TITLE: ATRAZINE BIODEGRADATION BY TWO BACTERIA ISOLATED FROM BRAZILIAN SOIL

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ABSTRACT

Atrazine is an herbicide used worldwide to control weeds in maize, soybean, sorghum and sugarcane crops. It is toxic, potentially carcinogenic and a hormonal disrupter. Moreover, it is classified as an environmental persistent chemical. The purpose of this work was to investigate the atrazine degradation ability of two bacteria isolated from different soil samples in São Paulo, Brazil. HPLC/UV and HPLC-MS/MS analysis were performed in order to determine degradation rate and identify metabolites resulting of atrazine biodegradation. SDS/PAGE electrophoresis was carried out to detect enzymes encoded by *atz* genes and responsible for atrazine cleavage. Northern Blot was performed to verify differences in *atz* gene expression before and after atrazine stimulation for 48 hours. Bacteria were identified as *Pseudomonas* sp. and *Achromobacter* sp. and showed potential to degrade atrazine in solid medium ATZ-R containing atrazine as a nitrogen source. HPLC/UV and HPLC-MS/MS analysis demonstrated that *Pseudomonas* sp. was capable to completely degrade atrazine in vitro after 48 hours of incubation. *Achromobacter* sp. showed a slower degradation profile, with the beginning of the degradation process after 24 hours. The three initial metabolites formed by atrazine degradation were detected in samples containing both *Pseudomonas* sp. as well as *Achromobacter* sp. SDS/PAGE electrophoresis analysis suggest the presence of atrazine chlorohydrolase, hydroxyatrazine chlorohydrolase and N-isopropylamelide isopropylamino hydrolase. Additional tests of two-dimensional electrophoresis are required to detect the enzymes responsible for the atrazine hydrolysis and its metabolites. *Pseudomonas* sp. ADP and *Pseudomonas* sp. showed significant differences in *atz*ABCD gene expression after 48 hours of atrazine stimulation by Northern Blot analysis. Therefore, this work reports two novel isolates with potential to degrade atrazine. The isolates may be used in the future as tools for bioremediation of atrazine-contaminated sites, as pure colonies or in bacterial consortia associated with other isolates.

Keywords: atrazine, biodegradation, bacterial consortium, *atz* genes

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