

Bioassay of secondary metabolites produced in an *Aspergillus terreus* semi-biosynthetic system

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In recent years, secondary metabolites produced by biosynthetic engineering are found to be greater potential and can replace synthetic chemicals. Filamentous fungi produce secondary metabolites with distinct structures and various biological activities for a wide range in biotechnology, food, and medical fields. A putative acetylaszonlenin-biosynthetic gene cluster was identified in the genome sequence of *Aspergillus terreus*. This cluster consists of three genes, *anaPS*, *anaPT*, and *anaAT*. We studied the semi-biosynthesis of acetylaszonalenin in *anaPS* deletion mutant in order to identify and/or produce more bioactive natural compounds from *A. terreus*. The precursor of acetylaszonalenin, benzodiazepinedione, catalyzed by *anaPS* in the biosynthetic pathway of acetylaszonalenin was chemically synthesized. *A. terreus anaPS* deletion strain was cultured with benzodiazepinedione. The semi-biosynthesis of acetylaszonalenin was studied in the feeding of precursor, benzodiazepinedione, and adaption of environmental factors such pH value, temperature, speed, etc. The results indicated that acetylaszonalenin was successfully obtained in *A. terreus anaPS* deletion strain after benzodiazepinedione-feeding. The dominant peak of interest, acetylaszonalenin, appeared in HPLC chromatogram at Tr 22.5 min. The increase of temperature could raise the yield of acetylaszonalenin. MTT assay was performed in various concentrations of crude extracts of benzodiazepinedione-treated *anaPS*-deletion *A. terreus* ferment. The results shown that there is around 60% cell viability after 24-hour treatment when colon cancer cells were tested with these crude extracts of mycelial liquid culture, but there is no cytotoxicity in HaCaT cells. In addition, crude extracts of *A. terreus* wild type, *anaPS* deletion strain, and benzodiazepinedione-feeding strain were shown the antioxidant capacity. The semi-biosynthetic system we successfully established should be useful for the application of elucidating the functions and the production of fungal secondary metabolites.

Key words: *Aspergillus terreus*; semi-biosynthesis; acetylaszonalenin; antioxidant; antitumor