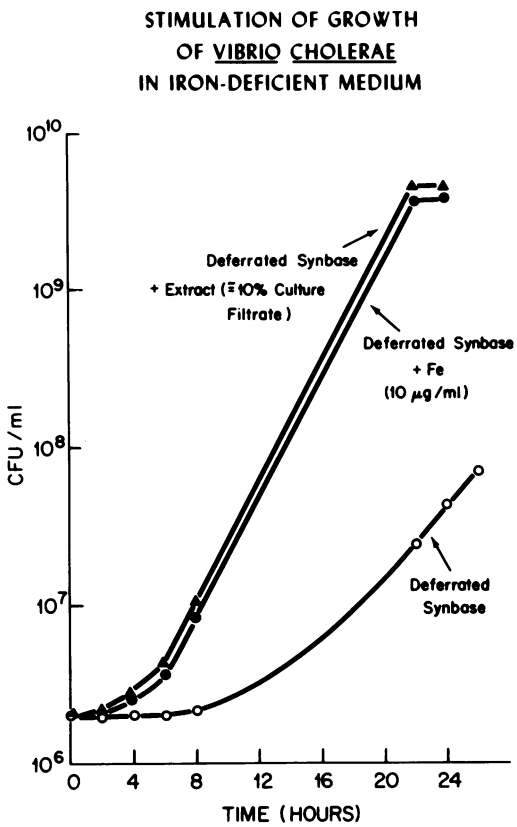

FIG. 1. *Stimulation of growth of V. cholerae in iron-deficient medium by iron (10 μg/ml) or an ethyl acetate extract of a culture grown in low-iron medium.*

whereas enterochelin or culture filtrates of *E. coli, Salmonella,* and *Shigella* were unable to stimulate growth of gonococci. Although gonococci were also able to respond to homologous and to meningococcal culture filtrates, gonococcal filtrates did not stimulate iron-limited growth of any of the other genera tested (S. M. Payne and R. A. Finkelstein, J. Clin. Invest., in press).

This investigation was supported by Public Health Service grants AI-08877 (under the U.S.-Japan Cooperative Medical Science Program) and AI-11278 from the National Institute of Allergy and Infectious Diseases. S.M.P. was a National Science Foundation Predoctoral Fellow.

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