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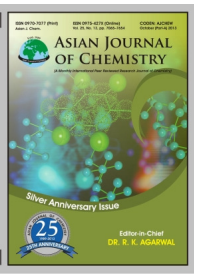

Chaetoglobosin A from Taxol-Producing Strain: Purification, Bioactivity and Structure Identification

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Fungi fermentation is a potential strategy for industrial production of taxol, an efficient anticancer medicine. A new taxol-producing strain has been recently isolated. A high-content, unknown secondary metabolite (herein referred to as "X") was observed from the fermentation broth. X was purified *via* organ extraction, high-performance liquid chromatography separation and crystallization. The purified compound X showed bio-toxic activity against *Drosophila*. Finally, X was identified as chaetoglobosin A through mass spectroscopy, ultraviolet spectroscopy, infrared spectroscopy and nuclear magnetic resonance with an overall consideration of the biological source.

Key Words: Chaetoglobosin A, Taxol, Fungi, *Drosophila* acute toxicity test.

INTRODUCTION

Taxol is a kind of diterpenoid compound with a highly efficiency in the treatment of intractable cancers[1]. Industrially, taxol is mainly purified from *Taxus chinensis* bark, which may destroys ecological environment and moreover, greatly impedes the mass production[2]. In 1993, a taxol-producing fungi strain was isolated[3]. Henceforth, an increasing number of taxol-producing strains were gradually discovered[4,5]. Fungi fermentation is suitable for massive production and has the potential applications in industry.

Recently, a new taxol-producing fungus was identified by Prof. X. Zhu (Nankai University) in China (unpublished data). The screening for other metabolites from this strain revealed a high-content unknown compound (herein referred to as "X"). Organisms can usually produce a wide range of secondary metabolites with distinctive bioactivities[6-8]; thus, in-depth research on X is required for the further utilization of this strain. In the current report, X was purified, tested for the bioactivity. Finally, X was identified as chaetoglobosin A.

EXPERIMENTAL

High-performance liquid chromatography conditions: C_{18} (5 μm) columns (Tianjin Chromatography Company, China) were used as followed, ϕ 4.6 mm × 250 mm in the analytical HPLC and ϕ 10 mm × 250 mm in the semi-preparative HPLC. The mobile phase was 70 % CH_3OH (v:v). The flow rates were 1.0 mL/min for analytical HPLC and 3.0 mL/min for semi-preparative HPLC.

Isolation and purification of X: The fermentation broth was concentrated into dried powder and soaked by different solvents in the same condition, respectively. The supernatants were subjected to HPLC analysis to measure the peak areas of X, on the purpose to select the most appropriate extracting solvent.

The semi-preparative HPLC was used to isolate X from the extract. The effluent products of X were manually collected followed with concentration by spinning at room temperature. After removal of excess solutions, purified X was precipitated and collected for downstream experiments.

***Drosophila* acute toxicity test:** 4% sucrose water was used to dissolve X with different concentrations as contamination solution. Three-day old *Drosophila* was divided into females and males and 12 groups with 25 individuals each further. After that, contamination solutions with 1 mL volumes and indicated concentrations were applied to each group before a starvation for 4 h. The mortality rate of *Drosophila* in each group was counted after 24 h and the changes in living individuals were also observed. Three groups of repetitive experiments were conducted.

Structure identification: Mass spectrum analyses with ion trap (Thermo LCQ Advantage) and Fourier Transform (Varian 7.0T) mass analyzers were performed. X powder was used in infrared spectroscopy (Bruker 550) and methanol solution was applied to obtain the ultraviolet spectrum (Shimadzu UV-315). X was dissolved in deuterated chloroform for nuclear magnetic resonance (Bruker 400) experiments.

RESULTS AND DISCUSSION

Selection of the extracting solvent: Compared with other solvents, ethyl acetate presented the highest efficiency in the extraction of X (Fig. 1) and was also advantageous in terms of excellent stability and low toxicity. Thus, ethyl acetate was selected as the extracting solvent for X.

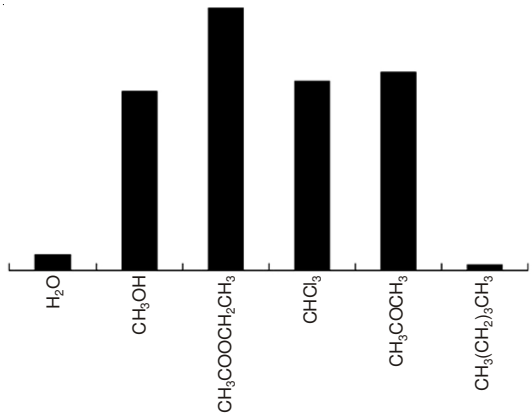

Fig. 1. Comparison of different extraction solutions for X

Purification results: Purified X presented single main peak in analytical HPLC. Based on the area percentage, the purity of X was estimated to exceed 95 %, which meets the requirements of subsequent experiments.

***Drosophila* acute toxicity test:** After the contamination of X for 24 h, each group of *Drosophila* exhibited death, meanwhile, living individuals also showed morbid symptoms such as delayed reaction and weakened ability to control behaviours. Generally, the death percentages (Fig. 2) and intensity of morbid symptoms were positively correlated with the concentration of X. These results indicated the bio-toxic activity of X.

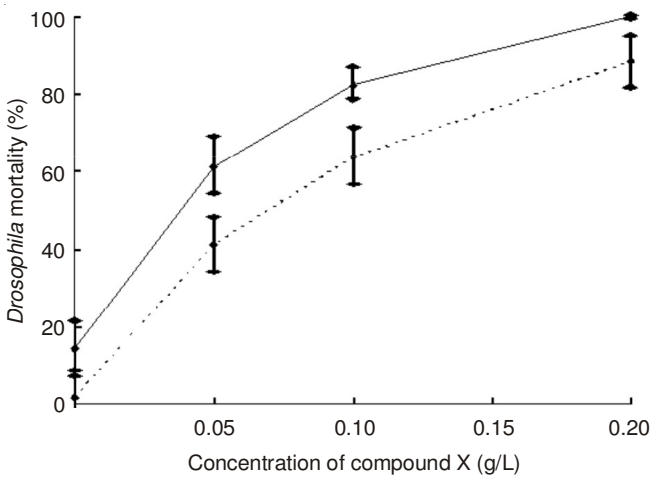

Fig. 2. Relationship between *Drosophila* mortality and the concentration of X

Identification of the molecular formula: The ion trap mass spectrographic analysis of X (Fig. 3A) revealed the possible relative molecular mass of X as 528.

After that, Fourier transform mass spectroscopy was performed to determine the precise molecular weight of X (Fig. 3B). Based on the exact mass-to-charge ratio of $[X + H]^+$ (529.2692), $[X + Na]^+$ (551.2513) and $[X + K]^+$ (567.2274),

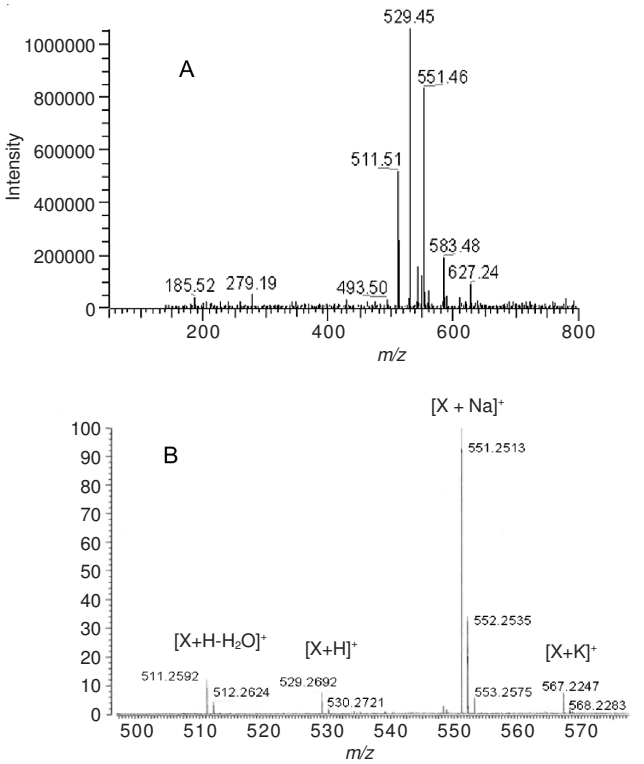

Fig. 3. Mass spectrum analysis of X with ion trap (A) and Fourier transform (B) mass analyzers

the molecular formula of X was identified as $C_{32}H_{36}N_2O_5$. The carbon atoms amount in the molecular formula also matched the relative abundance of isotopic peaks (Fig. 3B).

Identification of structural formula: The fungi strain has been classified as *Chaetomium globosum* (unpublished data). $C_{32}H_{36}N_2O_5$, the molecular formula of X, was searched for related candidates. Three metabolites of *C. globosum* were found, namely, chaetoglobosin A, B and C[9,10].

The absorption peaks of X in ultraviolet spectrum were observed at 220 and 280 nm, which indicates a highly conjugated system. Infrared spectroscopic analysis (Table-1) verified the existence of amino group, hydroxyl group, benzene ring, carbonyl group and *trans*-carbon-carbon covalent bonds in X. All the chemical structures above excited in chaetoglobosin A and B. The ^1H and ^{13}C nuclear magnetic resonance results of X (Fig. 4) were completely consistent with chaetoglobosin A but not others as reported[9,11].

TABLE-1
INFRARED SPECTROSCOPIC DATA
OF X AND CHAETOGLOBOSIN A, B, C

Compounds	Peaks (cm^{-1})						
X	3457	3275	1686	1623	984	968	756
Chaetoglobosin A	3438	3259	1689	1615	983	969	760
Chaetoglobosin B	3440	–	1690	1621	1153	972	746
Chaetoglobosin C	3445	3305	1697	1642	986	–	745

With the overall consideration of the chemical properties as well as the biological source of X, especially the NMR spectrum results, X was identified as chaetoglobosin (Fig. 5).

Conclusion

This study indicated a fungi strain produces bioactive substance chaetoglobosin A aside from the anticancer medicine

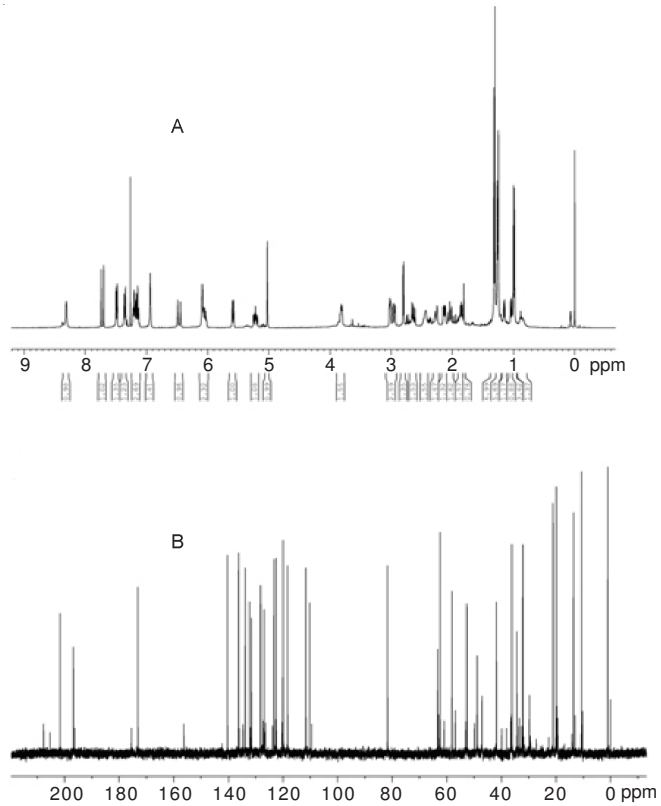

Fig. 4. ^{1}H (A) and ^{13}C (B) nuclear magnetic resonance results of X

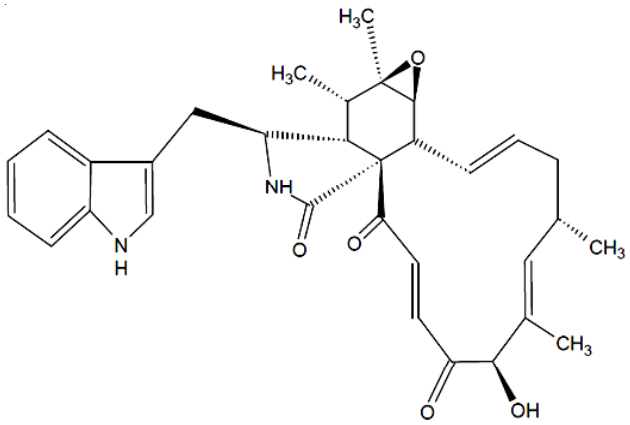

Fig. 5. Molecular structure of chaetoglobosin A

taxol, which further increases the practical values of the strain. Meanwhile, the strategies in purification, bioactivity test and structural identification presented particular values for reference.

ACKNOWLEDGEMENTS

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