Sporicidal Activities of Disinfectants on *Paenibacillus larvae*

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**ABSTRACT.** Sporicidal activities of glutaraldehyde, sodium hypochlorite, povidone iodine, ethylene oxide gas, chlorhexidine gluconate, and didecyl dimethylammonium chloride on wet and dry spores of *Paenibacillus larvae* (basonym: *Bacillus larvae*) were evaluated for control of honeybee American foulbrood. Glutaraldehyde was found to have a strong and rapid effect on both the wet and the dry spores among the disinfectants tested. — KEY WORDS: disinfectant, *Paenibacillus larvae*, sporicidal activity.

*Paenibacillus larvae* (basonym: *Bacillus larvae*) is the causative agent of American foulbrood (AFB), which is a serious infectious disease of broods of honeybees. It is difficult to eradicate the AFB because *P. larvae* forms spores. Although the vegetative rods of *P. larvae* were susceptible to many kinds of antibiotics [11], the spores which were sources of the infection were considered to be resistant to antibiotics. Therefore, the effective disinfectants against spores of *P. larvae* are necessary for the control of the AFB. Halogens and alkylators have so far been found to be sporicidal to *Bacillus* species [3, 4, 6, 8, 13, 15], however, their activities against *P. larvae* spores have not yet been confirmed. The present paper describes the sporicidal activities of several disinfectants against *P. larvae* spores.

The aqueous disinfectants used in this study were as follows: 25% glutaraldehyde (International Non-proprietary Name: glutaral), sodium hypochlorite (NaOCl) containing 5% available chlorine (AvCl), 10% povidone iodine (Popiyodon®) containing 1% available iodine, 5% chlorhexidine gluconate (Hibitane®), and 10% didecyl dimethylammonium chloride (Cleakil®-100). These disinfectants were prepared as stock solutions and diluted with distilled water to appropriate concentrations as indicated below, except that the glutaral was diluted with alkaline buffer (0.018 mM Na2CO3 and 0.035 mM NaHCO3, pH 8.0). Ethylene oxide (EO) gas which was a mixture of 20% (W/W) EO and 80% (W/W) CO2 was also used. All disinfectants were freshly prepared before use.

An isolate of *P. larvae*, designated RIAS-No.2, was chosen from our laboratory stock, since no obvious difference in the susceptibilities to the disinfectants had been found among the strains in the preliminary experiment. The bacteria were cultured on J-agar [1] at 37°C for 14 days under 5% CO2 to give an sporulation of approximately 80%. The difference in the susceptibilities to the disinfectants had been chosen from our laboratory stock, since no obvious disinfectants were freshly prepared before use.

**RESULTS.**

The sporicidal activities of disinfectants tested in this study are summarized in Fig. 1. The glutaral showed the most remarkable sporicidal activity on the spores of *P. larvae*, especially the dry spores were more susceptible than the wet spores. No viable spore was detected in the wet spores after exposure to 2% glutaral in 20 and 5 min, respectively. Similar results have already been described for *B. anthracis* and *B. pumilis* [13, 15]. These results suggest that the concentrations of 2% or more of glutaral are recommended for the practical use.

Similarly, the dry spores were more susceptible to NaOCl than the wet spores. No viable cell was detected in the dry spores after 40-min exposure to NaOCl at every concentrations tested, whereas many viable cells still remained in the wet spores at the concentrations below 0.0125% AvCl. On the basis of the data, NaOCl containing...
0.025% or more concentrations of AvCl were recommended for the practical use. However, NaOCl seemed to act slower than glutaral at the each recommended concentration. Contrary to glutaral and NaOCl, the povidone iodine showed a strong effect on the wet spores of *P. larvae* compared with the results in the dry spores. However, sporicidal effect of povidone iodine was slow acting and the dose dependency was obscure. Furthermore, the EO gas also acted slowly against the dry spores; it was necessary to incubate for a long time (more than 4 hr) to eliminate all the dry spores tested (data not shown). These results are in consistent with previous reports [7, 10].

On the other hand, no obvious reduction of viable spores was observed in both the wet and the dry spores exposed to either chlorhexdine gluconate or didecyl dimethylammonium chloride (data not shown). Since the spore coat is composed of proteins such as keratin, biguanides and quaternary ammonium compounds that primary attack the cytoplasmic membrane can not penetrate to the spore coat and generally show little or no sporicidal activity [9]. The present data completely agreed with the previous findings.

For the control of the AFB, sanitation of hives and nearby ground seemed to be essential. AFB affects only the larvae younger than 2 days by ingesting food contaminated with spores of *P. larvae* [1, 2, 12, 16]. The spores germinate soon after they enter the larval gut, and the vegetative rods proliferate in the tissues of larvae before pupation [1, 2, 12]. Finally, the larvae die down to spore-contaminated foulbroods. The foulbroods containing many spores are eaten by cleaning bees to remove to outside of the hives. For this reason, disinfection of the nearby ground by spraying is desired besides hive disinfection for the control of AFB. From the results of this experiment, glutaral would be the most suitable disinfectant in field use because of its strong and rapid effect on the spores of *P. larvae*.

**REFERENCES**


**Fig. 1.** Kinetics of the numbers of viable cells in wet (A, C, E) and dry (B, D, F) spores after exposure to several disinfectants. Cells were mixed with the disinfectants and incubated at room temperature without agitation. At different times of incubation, the spores were harvested and the numbers of viable cells were determined. CFU: colony forming units, ND: not detected, AvCl: available chlorine, AvI: available iodine.