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Note

Isolation and Characterization of a Stilbene-degrading Strain of *Pseudomonas fluorescens*, and Production of Antioxidant Compounds by Stilbene Metabolism

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In this study, we consider the use of hydrocarbon-degrading bacteria that degrade *trans*-stilbene as a novel approach for synthesizing potentially bioactive hydroxylated stilbenes. A *trans*-stilbene-degrading bacterium, MN2, was isolated from activated sludge through enrichment culture, and identified as *Pseudomonas fluorescens* using conventional techniques. Degradation of *trans*-stilbene by this strain yielded two metabolites that had significant antioxidant activity.

Key words: antioxidant; *Pseudomonas fluorescens*; stilbene

Hydroxylated stilbenes (1,1’-[1,2-ethenediyl]bis[benzenes]) are naturally occurring plant metabolites that are produced by a wide range of spermatophytes in response to injury or infection, and are classified as phytoalexins.1 Some of these compounds, including resveratrol (3,4’-dihydroxy-*trans*-stilbene) and piceatannol (3,3’-dihydroxy-*trans*-stilbene), have been shown to have pharmaceutically useful properties such as anticancer,2 antioxidant,3 cardioprotective,4 and vasodilatory5 effects. Since stilbene has previously been shown to be degraded by naturally-occurring isolates of *Pseudomonas* spp.,6 and bacterial stilbene degradation is presumably mediated through an oxygenase-mediated hydroxylation of the ethylene or aromatic ring(s), we hypothesized that bacterial fermentations involving stilbene metabolism could represent a novel route for the large-scale production of hydroxylated stilbene compounds. In this context, we demonstrate the feasibility of using stilbene-degrading bacteria for synthesis of bioactive stilbene metabolites.

For this study, an enrichment culture technique7 was used to isolate bacteria capable of degrading stilbene from activated sludge. One isolate, designated MN2, grew on stilbene as a sole carbon source and was characterized further. Interestingly, MN2 was found to be incapable of growth on *cis*-stilbene, biphenyl, bibenzyl, benzene, toluene, ethylbenzene, *p*-xylene, *o*-xylene, *m*-xylene, naphthalene, or phenanthrene, suggesting that the pathway for the metabolism of *trans*-stilbene in this organism was relatively specific. MN2 produced a fluorescent pigment on TNA plates and was found to be a motile, obligately aerobic Gram-rod. It grew in TNB at 20–42°C but not 4°C, and at pH 5–7 but not at pH 3 or pH 9. Using methods described by Benson,8 together with a BBL CRYSTAL Enteric/Nonfermenter ID Kit (Becton Dickinson, Sparks, Md., U.S.A.), the organism was identified as *Pseudomonas fluorescens* biovar III.

Potentially bioactive stilbene metabolites were purified from *trans*-stilbene-grown cultures of MN2, using the following methods. A 1-liter starter culture, which was grown for 3 days at 30°C and 200 rpm, was used to inoculate 8 liters of a basal medium (BM9) amended with 10 mM stilbene in a 10-liter Bioflo 3000 Bioreactor (New Brunswick Scientific, Edison, NJ). The bioreactor culture was incubated at 30°C for approximately 24 hours, with an impeller speed of 500 rpm and an oxygen concentration of 28% saturation. The culture was centrifuged at 10,000 rpm and 4°C for 10 minutes, and the supernatant was extracted with dichloromethane. Analytical thin-layer chromatographic analyses of dichloromethane extracts found untransformed *trans*-stilbene and six polar products, none of which were observed in extracts of citrate-grown cultures of the same strain. The metabolites were purified from the extract using preparatory thin-layer chromatography (TLC), and the purity of the separated compounds tested using analytical TLC.

None of the pure compounds were found to have cytotoxic or antimicrobial activity by appropriate methods,10,11 but Products 1 and 2, the most nonpolar of the metabolites, had significant antioxidant activity in a DPPH screen of a TLC plate12 (Fig. 1). On analytical TLC plates, Product 1 had an intense blue fluorescence characteristic of hydroxylated stilbenes.13 Further characterization of the structure of
Product 1 by $^1$H-NMR and $^{13}$C-NMR spectroscopy was incomplete (due to cross contamination by other metabolites), but these analyses clearly indicated that one of the ethylene carbons and at least one of the aromatic carbons were hydroxylated.

In summary, an enrichment culture technique was used to isolate a strain of *Pseudomonas fluorescens*, MN2, which could use trans-stilbene as a sole source of carbon and energy. This strain could not use other structurally related aromatic compounds, suggesting that the metabolic pathway for stilbene degradation was relatively specific, and that the organism may have encountered stilbene derivatives as pollutants.14) Metabolism of trans-stilbene by MN2 yielded six metabolites, two of which had significant antioxidant activity. The most non-polar of the metabolites was a hydroxylated stilbene, and likely an early intermediate in a metabolic pathway initiated by a oxygenase. Based on our findings, it can be concluded that stilbene-degrading bacteria can be used to metabolize stilbene for the production of bioactive compounds.

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**References**


