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The Effect of Incubation Temperature on the Growth Requirements of *Proteus vulgaris* and *Salmonella typhi*

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SUMMARY: *Proteus vulgaris* and *Salmonella typhi* require for growth at temperatures above normal complex nitrogenous substances additional to the media suitable for growth at normal temperature.

It was shown (Ware, 1951) that several strains of *Escherichia coli* and *Aerobacter aerogenes* which were able to grow at 37° in a defined medium were unable to grow in the same medium at higher temperatures. The addition of glutamic acid and nicotinamide to the medium supported growth up to 44°. This paper shows that organisms of other genera become nutritionally more exacting on increase in growth temperature.

METHOD

The following modification of Gale's (1947) medium devised by Bigger (1950) was used: Glucose, 3.0 g.; $(\text{NH}_4)_2\text{HPO}_4 \cdot 2\text{H}_2\text{O}$, 5.0 g.; NaCl, 1.0 g.; $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$, 0.3 g.; $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, 0.7 g.; KH_2PO_4 , 0.5 g.; K_2HPO_4 , 5.0 g.; water to 1000 ml.; pH value adjusted to 7.4.

The substances added to the medium were dissolved in distilled water, adjusted to pH 7.4, sterilized by boiling and added to the medium to give the final concentration required. The test organisms were grown in the basal medium for 24 hr. at 37° and the inoculum prepared by dilution in buffered saline. Five ml. quantities of the test medium were inoculated with 1 drop of the inoculum from a Pasteur pipette which delivered 22 drops/ml. An incubator was used for 37° and below and a water-bath having a stability $\pm 0.2^\circ$ for higher temperatures. The alternative methods of incubation did not influence the result.

RESULTS

The effect of temperature on the growth requirements of Proteus vulgaris

Our strain of *Proteus vulgaris* grew well in Lab-lemco broth between 25° and 44°. In the test medium growth occurred between 25° and 40° only when 5 mg. nicotinamide/100 ml. was added, and was enhanced by the further addition of one of several nitrogenous substances (Table 1). At 41° addition of any one of a number of these substances was essential for growth; at 42° only four of these compounds were capable of supporting growth, namely: L-glutamic acid, DL-methionine, L-cystine, thiamine; and at 43° only L-glutamic acid and DL-methionine; at higher temperatures growth did not occur. The temperature at which growth occurred was limited in some instances by the concentration of the added substance; L-leucine, however, was inhibitory in high concentration.

Table 1. *The effect of various substances on the growth of Proteus vulgaris and of Salmonella typhi in a defined medium at various temperatures*

The amounts of growth were read at 48 hr. for *Proteus vulgaris* and at 24 hr. for *Salmonella typhi*.

	<i>P. vulgaris</i>							<i>S. typhi</i>	
	Temperature of growth								
	40°	41°		42°		43°		40°	
	Concentration (mg./100 ml.)								
Substance added to defined medium	0.01	10-100	1.0	10-100	1.0	10-100	1.0	10-100	1.0
L-Alanine	X	0	0	0	0	0	0	0	0
L-Arginine	X	X	X	0	0	0	0	X	X
L-Asparagine	X	X	X	0	0	0	0	0	0
L-Aspartic acid	sl.	X	0	0	0	0	0	0	0
L-Cystine	X	X	X	X	X	0	0	0	0
L-Glutamic acid	X	X	X	X	X	X	0	X	X
Glycine	sl.	0	0	0	0	0	0	0	0
L-Histidine	X	X	X	0	0	0	0	0	0
L-Isoleucine	X	X	X	0	0	0	0	0	0
L-Leucine	X	0	0	0	0	0	0	0	0
L-Lysine	X	X	X	0	0	0	0	X	X
DL-Methionine	X	X	X	X	X	X	0	0	0
Nicotinamide	—	—	—	—	—	—	—	0	0
Thiamine	X	X	X	X	0	0	0	X	0
DL-Tryptophan	X	X	X	0	0	0	0	0	0
L-Valine	X	0	0	0	0	0	0	0	0
Controls									
Defined medium + 5 mg./100 ml. nicotinamide	sl.	0		0		0		0	
Defined medium	0	0		0		0		0	
Lemco broth	X	X		X		X		X	

0 = no turbidity; X = turbidity; sl. = slight turbidity.

The effect of temperature on the growth requirements of Salmonella typhi

The growth of our strain of *Salmonella typhi* in 24 hr. at 37° in the test medium was enhanced by the addition of any one of a number of nitrogenous substances in concentrations of from 1 mg./ml. to 1 mg./100 ml. The only substance which had an adverse effect on growth was nicotinamide. There was no growth after incubation at 40° for 24 hr. unless one of the following substances was added: L-glutamic acid, thiamine, L-lysine, L-arginine. At 43° no single addition tested was able to support growth, but L-glutamic acid + L-arginine with either thiamine or L-lysine showed growth in 24 hr.; without L-arginine growth was delayed for 48 hr. The addition of DL-methionine either to L-glutamic acid + L-lysine or to L-glutamic acid + thiamine inhibited growth, but there was no inhibition when DL-methionine was added to L-glutamic acid + thiamine + L-lysine; thiamine or L-lysine could, in this combination, be

replaced by L-arginine which also overcame the inhibitory effect of DL-methionine. L-Glutamic acid was always essential for growth. Although active at 10 mg./100 ml. the optimum concentration of each substance was approximately 200 mg./100 ml.

DISCUSSION

Hills & Spurr (1952) and Ware (1951) suggested that increase in incubation temperature caused changes in certain nutritional enzyme systems; it appears that enzymes responsible for L-glutamic acid formation may be among the first to be affected by increased temperature.

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