PSEUDOMONAS NATRIEGENS, A MARINE BACTERIUM WITH A GENERATION TIME OF LESS THAN 10 MINUTES

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ABSTRACT

EAGON, R. G. (University of Georgia, Athens). *Pseudomonas natriegens*, a marine bacterium with a generation time of less than 10 minutes. J. Bacteriol. **83**:736–737. 1962.—*Pseudomonas natriegens*, a marine microorganism, was demonstrated to have a generation time of 9.8 min. This is the shortest generation time reported to date. Optimal growth occurred at 37 C in brain heart infusion broth supplemented with 1.5% sea salt.

During studies in this laboratory on a marine bacterium, *Pseudomonas natriegens* sp. n. (Payne, Eagon, and Williams, 1961), it was observed that this microorganism has an unusually short generation time. The experiments reported herein, therefore, were done to determine the generation time of *P. natriegens* in readily available media.

METHODS AND RESULTS

P. natriegens is known to grow aerobically in various media supplemented with 1.5% sea salt. Results from experiments to determine the optimal medium and temperature are shown in Table 1. The microorganisms were cultivated on rotary shakers in Ryan flasks containing 15 ml of media. Growth was estimated turbidimetrically at 650 mμ, using a Coleman Junior spectrophotometer and a light path of 1.5 cm. One loopful of a stationary-phase culture was used as the inoculum. Of the four media used, it was observed that both the rate of multiplication and the total cell density were greatest and that the lag phase was shortest when *P. natriegens* was cultivated on brain heart infusion broth at 37 C. Growth fell off rapidly above 37 C and no growth was observed at 42 C.

Figure 1 illustrates the growth curve of *P. natriegens*. When a small inoculum was used (curve A) there was a typical lag phase, after which the microorganisms entered into a rapid and short logarithmic phase. When a larger inoculum was used (curve B) there was no noticeable lag phase, and the maximal stationary phase was reached after 3 hr.

DISCUSSION

The generation time was calculated from the data of Fig. 1, using the following equations (Lamanna and Mallette, 1959):

$$n = \frac{\log_{10} y/x}{\log_{10} 2} \quad (1)$$

$$g = \frac{t}{n} \quad (2)$$

where n = number of generations occurring in a time interval; x = number of organism at the beginning of a time period; y = number of organisms at the end of a time period; t = time; and g = generation time.

Using data from the 15-min interval between 3.50 and 3.75 hr (Fig. 1, curve A), and substituting in equation 1,

$$n = \frac{8.08 - 7.62}{0.301} \quad (3)$$

$$= 1.53 \text{ generations/15 min}$$

and then in equation 2,

$$g = \frac{15}{1.53} = 9.8 \text{ min} \quad (4)$$

Thus, *P. natriegens* has been shown to have a generation time of 9.8 min during its most active period of multiplication.

It is interesting that a longer generation time was noted when a large inoculum was used (Fig. 1, curve B). A value of 14.1 min was calculated from the growth rate during the 15-min interval between 0.50 and 0.75 hr. Possible explanations are that toxic or inhibitory products were carried over with the inoculum or that the large number

TABLE 1. *Cultural responses of Pseudomonas natriegens to different media and temperatures*

Medium	Rate*			Final optical density			Lag (hr)		
	25 C	30 C	37 C	25 C	30 C	37 C	25 C	30 C	37 C
Brain heart infusion	48	55	58	1.12	1.14	1.35	3	2.5	2.5
Nutrient broth	30	33	35	0.85	0.89	0.89	4	4	4
Nutrient broth + yeast extract	37	51	54	1.05	1.22	1.28	4	3	3
Trypticase soy	35	39	41	0.86	0.86	0.89	3	2.5	2.5

* Rate is defined as Δ optical density units/hr × 100 during the logarithmic phase.

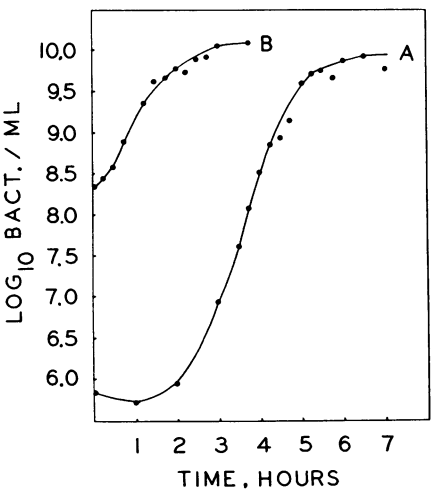

FIG. 1. *Growth curve of Pseudomonas natriegens. Numbers of microorganisms were estimated by the plate count method. Curve A: two loopfuls of a stationary-phase culture were used as inoculum for 30 ml of brain heart infusion medium containing 1.5% sea salt in 500-ml Erlenmeyer flasks on a rotary shaker at 37 C. Curve B: 5 ml of a logarithmic-phase culture were used as inoculum for 50 ml of medium.*

of microorganisms produced antagonistic substances within a short time.

The generation time of *P. natriegens* determined herein is the shortest reported to date. Mason (1935) compiled the generation times of several species of bacteria growing under optimal conditions. The most rapidly growing organisms were representatives of the coli-aerogenes group, with generation times of less than 20 min. Most *Escherichia coli* strains had generation times of 16 to 17 min, although one strain isolated from milk had a generation time of 12.5 min.

It is of further interest that a microorganism isolated from a marine environment should demonstrate the shortest generation time observed to date. Furthermore, it is likely that the generation time of 9.8 min reported herein could be shortened further by the use of conditions especially designed for optimal growth of this microorganism.

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