EFFECT OF INCUBATION TEMPERATURE ON BIOCHEMICAL TESTS IN THE GENERA *PSEUDOMONAS* AND *ACHROMOBACTER*

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The influence of temperature on both the growth of microorganisms and the activity of their enzymes is well known, but its effect on the synthesis of different enzymes has not been extensively investigated. Gale (1940), in studies on decarboxylation of amino acids, observed that washed suspensions of *Escherichia coli* grown at 27 C were more active than those from cultures grown at 37 C. The loss by a pseudomonad culture of its ability to liquefy gelatin when the temperature was raised has been reported (Greene and Jezeski, 1954). Nashif and Nelson (1953) found little lipase activity in cultures of *Pseudomonas fragi* which were grown at 30 C even though high counts were obtained. Alford and Elliott (1960) have shown that elevated temperatures inhibit the production of lipase rather than its activity since the enzyme has its optimal activity around 45 C. Stuart *et al.* (1942) found that many coliforms grew in the Eijkman test at 45.5 C without acid or gas production, and Tittsler (1931) found that temperatures above 34 C inhibited H_2S production by *Salmonella pullorum*.

In spite of these indications of a temperature effect on the synthesis of enzyme systems, the usual recommendation for incubation of routine tests for the identification of bacteria is that they be held at or near their optimal temperature (SAB, 1957). This temperature is usually defined as the one at which most rapid growth occurs (Oginsky and Umbreit, 1959). The recent development of quicker methods for the identification of bacterial cultures (Smith and Goodner, 1958), which depend upon rapid growth for their maximal usefulness, emphasizes the need for more detailed information on the effect of temperature on biochemical activity. The work reported here indicates that with several biochemical tests a false negative reaction may occur at optimal incubation temperatures.

MATERIALS AND METHODS

Cultures. Sixty strains of bacteria identified as belonging to the genera *Pseudomonas* and *Achromobacter* were obtained from different laboratories and from our own collection. Most of the strains were members of the genus *Pseudomonas*, although at least 5 were *Achromobacter* species. Twelve of the *Pseudomonas* and 2 of the *Achromobacter* strains were received with species designations, representing a total of 11 different species. No attempt was made to identify the other strains beyond their genus; with an occasional nonmotile rod, even this differentiation was not clear-cut.

Test procedures and incubation. The media and test procedures were those recommended in the *Manual of Microbiological Methods* (SAB, 1957) with the following exceptions. Lipolysis was determined on agar slants of lard-Nile blue sulfate medium prepared as described by Goldman and Rayman (1952). Plate determination of gelatin liquefaction was as described by Smith and Goodner (1958). The test for oxidative-fermentative metabolism of glucose was performed as described by Hugh and Leifson (1953). All media in tubes were incubated in water baths with a temperature control of ± 0.1 C and the gelatin plates in air incubators with a temperature control of ± 1.0 C.

RESULTS

Table 1 shows the effect of increasing temperatures of incubation on several biochemical tests. It should be emphasized that in all of these tests, growth, as evidenced by turbidity or culture development, was apparently unaffected by the temperature. Citrate utilization was an exception, of course, since visible growth is the evidence of a positive test. However, all of the bacteria included in the citrate utilization study grew well in other media at the indicated tempera-

TABLE 1

Effect of temperature of incubation on the reaction to various biochemical tests by strains of the genera Pseudomonas and Achromobacter

Biochemical Tests*	Number of Strains Showing Positive Reaction† at:		
	20 C	31–33 C	35–37 C
Citrate utilization	28	26	9
Lipolysis	20	19	9
Gelatin liquefaction (tube)	15	14	10
Gelatin liquefaction (plate)	10	9	6
Arabinose	25	24	19
Xylose	25	25	19
Litmus milk‡	9	5	4

* No temperature effect on reaction could be shown with the following tests: Nitrate reduction, ammonium phosphate, oxidative-fermentative metabolism, lactose, sucrose, glucose, salicin, glycerol, mannose.

† Growth evident in all tubes except negative citrate tubes.

‡ Proteolysis or coagulation recorded.

TABLE 2

Comparison of growth in nutrient broth at 20 C and 34 C of a Pseudomonas species that shows inhibition of 6 biochemical tests at 34 C

Time	Klett* Reading at:	
	20 C	34 C
0 hr	05	04
3 hr	06	52
6 hr	26	175
23 hr	198	242
30 hr	263	260
47 hr	345	325
71 hr	465	360
95 hr	495	355
7 days	590	455

* Turbidity reading on Klett-Summerson photoelectric colorimeter with a blue filter (approximate wave length, 4000 to 4600 A).

tures. Of the 60 strains examined, only 26 showed an effect of temperature on one or more tests. Of these 26, only 2 showed an effect on all six tests. With one exception, wherever a temperature effect was shown on a culture for arabinose it also was apparent for xylose. There also was a close, but not complete, correlation of temperature effect among tests for gelatin liquefaction, proteolysis of litmus milk, and lipolysis by the same strain. A study of the cultures of known origin indicated there was little or no apparent correlation between source and whether or not an effect of temperature was shown.

The data in table 2 indicate further that the amount of growth was not the limiting factor in the negative tests. Total cell population at 34 C was equal to or greater than that at 20 C for the first two days; by this time the cultures were beginning to show positive reactions.

Some of the tests developed positive reactions much sooner at the lower temperatures than at the higher temperatures. For example, the fermentation of xylose sometimes took 3 to 5 days at 33 C, whereas it was evident in 1 to 2 days at 20 C. It also was noted that with repeated transfer, some of the strains apparently were able to adapt their synthesis of the enzymes to the higher temperature without any increase in growth maximum. Thus, the apparent effect of temperature on the nonvital enzyme system disappeared.

DISCUSSION

The fact that the inhibitory effect of temperature on biochemical activity occurs sporadically and is somewhat transient within any given group of cultures does not simplify the taxonomic problem. On the contrary, the occasional nature of the inhibition may cause it to be overlooked. The data indicate that within the range of 31 to 37 C the greatest effect of temperature on biochemical activity was observed. Many laboratories employ incubators in this range for routine bacteriological analyses of milk and other food products, and it would appear to be a logical procedure to carry out any taxonomic studies on the organisms at the temperature at which they were initially isolated. However, for certain of the tests a partially incorrect characterization of the bacterium might result.

The monophosphate shunt is usually regarded as the schema by which pentoses are metabolized (Oginsky and Umbreit, 1959). It is possible that lability of one or more of the enzymes involved in this schema may account for the inability to ferment xylose and arabinose without an effect on fermentation of mono- and disaccharides. However, growth without acid or gas in the Eijkman procedure cannot be explained in this

manner. Regardless of the mechanism involved, it is apparent that "false negative" reactions may be encountered if one follows the usual procedure of incubating at temperatures at which most rapid growth occurs.

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SUMMARY

The biochemical activity of several cultures of the genera *Pseudomonas* and *Achromobacter* was determined at different temperatures in 16 different media. The results indicated that with some of the cultures a few degrees increase in temperature may inhibit gelatin liquefaction, lipolysis, citrate utilization, fermentation of xylose and arabinose, and action on litmus milk without apparently affecting the growth. The significance in taxonomic studies of this inhibition of the synthesis of nonvital enzyme systems is discussed.

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