Distribution of Dipicolinic Acid (Pyridine-2, 6-Dicarboxylic Acid) in Mature Spores of a Strain of *Bacillus cereus**

Since a report by Powell (1953) it has been generally admitted that large quantities of dipicolinic acid (DPA) are present in bacterial spores and that the bulk of this compound in ungerminated spores is believed to exist in a bound form (Foster, 1956). During the course of our investigation on the chemical composition of mature spores of a strain of *Bacillus cereus*, it was found that DPA could easily be released from the mature spores when the organisms were mechanically disrupted for a relatively short time (unpublished data). In a further investigation on the distribution of DPA in the disintegrated spores, we found that this compound is mostly detected in the risidual supernatant after ammonium sulfate fractionation.

Spores of *Bacillus cereus* No. 2 from the collection of our Institute were grown on broth agar plates at 35°C for a week. At the end of incubation, the whole culture was harvested, washed three times with chilled distilled water by centrifugation and resuspended in M/100 phosphate buffer (pH 7.0). The suspension was then incubated at 35°C with shaking until almost no vegetative cells were detectable. This aerated suspension was again washed three times by centrifugation and the packed, washed spore preparation obtained was mechanically disintegrated as follows; packed spores corresponding to 5 grams dry weight, were mixed with 30 grams of quartz powder and ground in a mortar for 3 hours in the cold. The whole resultant preparation was suspended in 100 ml of M/100 phosphate buffer (pH 7.0) and centrifuged at $10,000 \times g$ for 20 minutes. The precipitate thus obtained was thoroughly washed by centrifugation at the same speed in fresh phosphate buffer and the whole supernate (Fraction S−1), which had a total volume of 200 ml, was fractionated with ammonium sulfate (Fractions, S−2, S−3, S−4 and S−5). The DPA content of these fractions and of the washed precipitate and also of intact spores were determined by Janssen's method (1958) sometimes with a slight modification. In the case of both the washed precipitate and the intact spores, DPA was extracted by autoclaving the materials while the other fractions were reacted directly with Mohr's iron salt.

The results are summarized in the accompanying table. The DPA content of intact spores was 152.1 μg/mg dry weight. Disintegration of the spores followed by centrifugation revealed that the bulk of the DPA from the spores had been released into the supernatant. As can be seen from the table, 96.8 per cent of the total DPA was in the supernatant while only 3.2 per cent was detectable in the washed precipitate. Furthermore, it was shown that the DPA in the supernatant was not precipitated by ammonium sulfate but dialyzed through a semipermeable membrane. Thus in the table, the DPA in the supernatant is only detectable in the final fraction, Fraction S−5. These results indicate that the bulk of the DPA in ungerminated spores of *Bacillus cereus* No. 2 is not in such

* Aided by a grant from the Ministry of Education, Japan.

Table 1. Distribution of dipicolinic acid in spores of *Bacillus cereus* No. 2.

		Materials	DPA content (% dry weight)*	DPA content yield (%)
		Intact spores	15.21	100
		Insoluble fraction	—	3.2
		Total supernatant (S-1)	—	96.8
Ammonium sulfate fractionation	A	S-2 (0-30%)	—	0
		S-3 (30-50%)	—	0.42
		S-4 (50-80%)	—	0.28
	B	S-5	—	96.1

* Dry weights of organisms were estimated by drying measured volumes to constant weight at 120°C.
A ; Precipitated with ammonium sulfate.
B ; Not precipitated with ammonium sulfate.

bound form (Foster. 1956) that an extraction in boiling sulfuric acid or sodium hydroxide is essential to the release of the compound. The pattern of the distribution of DPA in spore disintegrates also suggests the localization of this compound in the core of the spores, since only a small amount of DPA is extractable from the washed precipitate which mostly consists of spore coat debris. Such DPA in the precipitate may be partially accounted for by DPA in a few intact spores still remaining in the material.

Although the true form of DPA in ungerminated spores is still unknown, it seems from our present data that DPA or Ca—DPA (Powell, 1953) is present in a free form or at least in such a loosely bound form, that the compound is easily released into ordinary physiological solvents. Sudden changes in spore coat permeability caused by mechanical shock might be followed by a simultaneous exchange of water in the medium with DPA in the spore core. Our preliminary observation suggests that small mechanical injuries to the spore coats, not visible under a light microscope, may result in the release of large quantities of DPA from intact spores. The intrinsic mechanisms involved, however, must await further analyses of the kinetics of the release of DPA from the spores. Further studies are now in progress and details will be published elsewhere.

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Received on December 9, 1959