Growth and Pigmentation of *Sarcina aurantiaca* at Various Temperatures

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**INTRODUCTION**

The optimum temperature for growth of *Sarcina aurantiaca* is said to be 30° (Breed, Murray, & Smith, 1957). Variation of pigmentation with time and temperature has not been studied, and the pigments themselves have received only scant attention (Reader, 1925; Sobin & Stahly, 1942; Strang & Thirkell, 1969). This work reports the effects of temperature and time of incubation on the growth and pigmentation of *S. aurantiaca*.

**METHODS AND RESULTS**

*Sarcina aurantiaca* Flügge ATCC 146 was subcultured at weekly intervals on nutrient agar + 2% glycerol (w/v) at 30°. Otherwise the bacteria were grown in nutrient broth + 2% glycerol (w/v) (volumes indicated below) on a New Brunswick G25 orbital incubator-shaker. All inocula were prepared from organisms grown in this medium (125 ml.) for 24 h. at 30°. The micro-organisms tended to clump heavily in nutrient broth, whereas clumping did not occur when glycerol was added (Sobin & Stahly, 1942).

Medium (500 ml.) was inoculated with 1 ml. of a suspension containing 10⁷ organisms/ml. (standard inoculum) and incubated at 20°, 25°, 28°, 30°, 35° and 40°. Samples were taken at intervals and the bacterial population estimated by (a) turbimetric measurement at 600 nm. (a wavelength at which there would be no interference from any pigments in the organisms) and (b) counting of viable organisms. Serial 1/10 (v/v) dilutions were made and 0.1 ml. from each dilution was plated on nutrient agar + 2% glycerol (w/v) and incubated at 30°. Colonies on plates that had between 20 and 200 colonies were counted on a Gallenkamp colony counter. The optimum temperature (for highest yield of organisms) was 28° and similar results were obtained by both techniques. This is 2° lower than the optimum temperature for growth quoted by Breed et al. (1957).

To investigate pH tolerance, medium was prepared at pH values of 4.4, 5.4, 6.4, 6.6, 7.2, 8.1 and 9.4 by titrating with either HCl or KOH, and the pH was checked after autoclaving. Each medium (250 ml.) was inoculated with 0.5 ml. of the standard inoculum, incubated at 28° and the growth assessed at regular intervals from turbimetric readings. Growth occurred between pH 6.5 and 7.5 with an optimum yield slightly on the acid side of neutrality. This narrow pH tolerance is in marked contrast to the wide tolerance reported for *Sarcina maxima* and *S. ventriculi* by Canale-Parola (1970). However, although there was a sharp cut-off in growth on either side of the pH range above, buffering may not have been equally good over the entire pH range investigated. Nevertheless, in any medium where growth had occurred no pH change greater than 0.2 pH unit was detected.

To determine the degree of pigmentation at different temperatures, cultures (500 ml.)
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were incubated at 20°, 25°, 30°, 35°, and 40° after inoculation with 1 ml. of the standard inoculum. Samples were taken at intervals and the organisms recovered by centrifuging. The pellets were washed twice with distilled water, suspended in methanol and subjected to ultrasonication for 10 min. The suspensions were made to contain 10% (w/v) KOH, gassed out with nitrogen and left in the dark at room temperature to saponify overnight. The supernatant liquids were recovered, made to constant volume with 10% (w/v) KOH in methanol and the extinctions read at 438 nm. The solid residues were recovered, dried to constant weight and the relative amount of pigment produced per unit weight of residue calculated. The temperature of incubation that led to the highest extraction of pigments was 30°, and as with the growth curves this was a sharp maximum. The finding that the optimum temperature for growth and pigmentation is virtually the same is unusual. Most pigment-producing non-photosynthetic bacteria tend to produce more carotenoids at a lower temperature than is optimum for growth (Goodwin, 1963) and this is the case in Sarcina flavia (Thirkell, Strang & Carstairs, 1965). The yield of bacteria obtained at 40° was too low for accurate determinations to be made, but at each of the other temperatures there was a substantial delay before the peak of pigmentation was reached (at approximately 90 h. compared with 20 to 30 h. for maximum numbers of organisms). The highest pigmentation values did not remain constant, but declined until 140 h. when the final determinations were made (Fig. 1). This decline in the pigmentation levels between approximately 95 and 140 h. could be due to (a) turnover and degradation of the pigments, (b) accumulation of storage compounds (grandules) during the stationary phase, or (more likely) (c) to the presence of pigmentless debris as a result of autolysis during the decline phase.

Fig. 1. Pigmentation of Sarcina aurantiaca at various temperatures: ▲, 20°; ×, 25°; ○, 30°; ●, 35°. (See text for explanation.)

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