Abstract

Cypermethrin (CYP) and imidacloprid (IMI) are toxic insecticides that are widely used in agriculture. Still, the most serious problem is their persistence in soil, which poses a significant threat to plants and human's health. Biofilm-mediated bioremediation of pesticides is emerging as an environmentally friendly method. In total, four biofilm-forming strains were isolated using Congo red agar from four soil samples and purified. Morphological and biochemical characterization showed that out of four biofilm forming strains, three (2A, 5A, and 6B) belong to *Bacillus* sp. and one (4A) to *Enterobacter* sp. Based on the 16S rRNA gene sequencing, strains 2A, 4A, 5A, and 6B showed maximum sequence similarity with *Bacillus thuringiensis*, *Enterobacter hormaechei*, *Bacillus* sp., and *Bacillus cereus* respectively. Biofilm time kinetics study revealed that these strains showed mature biofilm formation on the 5th day of incubation at 37 °C using the crystal violet test tube method. Mature biofilm of strains individually and in combination was subjected to the degradation of CYP and IMI. The degradation of CYP and IMI was analyzed by UV visible spectrophotometer and Fourier transform infrared spectroscopy (FTIR). UV-vis analysis showed the optical density (OD) of CYP at 224nm and at 258nm for IMI. The highest degradation (46.2 ± 0.3 %) of CYP (100µl) was shown by the biofilm of G7, which was the consortia of *B. thuringiensis* + *Bacillus* sp. IMI (100µl) was highly degraded (70.0 ± 0.5 %) by the biofilm of G11, which was the consortia of biofilm of all strains. One-way ANOVA followed by post hoc Tuckey test revealed that these results were statistically significant ($P \leq 0.05$). Each strain showed significantly different ability of pesticides degradation efficiency. FTIR analysis further confirmed the degradation of CYP and IMI. The spectrum obtained after treating CYP with a biofilm of *B. thuringiensis* showed that the major peak at 1373cm^{-1} linked with ether-cyanate in control was not detected after treatment. FTIR spectra of CYP treated with a biofilm of *E. hormaechei* suggested the formation of many new peaks (*e.g.*, 966, 1199, 1458, and 2362cm^{-1}) and peaks at 3741cm^{-1} belonging to –C=C– was not detected after biodegradation. Shifting of many peaks, *e.g.*, 1128cm^{-1} in control shifted to 1188cm-$^{1.}$ and the formation of new peaks, *e.g.*, 1323 and 1830cm$^{-1.}$ confirmed the degradation of CYP by *Bacillus* sp. FTIR spectra of CYP treated with *B. cereus* showed peaks

were shifted from 945 to 974cm^{-1} and 1128 to 1166cm^{-1}. FTIR spectra of combination of *B. thuringiensis* + *Bacillus* sp. showed an increase in carbonyl signals at 1645cm^{-1} and in the combination of all strains, a new peak was formed at 1002cm^{-1} which corresponded to C-Cl bonding that clearly indicated the degradation of CYP. FTIR analysis of IMI degraded with biofilm of all strains individually and in combination {(*B. thuringiensis* + *Bacillus* sp. (G7) and *B. thuringiensis* + *E. hormaechei* + *Bacillus* sp. + *B. cereus* (G11)} showed that the peak at 3352cm^{-1} corresponded to O-H stretching vibration, and the peak at 1658cm^{-1} depicted the N=O stretching of nitrosoguanidine residue in control was shifted which indicated the degradation of IMI. The peak at 1517cm^{-1} in the spectrum associated with the vibration band of N=N in the imidazolidine was also found to be disappeared after treatment, showing the degradation of IMI. As a result of this study, it was concluded that biofilm of the *B. thuringiensis*, *E. hormaechei*, *Bacillus* sp. and *B. cereus* could be suitable agents for the bioremediation of pesticides. These biofilm forming strains had the potential to clean up CYP and IMI contaminated agricultural soil. These strains should be further explored to determine their biodegradation ability against other harmful pollutants. This study expressing an ecofriendly approach for the bioremediation of harmful contaminants from environment like pesticides.