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Research Article

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Induced formation and characterization of a citreoisocoumarin derivative by a new-isolated *Eupenicillium* sp. in the presence of dimethyl sulfoxide or acetone

Na Guo[1,2*], Ya Liu[1,2], Xing You[1,2] and Peng Yu[1,2*]

[1]*Key Laboratory of Industrial Microbiology, Ministry of Education, College of Biotechnology, Tianjin University of Science and Technology, Tianjin, P. R. China*
[2]*Tianjin Key Laboratory of Industry Microbiology, College of Biotechnology, Tianjin University of Science and Technology, Tianjin, P. R. China*

ABSTRACT

Certain organic solvents in low concentrations have big impacts on metabolism of some microorganisms, including alterations in the production of secondary metabolites. Here we would like to report the formation, isolation, and characterization of a new secondary metabolite which was induced by dimethyl sulfoxide (DMSO) or acetone in the newly isolated Eupenicillium sp. fermentation system. The structure of this metabolite was confirmed to be the citreoisocoumarin derivative ***1*** *(6, 8-dihydroxy-3-(4-hydroxy-2-oxopentyl)-1H-isochromen-1-one) by NMR and HR-ESI-MS, which has been reported as a component of the inseparable fermentation mixture. Here is the first report of this compound as a pure isolated product and by simple solvent-induction. The highly selective formation of compound* ***1*** *from Eupenicillium sp. make* ***1*** *as the only isolated product. This strategy might provide a valuable but neat way for the discovery of novel natural products.*

Key words*:* acetone; citreoisocoumarin; dimethyl sulfoxide; *Eupenicillium* sp.; induction

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INTRODUCTION

Some natural products produced by microorganism are important bioactive molecules. Various secondary metabolites including antibiotics have been isolated from different microorganisms. Microbial secondary metabolism could be affected by environmental factors, such as stress, starvation or supplementation with nutrients. Meanwhile, mutations have been introduced to enhance the yield of a particular product, such as antibiotic. Interestingly, some of alterations in metabolism both quantitatively and qualitatively can also be made by the addition of organic compounds such as ethanol to growing cultures, although the biochemical basis of this effect is not completely known yet [1-2]. This solvent-induced alteration on metabolism encouraged us to look for more selective and efficient fermendation system induced by certain organic solvents, and explore a simple and convenient way to generate valuable and novel natural compounds [3-5]. In contrast, genetically engineered recombinant strains are more suitable for a specific known microbial reaction. Here we would like to report a significant metabolic variation induced by the addition of low concentrations (0.5‰-5%, v/v) of DMSO or acetone to growing cultures of *Eupenicillium* sp. In addition to alterations in morphology such as spore formation and pigment production [6-7], more importantly, a new metabolite was produced, isolated and characterized.

EXPERIMENTAL SECTION

Microorganism
Twenty-three fungi were used in the screening experiment, ten of them, including *Eupenicillium* sp., were stored in

our own lab, other thirteen strains of fungi were purchased from China General Microbiological Culture Collection Center, Beijing, China.

Culture medium

The compositions (w/v) of the seed culture medium were as follows: 2% glucose, 20% potato in deionized water. The compositions of the fermentation medium were the same as the seed culture except for the addition of DMSO at different concentrations.

Fermentation, extraction and purification

A standard two-stage fermentation protocol was adopted for this experiment. Every flask (100 ml of media per 250-ml flask) was treated by adding a 1-ml sample of 2-day-old seed culture. After 48 h, a certain amount of DMSO (typically 100 μl) was added to each flask. After 7 days of incubation, the cells were filtered, the filtrate was extracted with EtOAc, the extract were combined, dried and concentrated, affording a brownish residue. The crude residue was purified by a chromatography column (silica gel, 400 mesh), eluted with petroleum ether/ethyl acetate (8:1; 5:1; 3:1, v/v), afforded compound **1** as white powder.

Analytical methods

The effect of DMSO amount on the product were investigated with Agilent 1100 HPLC apparatus, which was equipped with a C_{18} reversed-phase column with water/ methanol (40%, v/v) as mobile phase at 1 ml/min. Absorption at 240 nm was monitored.

RESULTS AND DISCUSSION

Characterization of the product

The new product (**1**) induced by DMSO was displayed in Fig. 1. Compound **1** possessed the molecular formula $C_{14}H_{14}O_6$ as determined by HR-ESI-MS at *m/z* = 277.0787 $[M-H]^-$ (calcd. For $C_{14}H_{13}O_6$: 277.0712), A close inspection of NMR data, including ^1H, ^{13}C, DEPT, HSQC, HMBC NMR, confirmed compound **1** to be 6, 8-dihydroxy-3-(4-hydroxy-2-oxopentyl)-1H-isochromen-1-one (Fig. 2a). ^1H and ^{13}C NMR data of compound **1** were listed as below and the HMBC correlations were showed in Fig. 2b.

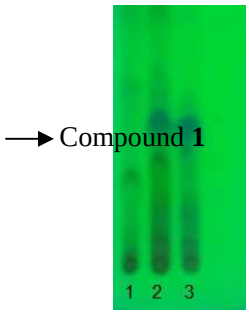

Fig. 1 Metabolic alteration of *Eupenicillium* sp. induced by DMSO (Lane 1. control sample; Lane 2. mixed sample; Lane 3. sample with DMSO added)

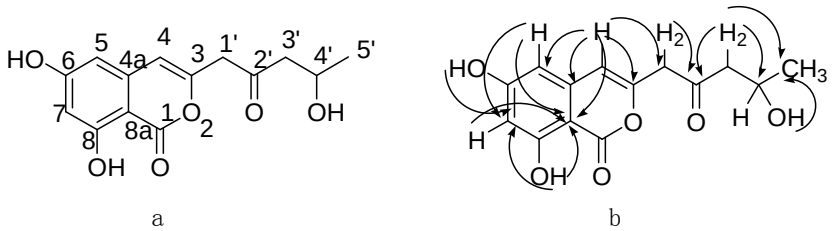

Fig. 2 Structure and HMBC correlations of compound 1

^1H NMR (DMSO-d_6, 400M): 1.09 (3H, d, *J* = 6.0 Hz, H-5'), 2.58 (2H, m, H-3'), 3.78 (2H, s, H-1'), 4.07 (1H, m, H-4'), 4.73 (1H, d, *J* = 5.2 Hz, OH-4'), 6.34 (1H, d, *J* = 2.0 Hz, H-7), 6.39 (1H, d, *J* = 2.0 Hz, H-5), 6.56 (1H, s, H-4), 10.89 (1H, s, OH-6), 10.92 (1H, s, OH-8).

^{13}C NMR (DMSO-d_6, 100M): 23.8 (s, C-5'), 46.9 (d, C-1'), 51.7 (d, C-3'), 62.9 (t, C-4'), 98.1 (q, C-8a), 101.9 (t, C-7), 103.0 (t, C-5), 107.0 (t, C-4), 139.3 (q, C-4a), 151.3 (q, C-3), 162.8 (q, C-1), 165.2 (q, C-6), 165.8 (q, C-8), 204.4 (q, C-2').

This compound has been reported once as a component of the inseparable mixture [8], but it has never been isolated before our work here. In that reported paper, it was found to be in a mixture together with its isomer (citreoisocoumarin) with exchanged 2'-keto and 4'-hydroxy groups, and those compounds were produced by the genetically engineered recombinant strain (*Aspergillus nidulans wA* gene). Therefore, in terms of isolation and production by solvent induction, our work is the first report.

Effect of DMSO concentrations on the formation of compound **1**

Fig. 3 showed that DMSO concentrations have significant influence on the formation of product. In general, low concentrations of DMSO could induce the formation of the secondary metabolite more productively. The best concentration of DMSO is 1‰ as shown in Fig 3.

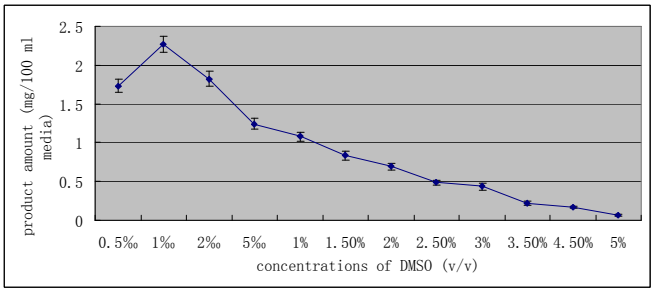

Fig. 3 Amount of compound 1 induced by different concentrations of DMSO

Product induced by acetone in the same microorganism system

In order to clarify whether other solvents exhibit similar induction as DMSO, several solvents, ethanol, acetone and N, N-Dimethyl formamide (DMF) were added in small amounts to fermentation culture of *Eupenicillium* sp. respectively. The results indicated that ethanol and DMF exhibited no induction on *Eupenicillium* sp. in our conditions, while acetone could induce *Eupenicillium* sp. to produce the same compound which was confirmed by NMR and LC-MS (data not shown).

CONCLUSION

In conclusion, DMSO and acetone could conveniently induce a new secondary metabolite in the *Eupenicillium* sp. culture system. The practical implication of this work is that the addition of small concentrations of an organic solvent (such as DMSO) elicits metabolic changes in the microbial cells, including formation of new secondary metabolites. This simple and convenient strategy might be valuable to expand the existing molecular diversity and increasing yields of minor metabolites from natural isolates or from genetically engineered recombinant strains.

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