

ABSTRACT

The microbial pesticide based on *Bacillus thuringiensis* (Bt) has been successfully used for the past 60 years worldwide to control agricultural pests and vectors of human diseases. The *cry* genes have been successfully incorporated into crop plants to develop inbuilt resistance against the pests. In recent times there has been a surge to isolate and utilize *B. thuringiensis* as a biopesticide against target insects and to develop transgenic crops. Rice is a staple food for more than 90 % of the Asian population. In Malaysia the major rice cultivating region is in the state of Kedah Darul Aman. The average yield of paddy was recorded around 3.5 - 4 metric ton ha⁻¹ from 2005 to 2010. However, the potential yield of paddy in Malaysia is around 10 tons ha⁻¹. The present investigation was aimed to isolate native strains of *B. thuringiensis* from the paddy soil ecosystem. Initially 200 soil samples were collected from different parts of Kedah Darul Aman, Kelantan Darul Naim and Perak Darul Ridzuan. The preliminary screening based on the colony morphology, vegetative cell morphology, sporulation and Coomassie Brilliant Blue (CBB) staining has resulted in identifying 93 *Bacillus thuringiensis* isolates. The isolates were also characterized by biochemical tests that are specific to *Bacillus thuringiensis* such as nitrate reduction test, Voges Proskauer test, methyl red test and starch hydrolysis test. In addition hemolytic, motility and antibiotic sensitivity test were also performed. The SDS- PAGE analysis was carried out for all the 93 isolates in order to study the variation in the protein profiling. This study also provides an insight in identifying the Bt siblings. A representative Bt isolate (Semeling 2/2) was used for growth curve analysis and it was shown that the isolate sporulated at 90th hour of incubation. The molecular characterization including RAPD, pathotype PCR and 16S rRNA were also conducted. The RAPD analysis was carried out on the selected 66 *B.*

thuringiensis isolates based on their SDS- PAGE results. In the RAPD- PCR analysis of the isolates, 3 different primers were used. It was found that primer 4 was considered the best in generating multiple bands and amplifying 58 out of 66 isolates. The dendrogram for all the three RAPD- PCR analysis was generated and the results were analyzed. This RAPD results indicated that all the 3 primers showed amplification and indicated good diversity of *Bacillus thuringiensis* isolates in Kedah Darul Aman. The RAPD pattern also showed more than 2 bands for each isolate, with molecular weight in the range of 0.3–1.5 Kb per primer. Based on the RAPD PCR results, the selected isolates (n=46) were subjected to pathotype PCR analysis, to predict the insecticidal potential of the isolates. The results indicated that all the 46 isolates may have anti-lepidopteran activity. The 16S rRNA studies were conducted to evaluate the genetic variation in the selected isolates (n=8). The results indicated the variation in the Bt isolates. This variation can be attributed to minor difference in the sequence or it may be a different subspecies. The crystal morphology of the selected isolates was delineated by scanning electron microscopy. The results indicated most of the isolates were having spherical crystal, an indication of dual insecticidal activity against lepidopteran and dipteran. Thus the detailed phenotypic and genotypic characterization of the Bt isolates distributed in the paddy ecosystem may be a good platform to further the studies in identifying *cry* genes specific to different paddy pests. In addition this study provides a basic idea in developing transgenic rice crops based on the distribution of *Bacillus thuringiensis* in paddy ecosystem.