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Optimizing Environmental Conditions for Amoxicillin-Resistant Bacterial Strains in the Degradation of Amoxicillin and Exploring the Degradation Mechanism

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Abstract

Amoxicillin is a type of penicillin antibiotic, which is commonly prescribed as a medication for bacterial infections, such as pneumonia, bronchitis, ear infections, and urinary tract infections. However, its excessive usages have resulted in unfavourable environmental consequences. The present study aims on analysing the effect of Nitrates and Phosphates on Amoxicillin degradation by *Micrococcus luteus, Bacillus cereus* (DJ080579.1)*, Bacillus subtilis, Lactobacillus bulgaricus, Lactobacillus* sp. (DI4388712.1)*, Enterobacter aerogenea, B. cereus* (EU678635), *Lactobacillus* sp. (HW413258.1) which were previously isolated as potential amoxicillin degraders and identify their degradation mechanisms. In the first study, the previously starved bacteria were exposed to three different concentration of Nitrate ($NaNO_3$) and Phosphate (KH_2PO_4) based on the environmental detection level (0.05 mg/L, 0.5 mg/L, 0.005 mg/L), while control samples were established, excluding bacterial suspension. The absorbance of samples was monitored at 595 nm over 14 days using ELISA. Based on ELISA assay absorbance values, the bacteria showed maximum Amoxicillin degradation potential at 0.05 mg/L and 0.5 mg/L nitrate concentrations. *B. cereus* (EU678635) showed a significant degradation potential at 0.05 mg/L nitrate while *L. bulgaricus* showed a highest degradation potential at 0.5 mg/L. When bacteria were enriched with phosphate, the optimal growth potential of the selected bacterial strains was found to occur in a phosphate concentrations range between 0.005 mg/L and 0.05 mg/L. *B. cereus* (EU678635) represented a noticeable ability for amoxicillin degradation at 0.005 mg/L and 0.05 mg/L phosphate concentrations. Significant degradation of amoxicillin was not detected in the controls. Results of this study revealed that the bacterial remediation of amoxicillin is controlled by nutritional status with special emphasis of nitrate enrichment in the environment. To identify the degradation mechanisms, eight selected bacteria which were activated and starved in 0.9% saline solution were separately exposed into the equal concentration of amoxicillin for 3 days. After incubation period, the filtrate and filter paper (Cell Extract) were separated through filtration and then analysed by HPLC. Considering the results of degradation mechanism, six out of eight bacterial strains, named *Micrococcus luteus, B. cereus* (DJ080579.1), *B. subtilis, L. bulgaricus, Lactobacillus* sp. (DI4388712.1)*, and E. aerogenea* were investigated possess an intracellular enzymatic mechanism for amoxicillin degradation. Conversely, two of the bacterial strains, namely *Bacillus cereus* (EU678635) *and Lactobacillus* sp. (HW413258.1), exhibit an extracellular enzymatic mechanism. This study provides a basic understanding of the practical application of bacteria on amoxicillin degradation and their responsiveness to variations in nutrient availability within the environment.

Keywords: Amoxicillin, Antibiotics, Amoxicillin-resistant bacteria, Extracellular enzymatic mechanism, Intracellular enzymatic mechanism