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BRIEF ARTICLE

Isolation of swarmer clostridia from soil samples

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Resumen: El fenómeno de agrupamiento dinánimo o "swarming" se ha descrito en el género *Clostridium* en *C. tetani, C. botulinum* G, *C. novyi, C. septicum* y *C. sporogenes.* Sin embargo, en nuestra experiencia ese fenómeno se presenta en otras especies de clostridios, por lo que se planteó emplearlo en su aislamiento. Se estudiaron 30 muestras de suelo de la Universidad de Costa Rica, aislándose 61 cepas de *Clostridium*, correspondientes a 12 especies, cuya frecuencia por muestra fue: *C. sporogenes* 16, *C. tetani* 13, *C. oceanicum* 7, *C. putrificum* 5, *C. sordellii* 4, y 2 de *C. glycolicum, C. fallax, C. beijerinckii, C. bifermentans, C. hastiforme* o *C. subterminale*, además de una cepa de *C. durum* y otra de *C. sardiniense.* Todas esas especies presentaron el agrupamiento, lo que extiende esa característica a diez especies más de las descritas originalmente.

Key words: *Clostridium* sp. swarming phenomenom, isolation, soil microbiology.

Five species of *Clostridium (C. tetani, C. botulinum* G *(C. argentinense), C. novyi, C. septicum,* and *C. sporogenes)* are known to swarm, *i.e.* they form film growth or medusa head-like colonies (Cato *et al.* 1986). Some morphological characteristics of the swarming behavior of *C. tetani* are similar to those described for *Proteus* (Hernández & Rodríguez 1992, 1993). The behavior was employed in the isolation of *C. tetani* from soil samples (Smith & Williams 1984).

The swarming phenomenom has been very common throughout our experience with clostridia, and not restricted to the species mentioned above. For this reason, we used it to isolate other species than *C. tetani* from 30 superficial soil samples (*ca.* 100 g by sample, extracted at 5 to 10 cm depth) collected in the University of Costa Rica campus (*ca.* 50 hectares. 84°3' W, 09°57' N). Soil specimens were analyzed according to standard methods, as described previously Rodríguez *et al.* (1993) as per the recomendations of Holdeman *et al.* 1977. Briefly, 1g of each sample was homogenized in 5 ml of sterile distilled water and 1.5 ml of that suspension was heated at 60 °C for 10 min and inoculated in a pre-reduced chopped-meat medium (CMM) and incubated for 7 d at 35 °C. A similar procedure was followed with another unheated aliquot.Two and seven days after incubation, samples of each tube were inoculated by a streak (3-4 cm long) near the periphery of a blood agar plate and incubated under anaerobic atmosphere (GasPak

system, BBL Microbiology System, Cockeysville Md, USA) at 35 °C for 24 h.

Swarming bacteria produced a thin spreading film and a sample taken from the border of that film was inoculated onto blood agar with 4% agar (that we called "hard agar") to inhibit the swarming phenomenom. The isolated strains were biochemically and cromatographically identified and possible *C. tetani* or *C. botulinum* strains were inoculated in mice. As a further corroboration those strains that killed mice were also neutralized with specific antibodies against tetanospasmin and botulinic toxins and inoculated again in mice, as described Holdeman *et al.* (1977).

Sixty one strains, distributed among 12 species, were isolated. The most common species were *C. sporogenes* and *C. tetani.* The former was isolated from 16 samples and the later from 13 (43.3%, five of them were not toxigenic), followed by *C. oceanicum* 7 samples, *C. putrificum* 5, and *C. sordellii* 4. Each of the following species were isolated from two: *C. glycolicum, C. fallax, C. beijerinckii, C. bifermentans, C. hastiforme* or *C. subterminale*, and one sample with *C. durum*, and another with *C. sardiniense.*

TABLE 1

Isolated strains of clostridia and effect of the incubation time and heat treatment of the soil samples (%)

Clostricia species	2 days		7 days		
	Heated	Unheated	Heated	Unheated	Total
1. *C. bifermentans*	0	0	1	0	1
2. *C. beijerinckii*	1	1	0	1	3
3. *C. durum*	0	0	0	1	1
4. *C.fallax*	0	0	1	1	2
5. *C. glycolicum*	0	0	1	1	2
6. *C. hastiforme*	0	0	1	1	2
7. *C. oceanicum*	1	3	2	2	8
8. *C. putrificum*	3	1	0	1	5
9. *C. sardiniense*	0	1	0	0	1
10. *C. sordellii*	1	2	1	0	4
11. *C. sporogenes*	3	5	3	5	16
12. *C. tetani*	6	5	2'	2	15
Total of strains	15 (25)	18 (30)	12 (20)	15 (25)	60

a. Both strains were isolated at two days too.

The method is simple and allows isolation of swarming clostridia, such as the species reported herein. The procedure apparently favoured the isolation of *C. tetani*: because in a previous paper, using the standard method, its frequency of isolation for the Central Valley of Costa Rica was 7% (Rodríguez *et al.* 1993), much lower than the 43% reported here.At least for the isolation of *C. tetani* and other swarmer clostridia from soil the sample should be divided in two aliquots, one of them for being heated before inoculation. Then, both would be inoculated and analyzed both at 48 hrs of incubation. The reason is that heat treatment favoured sporulation, as was our experience with 8 of our *C. tetani* strains, were isolated exclusively from heated samples. Nevertheless, some strains are heat sensitive (Cato *et al.* 1986), in our data 5 of them grew only from the unheated replicas of the samples. The most adequate incubation time was two days, because 11 of the strains was recovery at that time.

Bergey's Manual of Systematic Bacteriology describes swarming phenomenon for *C. tetani, C. botulinum G, C. novyi, C. septicum,* and *C. sporogenes* (Cato *et al.* 1986). Our report adds ten species.

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