Note

The Environmental Factors Affecting Sporulation of an Alkalophilic *Bacillus* Species

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There are many reports about the study of alkalophilic bacteria.¹⁻⁴⁾ These alkalophilic bacteria grew well under alkaline conditions, and NaCl (Na⁺) was necessary for their growth. Na⁺ ion also stimulated the transport of amino acids into the cells.³⁾ It was found that the optimum pH for germination of spores of alkalophilic *Bacillus* species was about 10 and Na⁺ ion stimulated the initiation of germination specifically (in perparation). But, there has been no report about the study of sporulation of alkalophilic *Bacillus* species. In this note, we will present the medium for sporulation of alkalophilic *Bacillus* species and the effect of environmental factors (pH, temperature and NaCl concentration) affecting sporulation.

An alkalophilic *Bacillus* no. 2b-2 isolated from soil was used throughout out the experiments because of its excellence in yield of spores. Alkalophilic *Bacillus* no. 16-2 and *Bacillus* no. 20 were used for comparative purposes. Taxonomic studies of these strains will be reported elsewhere.

As for as tested (exanixed), alkaline modified Schaeffer medium (AMS medium) was found to be the best medium for sporulation of alkalophilic *Bacillus* species, *Bacillus* no. 2b-2, *Bacillus* no. 16-2 and *Bacillus* no. 20. Therefore, we used the ASM medium for the experiments described below.

Table I shows the effect of pH on sporulation of *Bacillus* species. There seemed to be a general agreement that the optimum pH for sporulation was close to that for growth, but the range was narrow. The final pH in the medium became 9~9.5 irrespective of initial pH. It was also found that the sporulation rate was related to the final optical density. The optimum temperature for sporulation was almost the same as that for growth (Table II). The growth at 45°C was faster than that at 34°C, but the sporulation rate at 45°C was much lower than that at 34°C. Filamentous cells (5~6 times longer than the normal size) were observed at 45°C, and cell division found to be slightly inhibited. Considering that sporulation is a kind of cell division in a special condition,⁵⁾ the lower sporulation rate at 45°C may be related

TABLE II. EFFECT OF VARIOUS TEMPERATURE ON SPORULATION OF *Bacillus* No. 2b-2

Temperature (°C)	Final OD ₆₁₀ ^a	Growth Rate	Percent of sporulation
25	4.4	130	5%
34	4.4	45	95
37	4.4	40	95
45	3.0	43	1
55	0.0	—	—

AMS medium (pH 9.6) was used, and the details of the experiment are noted in Table I.

^a The final OD and the sporulation rate were measured after 42 hr of incubation.

TABLE III. EFFECT OF NaCl CONCENTRATION ON SPORULATION OF *Bacillus* No. 2b-2

NaCl concentration	Final OD ^a	Growth Rate	Percent of sporulation
0 M	2.9	54	0%
0.02	3.7	46	30-40
0.2	4.7	44	70
0.4	4.0	44	30-40
1	2.9	46	1

AMS medium (pH 9.6) was used, and the details of the experiments are noted in Table I.

^a The final OD and the sporulation rate were measured after 47 hr of incubation at 37°C.

to the inhibition of cell division at 45°C. Table III shows the effect of NaCl concentration on the sporulation of *Bacillus* no. 2b-2. The optimum concentration of NaCl for sporulation was similar to that for growth, but the range was narrow.

In conclusion, it was found that the optimum conditions for sporulation were almost the same as those for growth, and alkalophilic *Bacillus* no. 2b-2 had the characteristics that it sporulated very well in the AMS medium (initial pH 8~9.5) at 37°C, and 0.2 M NaCl was necessary for the optimum sporulation.

REFERENCES

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TABLE 1. EFFECT OF pH SPORULATION OF ALKALOPHILIC *Bacillus* SPECIES IN MODIFIED SCHAEFFER MEDIUM

Modified Schaeffer medium contained 6 g of beef extract, 10 g of polypeptone, 0.5 g of MgSO₄, 2 g of KCl, 236 mg of Ca(NO₃)₂, 19.7 mg of MnCl₂, 0.278 mg of FeSO₄ and 1 g of glucose per liter of distilled water. The pH was adjusted by the addition of Na₂CO₃ or NaOH, and the final concentration of Na⁺ ion was adjusted to about 0.2 M by the addition of NaCl.

Initial pH	<i>Bacillus</i> no. 2b-2			<i>Bacillus</i> no. 20			<i>Bacillus</i> no. 16-2		
	Final OD ₆₁₀ ^a	Growth rate ^b	Percent of sporulation ^c	Final OD ₆₁₀	Growth rate	Percent of Sporulation	Final OD ₆₁₀	Growth rate	Percent of sporulation
6.0	0.0	—	—	0.3	—	—	0.3	—	—
7.0	0.2	—	—	5.8	50	30	6.0	50	30
8.0	5.4	44 min	95%	6.2	50	50-60	6.0	50	30
8.5	5.4	44	95	6.2	50	50-60	6.0	50	30
9.0	5.4	44	95	6.1	50	50-60	6.0	48	30
9.5	5.4	44	95	6.1	50	50-60	5.0	50	10-20
9.8	4.0	50	50	3.4	60	30-40	4.0	60	10-20
10.5	3.2	ND ^d	10	3.4	60	30	3.2	65	10-20
11	0.1	—	0	4.3	ND	30	4.7	ND	10-20
12	0.0	—	—	0.1	—	—	0.0	—	—

^a The final cell growth was estimated by the absorbance at 610 nm with Shimadzu Spectronic 20 A after 40 h of incubation at 37°C.

^b Growth rate in represented by the doubling time (min) of the cells.

^c Percentages of sporulation were determined by the spore staining method. after 40 hr of incubation.

^d ND: Not determined.