LIII. THE HYDROLYSIS OF CERTAIN POLY-SACCHARIDES AND PROTEINS BY THE ENDOSPORES OF AEROBIC BACILLI.

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(Received January 19th, 1934.)

A STUDY of the enzymic content of the endospores of some twelve unidentified aerobic bacilli was made by Ruehle [1923]. He found that the spore preparations studied by him liquefied gelatin which contained 0.5 % of phenol and was covered by toluene, and this observation was confirmed by Cook [1931]. Ruehle also investigated the ability of certain of his preparations to hydrolyse fats and caseinogen and obtained negative, or at the best feebly positive, results. In a previous paper [Tarr, 1932] it was shown that almost every cell in a given culture forms spores when the aerobic bacillus being studied is cultivated under appropriate conditions. Later [Tarr, 1933] it was found that endospores of aerobic bacilli possess active respiratory catalysts which are quite different from the respiratory catalysts of the vegetative cells from which they are formed. In the present communication it is shown that endospores of aerobic bacilli possess an amylase and proteases.

EXPERIMENTAL.

The cultures employed (*B. subtilis* I; *B. subtilis* II and *B. mesentericus* I) were identical with those used in previous work [Tarr, 1932]. Endospore suspensions were prepared by the method previously described [Tarr, 1933] and were heated to 80° for 30 minutes immediately prior to use.

The amylase of bacterial endospores.

The presence of an amylase in bacterial endospores was verified by studying the hydrolysis of both starch and glycogen in the presence of toluene as antiseptic in the following manner. 250 ml. Erlenmeyer flasks received 50 ml. of 0.5 % solution of soluble starch (or glycogen), 10 ml. of *M*/5 phosphate buffer p_H 7.3, 15 ml. of water and 50 ml. of endospore suspension. The liquid in each flask was covered with 10 ml. of toluene, and the flask was corked and then incubated at 37°, shaking well each day. At suitable intervals portions of the solutions were removed for examination, the hydrolysis being followed in three ways: (1) the colour formed when a drop of the solution was mixed with a drop of *N*/100 iodine was noted; (2) the relative ability of 1 ml. of the experimental solution to reduce a drop of Benedict's solution was determined; (3) the amount of aldose present in a 5 ml. sample was estimated by the method of Willstätter and Schudel [1918], the results being reported in ml. of *N*/100 iodine per 5 ml. of solution.

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The results of this experiment which are recorded in Tables I and II show that heated spores of the organisms studied hydrolyse both starch and glycogen in the presence of toluene, for the typical reaction of the polysaccharide with iodine disappears in each case, the amount of reducing sugar increases, and the aldose titre increases. That the reducing aldose formed in each case is maltose and not glucose is shown by the following experiment.

Table I. *Hydrolysis of starch by bacterial endospores.*

Time in days	Iodine test			Reducing sugar test			Aldose formed (ml. N/100 I)		
	<i>B. subtilis</i> I	<i>B. subtilis</i> II	<i>B. mesentericus</i>	<i>B. subtilis</i> I	<i>B. subtilis</i> II	<i>B. mesentericus</i>	<i>B. subtilis</i> I	<i>B. subtilis</i> II	<i>B. mesentericus</i>
0	Blue	Blue	Blue	—	—	—	0.28 0.29	0.29 0.29	0.29 0.29
2	Red-brown	Blue	Red-brown	+	+	—	—	—	—
3	Red-brown	Red-brown	Neg.	+	++	—	—	—	—
5	Red-brown	Neg.	Neg.	++	+++	+	0.54 0.56	0.53 0.53	0.42 0.42
9	Neg.	Neg.	Neg.	+++	+++	+++	0.64 0.64	0.66 0.66	0.61 0.61
14	Neg.	Neg.	Neg.	+++	+++	+++	0.64 0.64	0.68 0.70	0.65 0.66

B. subtilis I 280 mg. dry wt. per exp. — No reduction.
B. subtilis II 480 " + Slight reduction.
B. mesentericus 410 " ++ Fair reduction.
 + ++ Pronounced reduction.

Table II. *Hydrolysis of glycogen by bacterial endospores.*

Time in days	Iodine test			Reducing sugar test			Aldose formed (ml. N/100 I)		
	<i>B. subtilis</i> I	<i>B. subtilis</i> II	<i>B. mesentericus</i>	<i>B. subtilis</i> I	<i>B. subtilis</i> II	<i>B. mesentericus</i>	<i>B. subtilis</i> I	<i>B. subtilis</i> II	<i>B. mesentericus</i>
0	Red-brown	Red-brown	Red-brown	—	—	—	0.28 0.29	0.30 0.30	0.30 0.30
2	Neg.	Almost neg.	Almost neg.	+	—	—	—	—	—
3	Neg.	Neg.	Neg.	+	+	—	—	—	—
5	"	"	"	++	+++	+	0.48 0.50	0.46 0.48	0.35 0.35
9	"	"	"	+++	+++	+	0.64 0.64	0.48 0.46	0.36 0.36
14	"	"	"	+++	+++	+++	0.64 0.64	0.48 0.48	0.48 0.48

B. subtilis I 280 mg. dry wt. per exp. — No reduction.
B. subtilis II 480 " + Slight reduction.
B. mesentericus 410 " ++ Fair reduction.
 + ++ Pronounced reduction.

After the conclusion of the above experiment 25 ml. of the remaining liquid in each flask were filtered through kieselguhr, the clear filtrate was evaporated at reduced pressure to about 3 ml. and the osazone prepared following the directions given by Cole [1933]. In each case typical maltosazone crystals appeared on cooling the liquid. Attempts to hydrolyse 0.2 % maltose solutions with endospore suspensions under toluene were unsuccessful; no increase in the aldose titre was obtained after 2 weeks at 37°.

The proteolytic activity of endospores.

The ability of bacterial endospores to hydrolyse various proteins was next investigated. 2 % solutions or suspensions of the proteins studied were prepared. 10 ml. of such a solution were placed in a 50 ml. Erlenmeyer flask together with

5 ml. of *M/5* phosphate buffer p_H 7.3, 5 ml. of spore suspension and 2 ml. of toluene. The flasks were corked and incubated at 37°, shaking thoroughly each day, and 1 ml. samples were removed at intervals for formaldehyde titration by the method of Brown [1923]. The results of this experiment which are given in Table III show that bacterial endospores hydrolyse caseinogen, gelatin, egg-

Table III. *Hydrolysis of proteins by bacterial endospores.*

No. of ml. of *N/20* NaOH required for 1 ml. of the following protein solutions using the formaldehyde titration method of Brown.

Time in days	Caseinogen		Egg-albumin		Blood-albumin		Edestin		Gelatin		Peptone (Witte)	
	<i>B. sub-tilis</i> I	<i>B. mesentericus</i>	<i>B. sub-tilis</i> I	<i>B. mesentericus</i>	<i>B. sub-tilis</i> I	<i>B. mesentericus</i>	<i>B. sub-tilis</i> I	<i>B. mesentericus</i>	<i>B. sub-tilis</i> I	<i>B. mesentericus</i>	<i>B. sub-tilis</i> I	<i>B. mesentericus</i>
0	0.08	0.08	0.08	0.08	0.08	0.08	0.08	0.08	0.08	0.08	0.18	0.18
7	0.14	0.10	0.08	0.08	0.12	0.09	0.07	0.05	0.10	0.06	0.22	0.20
14	0.19	0.12	0.12	0.10	0.21	0.10	0.08	0.06	0.16	0.14	0.34	0.26
21	0.18	0.14	0.11	0.13	0.20	0.10	0.12	0.05	0.20	0.14	0.33	0.34

The results given are the averages of duplicate experiments agreeing within 5 %.

B. subtilis I 66 mg. dry weight per exp.
B. mesentericus 42 „

albumin, blood-albumin, peptone (Witte), and, in the case of one species, edestin, the number of free amino-groups in the solution increasing in each case. No increase in the initial formaldehyde titration value was obtained when endospores or proteins alone were incubated under similar conditions.

SUMMARY.

Endospores of aerobic bacilli hydrolyse starch and glycogen to maltose, and various proteins with an increase in the number of free amino-groups in the solution.

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