Journal of Traditional Chinese Medical Sciences (2014) 1, 135–139

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Biotransformation of 6-deoxypseudoanisatin by *Synechocystis* sp. PCC6803

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Received 19 May 2014; accepted 3 September 2014

Available online 3 April 2015

KEYWORDS
Synechocystis sp. PCC6803;
6-deoxypseudoanisatin;
Seco-prezizaane-type sesquiterpene lactone;
Biotransformation;
Cyanobacterium

Abstract *Objective:* To explore the ability of *Synechocystis* sp. PCC6803 in transforming 6-deoxypseudoanisatin.
Methods: The experiment was performed by incubating 6-deoxypseudoanisatin with the freshwater cyanobacterium *Synechocystis* sp. PCC6803 under continuous white light at 30°C for 5 days. The crude converted product was detected using thin-layer chromatography (TLC) and further analyzed using high-performance liquid chromatography (HPLC) as well as HPLC with electron spray ionization mass spectrometry (HPLC-ESI-MS).
Results: TLC results showed that 6-deoxypseudoanisatin was converted into a less polar product. HPLC and MS data indicated that the retention time of the converted product increased in comparison with the standard of 6-deoxypseudoanisatin.
Conclusion: Thus, the study appears to demonstrate that *Synechocystis* sp. PCC6803 can transform 6-deoxypseudoanisatin. The polarity of the converted product is less than that of 6-deoxypseudoanisatin.

Introduction

The sesquiterpene lactone 6-deoxypseudoanisatin, the molecular formula of which is $C_{15}H_{22}O_5$ and the molecular weight of which is 282.33 (Fig. 1), belongs to the pseudoanisatin subtype of seco-prezizaane-type sesquiterpenes.[1–3]

Although reports on its biological activity are few, its characteristics such as its non-toxic property, sufficient content in plants, and that it is easily extracted from plants, makes it a desirable substrate to synthesize its structurally similar compounds with desirable pharmacologic activities. For example, Zhang et al.[4] attempted to

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Peer review under responsibility of Beijing University of Chinese Medicine.

http://dx.doi.org/10.1016/j.jtcms.2014.09.001

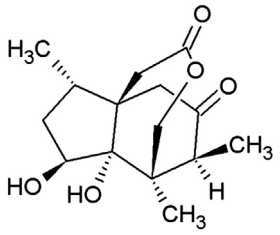

Figure 1 Structure of 6-deoxypseudoanisatin.

use 6-deoxypseudoanisatin instead of its structurally similar compound, pseudoanisatin, as a starting reactant for a series of chemical synthesis pathways to form merrilactone A, which has remarkably apparent neurotrophic activity. Though their efforts were unsuccessful, the potential of using 6-deoxypseudoanisatin to produce compounds via the effect of biologic reactors remains attractive. In this paper, we report on the use of a biological reactor to modify and transform 6-deoxypseudoanisatin to discover new compounds with inspiring bioactivities.

Although far less is known about its role as a biological reactor (bioreactor) than fungi, yeast, and other bacteria, cyanobacteria have been used as a bioreactor for biotransformation with some studies showing high transformation efficiency.[5,6] Cyanobacteria are microorganisms that use ubiquitous sunlight as their energy source, and some can even fix atmospheric nitrogen.[7] Thus, their large-scale culture can be simpler and cheaper than that of other microorganisms. These important advantages give cyanobacteria great potential to be biocatalysts. *Synechocystis* sp. PCC6803 is a well-studied cyanobacterium. In addition to the general advantages of cyanobacteria, when used as a biocatalyst the advantages of *Synechocystis* sp. PCC6803 include a clear genetic background, rapid growth rate, simple structure, and abundant enzyme systems.[8,9] Moreover, it can biotransform inorganic arsenic (As), that is, methylate arsenic to different As species.[10] However, its ability to transform organic compounds has not been evaluated. Thus, we used *Synechocystis* sp. PCC6803 to transform 6-deoxypseudoanisatin to confirm its ability as a biocatalyst and obtain compounds with inspiring bioactivity. We also performed thin-layer chromatography (TLC), high-performance liquid chromatography (HPLC), and high-performance liquid chromatography with electron spray ionization mass spectrometry (HPLC-ESI-MS) to analyze the converted product.

Materials and methods

The substrate 6-deoxypseudoanisatin was isolated from *Illicium macranthum* according to the previous protocol described by Ma et al.[11] *Synechocystis* sp. PCC6803 was kindly provided by Fang Huang (Institute of Botany, The Chinese Academy of Sciences, Beijing, China). The cyanobacterium was cultivated in aseptic BG-11 liquid medium[12] under 20 000 lx continuous white light at 30°C. The medium in the Erlenmeyer flask was incubated on a rotary shaker at 110 rpm. All chemicals were of analytic reagent grade.

Table 1 Gradient elution program for the converted product.

Time (min)	Water (%)	Acetonitrile (%)	Flow (mL/min)
0	98	2	1
5	85	15	1
5.5	85	15	0.5
24	84	16	0.5
25	80	20	1
60	30	70	0.5

Biotransformation

Synechocystis sp. PCC6803 was aseptically transferred to the BG-11 liquid medium for shake culture. When $OD_{750\ nm}$ was 0.4, 40 μl of a 6-deoxypseudoanisatin dimethyl sulfoxide solution (103 mg/mL) was mixed with 50 ml of medium to perform the biotransformation experiment. The entire transformation system was placed under 20 000 lx continuous white light at 30°C for 5 days on a rotary shaker at 110 rpm. Two control experiments were performed under identical culture conditions in the absence of either *Synechocystis* sp. PCC6803 or 6-deoxypseudoanisatin. The entire experiment was repeated 3 times to validate reproducibility.

Extraction and isolation of the converted product

After 5 days of incubation, the alga suspension was placed in an −80°C ultra-low temperature refrigerator to freeze for 4—12 h. The frozen mixture was then thawed in a 37°C water bath for 15—30 minutes. Repeated freezing and thawing were performed 4 times before the next process. The treated alga suspension was filtered through a 0.22-μm mixed cellulose ester microporous filtration membrane. The concentration of the filtrated aqueous solution was approximately 0.08 mg/mL (calculated based on the

Table 2 Mass spectroscopy parameters.

Range mode	Ultra scan
Ion polarity	Positive
Ion source type	ESI
Dry temp (Set)	350°C
Nebulizer (set)	40.00 psi
Dry gas (set)	10.00 L/min
Scan begin	100 m/z
Scan end	1000 m/z
Averages	5 spectra
Trap drive	41.4
Octopole RF amplitude	152.8 Vpp
Capillary exit	113.5 V
Skimmer	40.0 V
Oct 1 DC	12.00 V
Oct 2 DC	1.70 V
Max. accu time	200 000 μsec
Ion charge control target	200 000
Charge control	On