[SHORT COMMUNICATION]

*Promotion of Spore Attachment of
Pasteuria penetrans
to Meloidogyne spp.
by a Combination of Sonication
of Spores and Storage
of Spores in Water*

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Pasteuria penetrans (THORNE) SAYRE & STARR is a host-specific parasitic bacterium on the root-knot nematodes *Meloidogyne* spp. (7). It is known that sonication of *P. penetrans* spores increases the number of spores attached per second-stage juvenile (J2) of *Meloidogyne* spp. (4, 8) without losing their host-specific characteristic (4).

Methods for identifying the major root-knot nematodes using the host-specificity of *P. penetrans* have recently been developed (3, 5). A large number of spores attached to J2 make the observation in these methods easy. It may become an important pretreatment for using *P. penetrans* to control *Meloidogyne* spp.

The object of this study was to examine the effect of storage conditions of spores and sonication of spores on spore attachment of *P. penetrans* isolates to J2s of *Meloidogyne* species.

MATERIALS AND METHODS

The origins of *M. incognita* (KOFOID & WHITE) CHITWOOD, *M. arenaria* (NEAL) CHITWOOD, and *M. hapla* CHITWOOD used in this study were obtained from tobacco in Chiba,

Okayama, and Akita Prefectures, respectively. These three populations were cultured on tomatoes (cv. Kyoryoku-beiju) in a greenhouse. The egg masses were hand picked from the roots, and J2s were obtained by incubating these egg masses for 3 days in sterile water at 25°C. Three isolates of *P. penetrans* used, i.e., MIP, MAP, and MHP, originated from the above populations of *M. incognita*, *M. arenaria*, and *M. hapla*, respectively.

Mass spore production of *P. penetrans* was conducted as described previously (4). Tomato seedlings (cv. Kyoryoku-beiju) inoculated with J2s encumbered with the spores were grown for 75-90 days in a greenhouse at 25°C. Then, infected adult females were picked from the roots, and were twice washed with sterile water in a dish. After the females were squashed with a pin in sterile water, the spore suspension was immediately adjusted to a concentration of 2×10^5 spores/ml with sterile water. These spore suspensions were stored under different storage conditions: storage temperatures of -20, 5, and 25°C and storage periods of 0, 20, and 40 days.

Seven ml of the stored spore suspension was sonicated for 30 min in an ice bath with an ultrasonic transducer (TA-5287: Kaijo Denki Co. Ltd.), which supplied 19.5 kHz modulated electrical output with a power setting of 300 W.

After the sonication, bioassay for spore attachment to J2 was immediately performed as follows: 50 μ l of McIlvaine buffer pH 8.0, and 50 μ l of a nematode suspension containing 50-100 J2s hatched within 3 days in sterile water at 25°C were pipetted into a well of a 96 well microplate (Nunc), followed by the addition of 100 μ l of the spore suspension (2×10^5 spores/ml), and the suspension was mixed in the well. After 4 hr at 25°C, the supernatant was discarded and replaced with equal volumes of McIlvaine buffer (pH 2.2) and 0.1% Brilliant Blue G solution to stain the spores (1). After staining the spores for about 2 hr at 5°C, the supernatant was

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Table 1. Effect of sonication of *P. penetrans* spores (isolates of MAP, MIP, and MHP) stored in sterile water under several different conditions on spore attachment to *Meloidogyne* J2.

Storage condition	Number of spores attached/J2 ^a					
	MAP to <i>M. arenaria</i>		MIP to <i>M. incognita</i>		MHP to <i>M. hapla</i>	
	— son	+ son	— son	+ son	— son	+ son
Before storage	0.2c	5.3d	0.3cd	9.8c	0.3a	3.0d
20 days at -20°C	0.5c	5.1d	0.8ab	8.8c	0.5a	4.0d
40 days at -20°C	0.5c	10.7c	1.1a	10.2c	0.7a	3.2d
20 days at 5°C	1.4b	12.2c	0.2cd	25.9b	0.5a	7.2c
40 days at 5°C	2.3a	25.0a	0.3cd	37.4a	0.7a	14.2a
20 days at 25°C	0.8bc	20.3b	0.6bc	17.4bc	0.5a	9.3bc
40 days at 25°C	1.6ab	22.8ab	0.2cd	16.5bc	0.6a	10.2b

^aData are the means of four replicates. For each replicate, 20 specimens were counted. Values are the mean numbers of spores attached per J2. '— son' and '+ son' mean non-sonicated spores and sonicated spores for 30 min, respectively. Means within each column followed by the same letter are not significantly different at the 5 % level by the Tukey's test.

discarded. The remaining nematode suspension was mounted on a slide glass, and covered with a cover glass. The number of spores attached per J2 was counted at a magnification of $\times 200$ or $\times 400$ for 20 individuals. Each treatment was replicated four times.

RESULTS AND DISCUSSION

Number of spores attached per J2 (No./J2) in the non-sonicated spores of the three isolates ranged from 0.2 to 2.3 in all storage conditions. Sonication of spores of the three isolates remarkably increased No./J2 regardless of the storage conditions (Table 1). Sonication of spores after storage at 5°C and 25°C tended to increase No./J2 more than that after storage at -20°C and before storage, especially significantly in MAP at 25°C , MIP at 5°C , and MHP at 5 and 25°C . Sonication of spores stored for 40 days at 5 and 25°C tended to increase No./J2 more than that for 20 days, especially significantly in the three isolates at 5°C .

Spore attachment was promoted by the sonication of spores after storage of spores in water. Accumulated temperature of 850 degree-days (above 10°C) at 25°C was required until mainly mature spores were produced in the development of *P. penetrans* in *M. javanica*

(TREUB) CHITWOOD (6). As the tomato seedlings inoculated J2s with the spores were grown for 75-90 days in a greenhouse at 25°C , accumulated temperature in this study was beyond 850 degree-days. Therefore, it seems that mature level of *P. penetrans* spores during storage is not related to spore attachment in this study. Removal of the sporangial wall appears to be an important factor in spore attachment to J2 (8). Storage of spores in water may promote removal of the sporangial wall by spore sonication because the water of the spore suspension during storage may physically modify the sporangial wall. After the surface of infected females by *P. penetrans* was slightly washed with sterile water, their females were squashed with a pin in sterile water. Therefore, it is possible that the body fluids of the females and microorganisms in spore suspension changed the nature of spore during storage. To prove this in further experiments, completely sterilized adult females and washed spores should be used.

The spores stored at the room temperature as a dry powder for 11 years did not reduce spore attachment to J2 of *M. javanica* though their infectivity significantly decreased (2). Spores stored after the sonication may also retain spore attachment. Effect of storage of soni-

cated spores on spore attachment to *Meloidogyne* J2 should be studied more closely in order to use these spores conveniently.

The identification methods for the major root-knot nematodes using the host-specificity of *P. penetrans* (3, 5) require a large number of spores attached to J2 in order to facilitate observation. The promotion method for spore attachment using sonication after storage of spores in water will contribute to these identification methods. Further, the sonication after storage of spores of MAP and MHP promoted infectivity to *M. arenaria* and *M. hapla* (ORUI, unpublished). This promotion method also may become an important pretreatment for using *P. penetrans* to control *Meloidogyne* spp.

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和文摘要

Pasteuria penetrans 胞子の水中保存と超音波処理の組み合わせによるネコブセンチュウに対する付着性の増進

大類幸夫

異なる温度条件下の水中で保存した *Pasteuria penetrans* 胞子の超音波処理がネコブセンチュウの第2期幼虫に対する付着性に及ぼす影響を明らかにした。*Meloidogyne incognita*、*M. arenaria*、および *M. hapla* から分離したそれぞれの *P. penetrans* MIP、MAP、およびMHPの3系統の摘出直後の胞子を供試し、保存温度（-20、5、25℃）と保存期間（摘出直後、20、40日）のそれぞれの保存条件を組合わせた実験を行った。それぞれの組合わせにおける30分間の超音波処理胞子および無処理胞子の第2期幼虫に対する付着性を比較した。その結果、MIP、MAP、およびMHPの3系統とも5℃または25℃で20日または40日保存後超音波処理したとき、摘出直後あるいは-20℃保存後に超音波処理するよりも付着性が増進した。