

FACTORS INDUCING MESOPHILIC BACTERIA TO GROW AT 55°C.*

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Organisms able to grow at temperatures higher than their biothermal range have been shown to require additional growth supplements (Maas and Davis, 1952; Hutner et al., 1957). Recently, we have shown that a strain of mesophilic bacteria was able to grow at 55°C. when yeast autolysate (Albimi Laboratory, Brooklyn, New York) was added to the culture medium (Sie, Baker and Sobotka, 1961). Further studies revealed that several other commercial preparations as well as the supernatant fluid from a thermophilic bacterial culture also contained factor(s) which supported growth at 55°C. of several other strains of mesophilic bacteria.

Methods

To obtain the supernatant, the thermophile, Bacillus stearothermophilus B-194, was grown without aeration at 55°C. for 7-9 hours in a chemically defined medium (Baker et al., 1953), supplemented with 1 percent casein hydrolysate. Three-hundred-fifty ml. portions of the thermophile medium were added to one liter Fernbach flasks; one ml. of a 16 hour culture was used as inoculum. The suspension was clarified by a refrigerated centrifuge at 50,000 r.p.m. and the supernatant was autoclaved the same day.

To test for the activity of temperature factor(s) in the thermophile supernatant, 5 ml. of test medium, consisting of supernatant and base, pH 6.5, was

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added to 10 ml. Fernbach flasks, covered with aluminum caps, and autoclaved for 30 minutes at 16 psi. They were inoculated with one drop of a distilled water suspension of the mesophilic bacteria, and incubated at 55°C. Assays were carried out in triplicate. Flasks with uninoculated and inoculated basal medium served as controls. Growth was recorded in optical density units as measured by a Welch Densichron, equipped with a red-sensitive probe (Baker et. al., 1953). Strains of mesophilic bacteria selected for the present study were maintained on nutrient agar slants.

Results and Discussion

Thirteen strains of mesophilic bacteria were included in this study:

Bacillus alvei, (ATCC) 662; B. brevis, 604; B. cereus, 7064; B. macerans, 7068; B. megatherium, 7056; B. megatherium (lysogenic); B. polymyxa, 842; B. subtilis, 58; B. subtilis 7067; B. subtilis-B₂ less; B. sphaericus, 7054; B. sphaericus, RA-91 (obtained from Dr. M. B. Allen); and Escherichia coli, 9637. At 55°C., growth was observed in four strains - B. megatherium (lysogenic); B. subtilis-B₂ less; B. subtilis 7067; and B. sphaericus, RA-91, with yeast autolysate or thermophile supernatant but not with regular thermophile medium or beef extract and proteose peptone (Table I). Unlike yeast autolysate, 7-hour thermophile supernatant did not support the growth of mesophiles at 55°C. without being supplemented with 0.5 percent beef extract and 0.5 percent proteose peptone. In yeast autolysate or thermophile supernatant plus a combination of beef extract and proteose peptone, good growth at 55°C. required 6 days. In the same media, when incubated overnight at 37°C., growth equalled or surpassed that at 55°C. (Table I). Growth of these four mesophiles, at 55°C., is directly proportional to the concentration of yeast autolysate alone; addition of thermophile medium increases the growth response. The effect is most pronounced at low concentrations of yeast autolysate (i. e. 1 percent); growth

TABLE I

GROWTH OF MESOPHILIC BACTERIA AT 37° C. AND 55°C.
IN THERMOPHILE SUPERNATANT AND IN YEAST AUTOLYSATE

7 hr. super- natant (v/v) (in percent)	Growth ¹ of							
	<u>B. megather-</u> <u>tium (lysogenic)</u>		<u>B. subtilis</u> ²		<u>B. subtilis</u> (ATCC 7067)		RA-91	
	55°C. 6 days	37°C. 15 hrs.	55°C. 6 days	37°C. 15 hrs.	55°C. 6 days	37°C. 15 hrs.	55°C. 6 days	37°C. 15 hrs.
In beef ex- tract + pro- teose peptone								
0	0.10	0.26	0.08	0.26	0.08	0.42	0.10	0.26
10	0.25	1.20	0.29	0.96	0.28	0.95	0.17	1.18
25	0.66	1.28	0.75	1.10	0.59	1.07	0.68	0.95
50	0.85	1.43	0.44	1.27	0.80	1.22	0.47	1.10
Yeast autolysate (w/v)								
In H ₂ O								
1	0.18	1.37	0.14	0.94	0.12	1.28	0.33	1.44
2	0.43	1.66	0.15	1.60	0.28	1.63	0.73	1.56
6	0.86	1.60	0.52	2.35	1.25	1.78	1.14	1.65
In thermophil medium								
0	0.00	0.00 ³	0.00	0.00 ³	0.00	1.75	0.00	0.00 ³
1	0.50	0.68	0.60	2.10	0.44	2.80	0.65	1.91
2	0.66	1.20	0.82	2.29	0.62	3.30	0.75	2.13
6	1.21	2.30	1.21	2.71	1.76	3.40	1.28	2.45
In beef ex- tract + pro- teose peptone								
0	0.10	0.22	0.08	0.26	0.08	0.42	0.10	0.26
1	0.38	1.17	0.27	1.47	0.50	1.60	0.24	1.22
2	0.61	1.58	0.53	1.88	0.52	2.00	0.47	0.52
6	1.14	1.33	1.05	2.21	1.12	1.70	1.94	1.33

¹ Growth was recorded in optical density units.

² A vitamin B₂ requiring strain.

³ Good growth (O.D. above 1.5) was observed after 48 hours of incubation.

is also increased by the addition of beef extract plus proteose peptone to yeast autolysate. In view of the recent finding by Tabor (1961) that spermine (10^{-4} to 10^{-7} M) stabilized DNA from heat inactivation, we have studied its effect on the growth of mesophiles at high temperature. Spermine did not maintain the growth of mesophiles at high temperature.

As shown previously (Sie, Baker and Sobotka, 1961), thermophily of mesophilic organisms is made possible by addition of yeast autolysate or by thermophile supernatant in combination with beef extract and proteose peptone. No growth occurs in subcultures in media containing no yeast autolysate or thermophilic supernatant plus beef extract and proteose peptone.

At 55°C., B. megatherium (lysogenic) and B. subtilis, 7067, can also grow in liver extract or yeast hydrolysate, suggesting that the temperature factor(s) may be present in many substances.

Temperature factor(s) in the yeast autolysate is dialyzable and stable to autoclaving. It is not precipitable by 2 volumes of EtOH at 4°C. Moreover, acid hydrolyzed yeast autolysate (1 N HCl, autoclaved 15 minutes, 16 psi) showed much greater activity (Table II).

Enzymatic hydrolysis of the temperature factor(s) in the yeast autolysate and its dialysate at 37°C. with 0.1 mg./ml. of either crystalline pepsin, trypsin, chymotrypsin or papain does not alter its effectiveness.

The isolation and identification of the factor(s) is underway.

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TABLE II

GROWTH OF MESOPHILIC BACTERIA AT 37°C. AND AT 55°C. IN 2 PERCENT YEAST AUTOLYSATE THAT WAS TREATED IN VARIOUS WAYS, AND IN DIFFERENT BASAL MEDIA

Treatment	Base	Growth of			
		<u>B. megatherium</u> (lysogenic)		<u>B. subtilis</u> (ATCC 7067)	
		55°C. 6 days	37°C. 15 hours	55°C. 6 days	37°C. 15 hours
None	H ₂ O	0.04	1.42	0.20	1.55
	B.P. ¹	0.10	1.49	0.50	1.20
	R.T.M. ²	0.66	1.10	0.89	1.10
Acid hydrolyzed and neutralized	H ₂ O	0.70	1.51	0.98	2.45
	B.P.	0.85	1.39	1.20	2.50
	R.T.M.	1.52	1.49	1.58	2.60
Concentrated dialysate (48 hours against 10 volumes of H ₂ O under continuous stirring at 4° C.)	H ₂ O	0.10	1.40	0.48	1.70
	B.P.	0.14	1.34	0.48	0.80
	R.T.M.	0.62	2.20	1.12	1.50
Concentrated dialysate, acid hydrolyzed and neutralized	H ₂ O	0.42	0.80	0.38	1.20
	B.P.	0.12	1.10	0.42	1.20
	R.T.M.	1.02	1.90	1.42	1.35

¹ Beef extract + protease peptone.

² Regular thermophile medium.

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