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Short Communication

Discrimination of *Bacillus sphaericus* strains by filtrate protein profiles

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A total of 19 strains of *Bacillus sphaericus* are compared both in vegetative and sporulated stages according to their filtrate protein profiles obtained by Native-PAGE and SDS-PAGE. When the strains are compared in the sporulated stage, filtrate protein profiles obtained by Native-PAGE differentiated the strains according to their phage and serogroups. On the other hand, the typing according to filtrate protein profiles is correlated with serotyping and phage typing. The discrimination of *B. sphaericus* strains by Native-PAGE is more useful.

Key words: *Bacillus sphaericus*, Microbial control of mosquitoes, classification, electrophoresis.

INTRODUCTION

Bacillus sphaericus is a species which includes isolates pathogenic to the larvae of a number of mosquito species (Yousten, 1984a; Lacey and Undeen, 1986). It has been recognized as a saprophytic microorganism which does not use glucose as carbon source for growth (Russell et al., 1989). The first *B. sphaericus* strain toxic to mosquito larvae was reported in 1965 (Kellen et al., 1965). Until now, a number of isolates were discovered. As the strains were isolated, it became apparent that there was a need to differentiate among the strains. Consequently, different methods were developed for classifying the strains and proving their identification. The first classification studies were mostly based on morphological and biochemical characteristics. Since the phenotypic properties of strains are insufficient to separate different strains, new methods were required to show strains′ differences. A big step was taken in this direction when Yousten (Yousten et al., 1980; Yousten, 1984a,b) developed a combination of bacteriophage for this purpose and de Barjac et al. (1980), used H-antigens to serotype the strains. The strains have been differentiated by a variety of techniques including DNA homology (Krych et al., 1980), cellular fatty acid analysis (Frachon et al., 1991), and bacteriocin activity (Çökmüş and Yousten, 1993). Statistical methods were also used for the classification of bacteria (Mercan et al., 2003; Alexander and Priest, 1990). Generally, the protein gel electrophoresis in microbial systematics has been used mainly as a sensitive technique for the seperation and comparison of cellular proteins of strains belonging to the same species or subspecies for several years (Kampfer, 1995).

In this study, a total of 19 strains of *B. sphaericus* (13 of which are mosquito pathogens) in vegetative and sporulated cultures were discriminated according to their filtrate protein profiles obtained by SDS-PAGE and Native-PAGE.

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MATERIALS AND METHODS

B. sphaericus strains used in this study were obtained from the Department of Biology, Ankara, University, Turkey. Bacteria were incubated at 30ºC in a shaker water bath in NY broth (Nutrient broth) and NYSM broth (NY broth supplemented with $7x10^{-4}$ M $CaCl_2$, $1x10^{-3}$ M $MgCl_2$, $5x10^{-5}$ M $MnCl_2$) (Myers and Yousten, 1978). Strains were left 2-3 days in NYSM broth until they are fully sporulated. Later, vegetative and sporulated cells were removed from NY and NYSM broth by centrifugation for 3 min at 15 000 rpm. The supernatants were filtered by millipore filter with a pore size of 0.45 µm and stored at -50ºC. Afterwards, the samples were freeze-dried, and homogenized in sterile distilled water in volume 15 times less than their initial volume.

The spot test was used for determination semiquantitative of proteins (Esen, 1978). Gel electrophoresis was performed according to Laemmli (1970). The proteins were electrophoresed on 10% Native-PAGE and 10% SDS-PAGE and gels were stained with Coomassie blue. Afterwards, the gels were destained until protein bands become clearly visible. The tests were repeated three times.

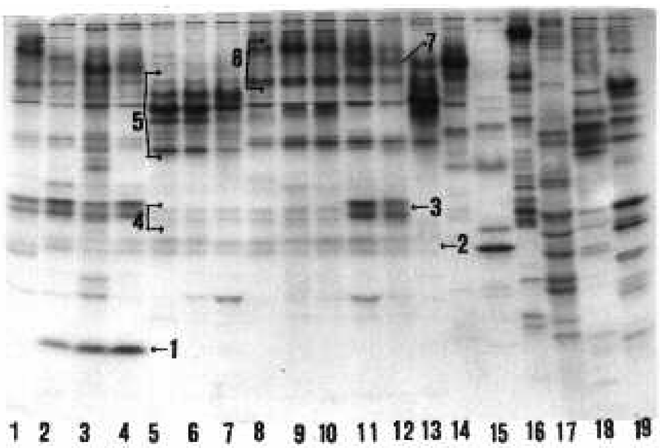

Figure 1. Native-PAGE profiles of sporulated cell proteins. Lanes 1: Kellen Q, 2: SSII-1, 3: 1883, 4: 1404, 5: 2362, 6: 1593, 7: 1881, 8: IAB 59, 9: IAB 460, 10: IAB 467, 11: 31-2, 12: 34-2, 13: 2297, 14: ATCC 7055, 15: ATCC 14577, 16: NRS 400, 17: NRS 592, 18: NRS 1198, 19: NCTC 9602.

RESULTS

Figure 1 presents sporulated cell filtrate protein profiles obtained by Native-PAGE for pathogenic and nonpathogenic *B. sphaericus* strains. The pathogenic strains belonging to the species show the similarity in their protein patterns (strains: Kellen Q, SSII-1, 1883, 1404, 2362, 1593, 1881, IAB 59, IAB 460, IAB 467, 31-2, 34-2, 2297). Although both mosquito pathogenic and nonpathogenic strains have some common protein bands, the nonpathogenic strains do not show the same profile among themselves. It is observed that the pathogenic strains are grouped by some protein bands which are specified by numbers. The protein band marked by 1 is common in SSII-1, 1883, and 1404 strains which are pathogenic to mosquito larvae, but this band do not exist in other pathogenic strains.

A pair of bands marked by "2" are common in all pathogenic strains can be used to distinguish pathogenic from nonpathogenic strains. A pair of bands marked by "3" are common in 6 of the 13 pathogenic strains. Three of protein bands marked by 4 are shown in strains which have high virulence. Three strains, 2362, 1593, and 1881, which are present in same serogroup are discriminated from IAB strains by seven of protein bands marked with 5. However, a pair of bands marked by "6" are common in IAB strains. Although these protein bands are present in 31-2 and 34-2 strains, a single protein band marked by 7 distinguishes these strains from IAB strains.

Although *B. sphaericus* strains which are cultivated in NY broth have some common bands in Native-PAGE with respect to protein profiles in vegetative stage, the bands are not discriminatory. The filtrate protein profiles of *B. sphaericus* strains both vegetative and sporulated cultures were also compared by SDS-PAGE, but no satisfactory result was obtained (Not shown).

DISCUSSION

As seen in Figure 1, pathogenic strains belonging to the species show similarity in their protein patterns. The low toxicity strains of serotype 2a2b (SSII-1, 1883, 1404) possess a single protein band (marked by 1) which is not present in serotypes 5a5b (2362, 1593, 1881) or 25 (2297) (de Barjac and Sutherland, 1990). A pair of bands (marked by 2) are common in all pathogenic strains and pathogenic strains are distinguished by these protein bands from nonpathogenic strains. A pair of bands (marked by 3) are common in serotypes 1a (Kellen Q), 2a2b (SSII-1, 1883, 1404) and 9a9c (31-2, 34-2) and these strains which have weak or intermediate virulance and present in different serogroups (de Barjac et al., 1980) are discriminated by these protein bands from the other pathogenic strains. Three of protein bands marked by 4 are only shown in strains which have high virulance (2362, 1593, 1881, IAB 59, IAB 460, IAB 467). Strains of serotypes 5a5b, 6, and 25 that produce binary toxin have readily been distinguished microscopially by the presence of a parasporal inclusion body in sporulated cells (Çökmüş and Yousten, 1994). Serotype 5a5b (2362, 1593, 1881) is discriminated by the presence of seven of protein bands marked by 5 from serotypes 6 (IAB 59, IAB 460, IAB 467) and 25 (2297). However, a pair of bands marked by 6 are common in IAB strains (serotype 6) and these strains are distinguished by these protein bands from serotype 5a5b (2362, 1593, 1881). Although these protein bands (marked by 6) are present in 31-2 and 34-2 strains (serotype 9a9c) that are weak pathogenic and present in different serogroup and phage group (Çökmüş and Yousten, 1991), 31-2 and 34-2 have been distinguished from serotype 6 by a single protein band marked by 7. It is possible to distinguish among these serotypes by examination of sporulated cell protein profiles.

As a result of Native-PAGE profiles of vegetative cell proteins, it is seen that there are no any distinguishing groups among strains. Protein profiles obtained by SDS-PAGE are used for characterization and discrimination of various microorganism species (Bruce and Jordens, 1991; Qhobela et al., 1991). We have used SDS-PAGE to compare vegetative and sporulated cultures protein profiles of 13 mosquito pathogenic and 6 nonpathogenic *B. spaericus* strains, but no satisfactory result has been obtained.

In summary, filtrate protein profiles obtained by Native-PAGE differentiated the strains even in each phage and serogroups when the strains are compared in fully sporulated cultures. Generally, it is seen that the typing according to filtrate protein profiles is correlated with serotyping. Vegetative cultures are less useful for distinguishing strains in this study. Furthermore, for classifying *B. sphaericus* strains, it is concluded that filtrate proteins in sporulated cultures are more used effeciently when compared to vegetative cultures. In addition, careful analysis of filtrate protein profiles can differentiate new mosquito pathogenic *B. sphaericus* isolates from nonpathogenic.

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