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Screening and isolation of Bacillus thuringiensis harboring new *cry2* genes toxic against *Ephestia kuehniella* (Lepidopteran:*Pyralidae*)

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In order to identify new toxic strain(s) against lepidopteran, the study of 29 crystal-producing B. thuringiensis isolates, present in the laboratory collection, was performed. The comparison of their plasmid patterns narrowed down the collection to 15 representative strains of the different classes. These later were subjected to morphological and molecular studies. The microscopic observation of the crystal produced by each strain showed that 13 of 15 contain one shape while the other two contain two shapes simultaneously. The PCR amplification using specific primers of the *cry2* gene allowed to select 6 strains (BUPM109, BLB30, BLB140, BLB200, BLB240 and MEB4) harboring a *cry2* gene of the 15 studied isolates. Further molecular study submitted to these strains using bioinformatics methods, PCR with additional specific primers and PCR-RFLP showed that they may have multiple subclasses of cry2 gene arranged in operon for some (BLB240 and MEB4) and not for the others. BLB140 was found to harbor a cryptic *cry2*Ab gene. The SDS PAGE profile indicated that all these isolates produces a 70-65 kDa protein, which corresponds to the typical weight of *Cry2*A protein, in addition to a 130 kDa protein corresponding to Cry1A protein, found in some (BLB140, BLB240 and MEB4). The Western Blot performed using a polyclonal antibody anti-Cry2Aa showed that only MEB4 and BLB240 have the Cry2Aa protein family, while for the others, no signal was found. Unlike BNS3 and BLB240, MEB4 didn't display a signal of 49 kDa. This band was found to be the result of an extra proteolytic cleavage by E.kuehniellamidgut juice and to be specific to *Cry2*Aa and neither to Cry1Ac nor Cry1Aa, resulting most probably the resistance of this larvae to *Cry2*Aa of BNS3. Therefore, the toxicity of the six selected strains was tested against E.kuehniella using BNS3 as reference. It was found that MEB4 presented the high toxicity suggesting that the absence of the extra cleavage may have a positive effect on the global toxicity.

Biography

Souad Rouis is an Associate Professor at Centre of biotechnology of Sfax, Tunisia. She completed her PhD at Center of Biotechnology, Sfax (sep. 2001) in laboratory of human molecular genetics Pr. H Ayadi. She is the entrepreneur and co-founder of Biotech Startup (BiotechRDP-2008). She has more than 10 research publications.

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