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Selenium-Enriched Medium for *Legionella pneumophila*

DAVID L. SMALLEY, PERCY A. JAQUESS, AND JOSEPH S. LAYNE*
Department of Biology, Memphis State University, Memphis, Tennessee 38152

We found that sodium selenate added to F-G cysteine iron agar enhanced the growth of *Legionella pneumophila* (Philadelphia 1 strain), with visible colonies at 18 h of incubation. The optimum enhancement of growth was found to occur at a concentration of 5 to 10 μg of selenium per ml as sodium selenate; enhanced growth, up to 22 times the number of colonies on F-G cysteine iron agar without selenate, was observed at 36 h.

Legionella pneumophila has been characterized as a fastidious organism that is unable to grow on many standard media. Several media have been recommended for primary isolation of *L. pneumophila* including charcoal-yeast extract agar, F-G agar, and F-G cysteine iron agar (3). Charcoal-yeast extract is an improved medium compared to F-G and F-G cysteine iron agars, yielding visible colonies usually after 2 days of incubation (2). Recently it has been reported that addition of sodium selenate to media such as the above enhances growth of *L. pneumophila* (Jaquess, Smalley, and Layne, J. Am. Med. Assoc., in press). This investigation was undertaken to determine the optimum concentration of sodium selenate required to stimulate growth of *L. pneumophila* and to determine whether other selenium compounds, such as sodium selenite or selenium dioxide, were also capable of enhancing growth of this organism. The investigation was also extended to determine whether the volatile selenium-containing product hydrogen selenide could be generated by *L. pneumophila*.

MATERIALS AND METHODS

Media preparation. The control medium in the experiment was F-G cysteine iron agar made as previously reported (3). Experimental media included F-G cysteine iron agar plus 1, 2.5, 5, 10, 25, 50, and 100 μg of selenium per ml in the form of sodium selenate; F-G cysteine iron agar containing 1, 10, 25, 50, and 100 μg of selenium per ml as sodium selenite; and F-G cysteine iron agar with 1, 10, 25, 50, and 100 μg of selenium per ml as selenium dioxide. All media had 0.4 g of L-cysteine hydrochloride and 0.25 g of soluble ferric pyrophosphate per liter and were adjusted to a pH of 6.9. Another medium tested had 50 μg of selenium per ml in the form of sodium selenate and 0.4 g of L-cysteine hydrochloride per liter in Mueller-Hinton medium (Difco Laboratories, Detroit, Mich.), but no ferric pyrophosphate.

Media inoculation. *L. pneumophila* (Philadelphia 1 strain), provided by the Center for Disease Control, Atlanta, Ga., was initially grown for 72 h on F-G cysteine iron agar at 35°C in a 2.5% CO_2 atmosphere. Two separate colonies were picked from the stock plates and used to streak each plate of experimental medium and control medium. All media were then incubated at 35°C in a 2.5% CO_2 atmosphere and observed for growth after 18, 36, and 48 h. Growth was rated at 36 h on a scale of 1+ to 4+. A score of 1+ represented growth of 1 to 20 individual colonies; 2+ represented growth of 21 to 50 colonies; 3+ represented growth of 51 to 150 colonies; and 4+ represented growth of 151 to 300 colonies.

Hydrogen selenide assay. To assay for hydrogen selenide production, *L. pneumophila* was grown on plates of F-G cysteine iron agar amended with sodium selenate (50 μg/ml). The organisms were then washed off the plate and suspended in sterile saline. Oxygen-free nitrogen was passed over the culture and through a 0.1 M silver nitrate solution for 1 h, so that any hydrogen selenide formed by the organisms could react with the silver nitrate to form black, insoluble silver selenide without being oxidized (1). Any precipitate formed was digested with concentrated nitric acid and evaporated to dryness, and the residue was solubilized in a 10% solution of hydrochloric acid. This solution was then analyzed by atomic absorption spectrophotometry for selenium.

RESULTS

L. pneumophila colonies were visible at 18 h on all media containing sodium selenate. The colonial characteristics of *L. pneumophila* were the same as seen on F-G or F-G cysteine iron agar, with slight browning of surrounding media.

At 36 h of incubation, growth was seen on all media. Enhanced growth was evident in all cultures grown on F-G cysteine iron agar supplemented with sodium selenate, as compared to control media (Table 1). Again, colonial morphology was typical of *L. pneumophila*, with characteristic browning even more prominent in the surrounding media. Media containing sodium selenite and selenium dioxide at concentrations of 25 μg/ml or higher had colonies which were reddish orange in appearance due to the formation of red amorphous elemental selenium as described by Nickerson and Falcone (4). Media containing selenate and L-cysteine hydrochloride but no ferric iron showed growth similar to that on F-G cysteine iron agar. Growth on

such media was scored as 1+.

At 48 h of incubation the growth of *L. pneumophila* on plates with sodium selenate was significantly enhanced (Fig. 1) as compared to control plates. Media containing 5 to 10 μg of selenium per ml in the form of sodium selenate showed the greatest enhancement of growth, with an average of 20 to 22 times the number of colonies that were produced on F-G cysteine iron agar. Enhanced growth was evident even in media with as little as 1 μg of selenium per ml, with an average of three times the number of colonies as compared to F-G cysteine iron agar (Fig. 1). All media with sodium selenite or selenium dioxide had about the same amount of growth as seen on the F-G cysteine iron agar plates.

DISCUSSION

The present study demonstrated that sodium selenate enhanced the growth of *L. pneumophila* at concentrations as low as 1 μg/ml (Table 1). However, the optimum concentration was found to be 5 to 10 μg/ml (Table 1). Sodium selenite and selenium dioxide did not enhance the growth of *L. pneumophila* as well as sodium selenate did. It was also found that *L. pneumophila* was capable of converting selenate to selenide, as shown by the silver nitrate trap and subsequent analysis by atomic absorption spectrophotometry.

TABLE 1. *Growth of L. pneumophila after 36 h of incubation in the presence of selenium compounds*[a]

Selenium concn (μg/ml)	Growth with compound:		
	Na_2SeO_4	Na_2SeO_3	SeO_2
1.0	2+	1+	1+
2.5	3+	−	−
5.0	>4+	−	−
10.0	>4+	1+	1+
25.0	4+	1+	1+
50.0	3+	1+	1+
100.0	2+	1+	1+

[a] Control (F-G cysteine iron agar) = 1+.

Nissen and Benson (5) have postulated a pathway for metabolism of selenate, in which selenate is reduced to selenide, analogous to the metabolism of sulfate. Although further studies are needed to prove that this is the pathway for incorporation of selenium into organic compounds in *L. pneumophila*, our finding that *L. pneumophila* does produce selenide is consistent with this view.

Experimental media with ferric pyrophosphate deleted that were tested in our study were able to support the growth of *L. pneumophila*, as previously reported by Warren and Miller (6). However, the amount of growth produced was markedly increased (from 1+ to 3+) in media containing 50 μg of sodium selenate per ml and ferric pyrophosphate. This may indicate that some ferric selenate complex is produced in such media which facilitates enhanced growth of *L. pneumophila*.

In summary, we found that sodium selenate added to F-G cysteine iron agar enhanced the growth of *L. pneumophila*, with up to 22 times the number of colonies being produced at 36 h of incubation as produced on F-G cysteine iron agar without selenate. The optimum enhancement of growth by selenate was found at sele-

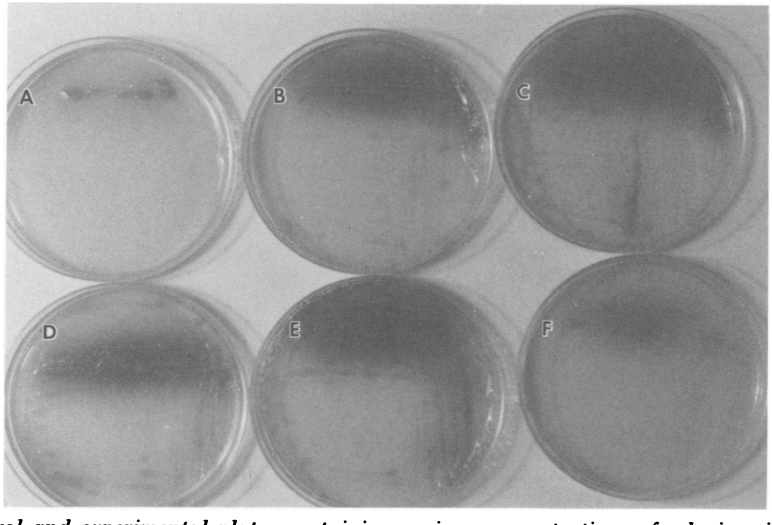

FIG. 1. *Control and experimental plates containing various concentrations of selenium in the form of sodium selenate after 48 h of incubation. (A) Control; (B) 1 μg/ml; (C) 2.5 μg/ml; (D) 5.0 μg/ml; (E) 10.0 μg/ml; (F) 25.0 μg/ml.*

nium concentrations of 5 to 10 μg/ml, with visible colonies after 18 h of incubation. The modified assimilatory sulfate reduction pathway is suggested as the metabolic pathway by which selenate is utilized. Because of the growth enhancement of *L. pneumophila* by media containing selenate, further studies are indicated to determine the selectivity and usefulness of such media as primary isolation media for this organism.

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