IMPROVEMENT OF STRAINS OF *Bacillus Sphaericus* 1593

FOR MOSQUITOES CONTROL

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SUMMARY

The improvement of the strain *B. sphaericus* 1593 (Cm\(^{7R}\)) was investigated by two techniques, protoplast fusion and conjugation-like process. At first, a mutant which was resistant to chloramphenicol was selected from the standard strain of *B. sphaericus* 1593 and designated as *B. sphaericus* 1593 (Cm\(^{7R}\)).

In the protoplast fusion experiment between *B. sphaericus* 1593 (Cm\(^{7R}\)) and *B. thuringiensis* var. *israelensis* A084-16, the protoplast was prepared under the optimal conditions for each strain in order to obtain 95-100% product. These protoplasts could be regenerated on appropriate regenerating medium with the frequency of 4 to 17%. Several conditions for fusion were investigated. It was found that 40% PEG 6000 in 10 mM CaCl\(_2\) was the most appropriate conditions. However, the regeneration frequency of the fusant was still low (1.47x10\(^{-4}\)).

In the case of conjugation-like process, the plasmid pBC 16 from *B. thuringiensis* var. *israelensis* A084-16 could be transferred to *B. sphaericus* 1593 (Cm\(^{7R}\)) with high frequency (5.42x10\(^{-2}\)). Conjugants # I, II and III showed good larvicidal activity but the conjugant # IV did not have larvicidal activity in which the plasmid that was responsible for producing parasporal crystal was lost.

The transipients, fusant and conjugants, showed distinct properties in morphological, biochemical and larvicidal activity. The fusant expressed both properties of the parents, *B. thuringiensis* var. *israelensis* A084-16 and *B. sphaericus* 1593 (Cm\(^{7R}\)), in biochemical tests. Crystal produced by fusant was quite large when observed under the phase contrast microscopy and could kill all groups of mosquito larvae (*Aedes, Culex* and *Anopheles*). On the other hand, the conjugants demonstrated the same characters as those of *B. sphaericus* 1593 (Cm\(^{7R}\)) in both morphological and biochemical properties. However, all of them could kill *Aedes, Culex* and *Anopheles* larvae except the conjugant # IV.

It was interesting to note that plasmid pBC 16 was able to replicate in *B. thuringiensis* var. *israelensis* A084-16 and *B. sphaericus* 1593 (Cm\(^{7R}\)). This finding suggest the possibility of using pBC 16 as vector for cloning mosquito toxin gene into *B. sphaericus*.