

Research Article

Gulab Said* and Farooq Ahmad

Effects of salt concentration on the production of cytotoxic geodin from marine-derived fungus *Aspergillus* sp.

<https://doi.org/10.1515/tjb-2022-0058>

Received March 10, 2022; accepted June 10, 2022;

published online August 4, 2022

Abstract

Objectives: Microorganisms produce optically single bioactive natural products; the process is cheap as compared to chemical synthesis and environmentally friendly. Geodin 1 was isolated from the soft coral-derived fungus *Aspergillus* sp. It has a broad range of biological activities such as antiviral, antimicrobial, glucose stimulator for rat adipocytes, enhancement of fibrinolytic and cytotoxic activities, and is a subunit of the first nonpeptide and inhibitory active galanin receptor. In this work, we studied the effects of sodium chloride (NaCl) salt concentration on the production of geodin 1 and improved its yield to a multi-gram quantity through media optimization from a marine-derived fungus *Aspergillus* sp.

Methods: The fungal strain was cultivated at various concentrations of NaCl salt in rice medium and extracted after different intervals of time.

Results: The yield of geodin 1 was 137.2 mg/L at optimal conditions. The optimal conditions for the high yield of geodin 1 were found as rice medium with 2.0% NaCl salt and 3 weeks incubation at room temperature.

Conclusions: The concentration of NaCl salt greatly affects the yield of geodin and hence its biosynthetic pathway.

Keywords: *Aspergillus* sp; geodin; media optimization; optically single; salt concentration.

Introduction

Fungi play an important role in drugs discovery, producing secondary metabolites having interesting bioactivities [1, 2]. Most fungi belong to terrestrial habitats and have been studied for the bioactive secondary metabolites such as antibiotics; however, those related to the harsh marine environment are recently under extensive study for the production of bioactive natural products [3, 4]. Fungi from the depth of the sea could be studied by culturing in the same artificial environment [4] and those isolated from the coastal areas of the sea produce a variety of valuable bioactive secondary metabolites such as polyketides, peptides, alkaloids, and terpenoids [5]. Fungi from the coastal regions are related either to coral or coral reefs, coastal plants such as mangroves, and rocks [6]. Filamentous fungi synthesize commercially beneficial byproducts like wine from rice by *Aspergillus oryzae*, and bean curd by *Monascus* while the pure bioactive secondary metabolites are used as medicines such as penicillin, cephalosporin, and lovastatin produced by *Aspergillus terreus* and cyclosporine isolated from *Tolypocladium inflatum* [7]. Furthermore, microorganisms also synthesize optically single bioactive natural products; the process is cheap and also environmentally friendly [8].

In our ongoing research on marine-derived fungi, we concentrate our study on a major secondary metabolite, geodin 1 (Figure 1) isolated from the soft coral *Simularia* sp. (SYM-02) derived fungus *Aspergillus* sp. (SYM-02-005) from the South China Sea collected in 2015. It has been reported to be isolated from a filamentous fungus *A. terreus* [9–12]. Geodin 1 has interesting biological activities such as antiviral [13], antimicrobial [14], glucose stimulator for rat adipocytes [15], enhancement of fibrinolytic activity [16], cytotoxic activity [17], and is an essential part of the first nonpeptide, having inhibitory activity of the galanin receptor subtype GALR1 [18, 19]. In our previous study, it showed moderate cytotoxic activities against various cancer cell lines such as breast cancer (BT474), large cell lung

*Corresponding author: Gulab Said, Department of Chemistry, Women University Swabi, Swabi 23430, Pakistan; and Department of Biochemistry, Women University Swabi, Swabi 23430, Pakistan, E-mail: gulabouc@gmail.com. <https://orcid.org/0000-0001-9839-018X>

Farooq Ahmad, Department of Biochemistry, Women University Swabi, Swabi, Pakistan, E-mail: farooq.amazai@gmail.com. <https://orcid.org/0000-0002-7472-519X>



cancer (NCI-H460), non-small cell lung cancer (H-1975), lung cancer (A549), chronic myelogenous leukemia (K562) and prostate cancer (DU145) cells [20].

Materials and methods

The fungal strain was cultivated in 1,000 mL Erlenmeyer flasks containing about 60 g sterilized rice medium and the respective concentrations of sodium chloride (NaCl) salt at 0.0%, 1.0%, 1.5%, 2.0%, 2.5%, 3.0%, 5.0%, 8.0%, 10.0%, 15.0%, 20.0%, and 30.0%. The flasks were fermented at room temperature for 1, 2, 3, 4, and 5 weeks, and extracted thrice times with ethyl acetate (EtOAc). Each crude extract was evaporated under vacuum to dryness and subjected to normal phase silica gel column chromatography (mesh 200–300), eluting with linear gradients of petroleum ether (PE) – EtOAc. Friction 4, eluted with 40% PE-EtOAc, was further subjected to the re-crystallization in methanol and dichloromethane. As a result of re-crystallization, geodin 1 was obtained in pure form as a white crystalline solid. The electrospray ionization mass spectrometry (ESI-MS) spectra were measured on Ultra Performance Liquid Chromatographic Mass Spectrometer (Waters UPLC® system using a C18 column [ACQUITY UPLC® BEH C18, 2.1 × 50 mm, 1.7 µm; 0.5 mL/min]). Nuclear magnetic resonance (NMR) spectra were recorded on an Agilent DD2 NMR spectrometer (500 MHz for ¹H and 125 MHz for ¹³C NMR) and tetra-methyl saline (TMS) as an internal standard while deuterated chloroform (CDCl₃) as a solvent. The structure of compound 1 was confirmed based on ESI-MS *m/z* at 398.9 [M+H]⁺ and NMR spectral data (Table 1) [20, 21]. The fungal strain was identified as *Aspergillus* sp., based on its morphology and molecular identification. The 512 base pair ITS sequence had 98% similarities with *Aspergillus* sp. NRRL58570 (HQ288052.1). The sequence data were submitted to GenBank with accession number KY235298 and the strain was stored in the Key Laboratory, School of Medicine and Pharmacy, Ocean University of China, Qingdao, P.R.China, with Genbank accession number KY235298.

Results and discussion

Geodin was discovered by Raistrick & Smith in 1936 from *A. terreus* and its structure with relative configuration was elucidated by Barton and Scott in 1958 [9, 22]. It was reported about 16 mg/L by Bizukojc and Ledakowicz [8] and 74.3 mg/L by Bizukojc and Pecyna [23] from *A. terreus*

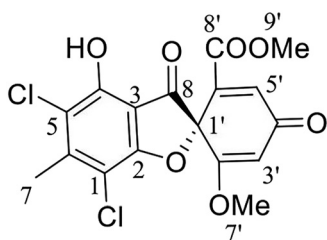


Figure 1: Chemical structure of geodin 1.

Table 1: Comparison of experimental NMR data with the literature [21] for geodin 1.^a

Positions	Data from literature		Data from experiment	
	δ_C	δ_H	δ_C	δ_H
1	108.8		108.8	
2	165.4		165.5	
3	109.4		109.4	
4	149.3		149.4	
5	114.7		114.7	
6	146.6		146.6	
7	18.7	2.57 (3H, s)	18.7	2.57 (3H, s)
8	193.3		193.2	
1'	84.5		84.5	
2'	137.0		137.0	
3'	137.5	7.15 (1H, d, 1.5)	137.5	7.14 (1H, d, 1.5)
4'	185.0		185.0	
5'	104.4	5.83 (1H, d, 1.5)	104.4	5.82 (1H, d, 1.5)
6'	167.9		167.9	
7'	57.0	3.75 (3H, s)	57.0	3.75 (3H, s)
8'	163.4		163.4	
9'	53.1	3.71 (3H, s)	53.1	3.70 (3H, s)

δ_C , chemical shift of carbon; δ_H , chemical shift of proton; NMR, nuclear magnetic resonance. ^aMeasured in deuterated chloroform (CDCl₃).

through lactose and glycerol as a medium. Abd Rahim et al. used a cheap industrial crude glycerol and reported about 58.9 mg/L in shake flask; they also studied the effects of viscosity, friction, and sonication and obtained about 56.9 mg/L geodin from *A. terreus* [12, 24]. It is for the first time to study the effect of salt concentration on the yield of geodin 1 isolated from the soft coral-derived fungus *Aspergillus* sp. and its yield was improved to multi-gram quantity through a cheap fermentation medium.

Broth media such as potato dextrose broth (PDB), glucose peptone yeast (GPY), Chashi, and Starch were used under shaking and unshaking conditions, containing a series of NaCl salt concentrations. However, no improvement in the yield of geodin was observed. Furthermore, rice medium having different concentrations of NaCl salt and supplements such as MgSO₄, NaCOOCH₃, FeSO₄, CuCl₂, and KCl were also studied. However, the optimal conditions for the high yield of geodin were found as rice medium with 2% NaCl salt, room temperature, static condition, and 3 weeks incubation period. NaCl salt enhances the biosynthesis of geodin, controlling the osmotic pressure. A high concentration of NaCl salt increases osmotic pressure and thus the fungal cells expand, and hence numerous cells rupture which results in low production of metabolite [12]. Moreover, Na⁺ ions are comparatively uncreative and are responsible for metabolite production [25]. The less amount of water in the medium may interfere

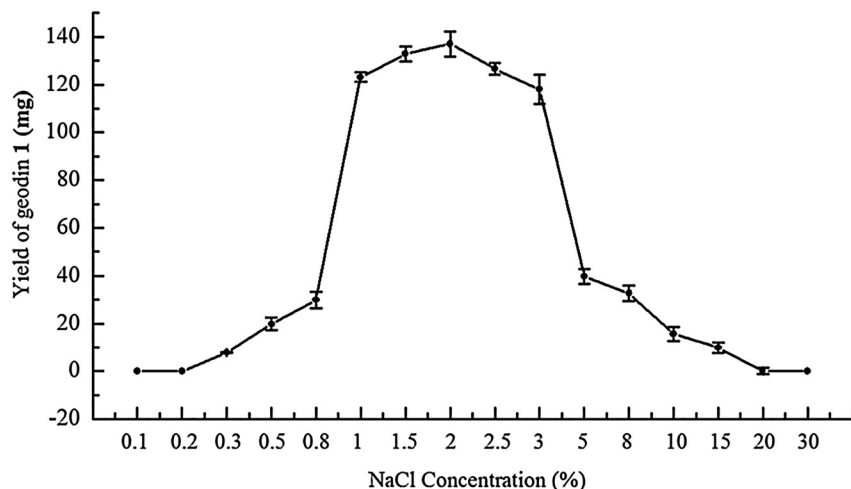


Figure 2: The yield of geodin 1 at various concentrations of NaCl after 3 weeks of incubation at 25 °C.

with physiological functions such as increasing membrane permeability and producing enzymes in the fungal cell [26]. Similarly, as the fungal cells are delicate and therefore in a static condition, porous and less compact mediums such as rice give rise to a high yield of the respective metabolite.

The yield of compound 1 was 137.2 mg/L after 3 weeks incubation period at 25 °C at optimal fermentation medium in static condition (Figure 2), while its yield was 3.4 mg/L in broth media in a shake flask. The factor of incubation period was also studied and it was found that the yield of geodin 1 increases with time, but after 3 weeks the gradual decrease in yield occurred and disappeared after 2 months. Furthermore, in the absence of NaCl salt, no chlorinated compounds were produced as we had isolated previously [20]. Thus, NaCl salt played an important role in the production of chlorinated secondary metabolites produced by

this *Aspergillus* sp. SYM-02-005. Similarly, the hypothetical biosynthetic pathway [27] for geodin 1 was also confirmed (Figure 3).

Research funding: This work was supported by the Program of the National Natural Science Foundation of China (No. 41606172), the Fundamental Research Funds for the Central Universities (No. 201841004), Key Laboratory of Tropical Medicinal Plant Chemistry of Ministry of Education, Hainan Normal University, the Project of State Key Laboratory for the Chemistry and Molecular Engineering of Medicinal Resources (Guangxi Normal University), Ministry of Education of China (Nos. CMEMR2016-A06 and CMEMR2016-B04).

Author contributions: All authors have accepted responsibility for the entire content of this manuscript and approved its submission.

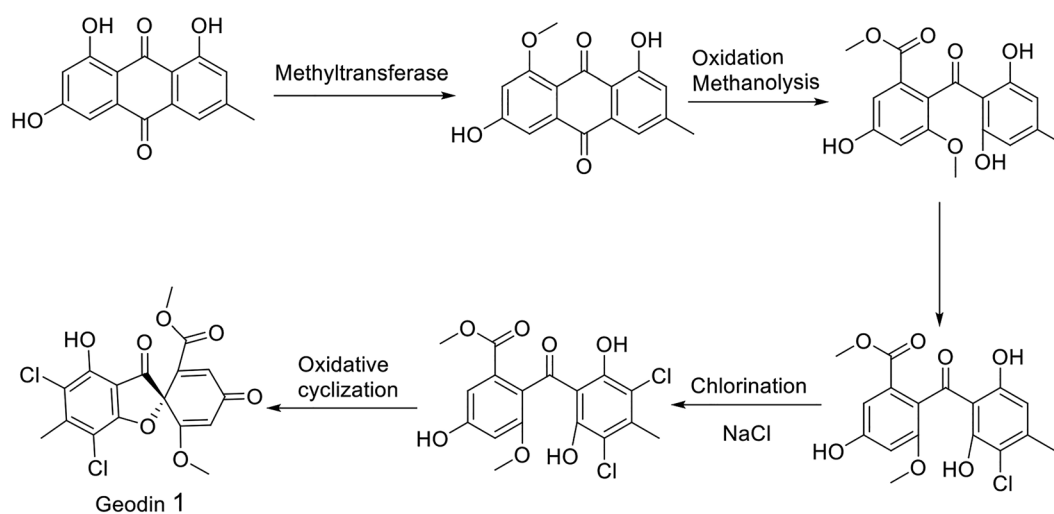


Figure 3: Biosynthetic pathway of natural product geodin 1 [27].

Competing interests: The authors state no conflict of interest.

Informed consent: Informed consent was obtained from all individuals included in this study.

Ethical approval: The local Institutional Review Board deemed the study exempt from review.

References

- Wei MY, Wang CY, Liu QA, Shao CL, She ZG, Lin YC. Five sesquiterpenoids from a marine-derived fungus *Aspergillus* sp. isolated from a gorgonian *Dichotella gemmacea*. *Mar Drugs* 2010; 8:941–9.
- Yurchenko AN, Smetanina OF, Kalinovskiy AI, Pivkin MV, Dmitrenok PS, Kuznetsova TA. A new meroterpenoid from the marine fungus *Aspergillus versicolor*. *Russ Chem Bull* 2010;59: 852–6.
- Zin WWM, Prompanya C, Kijjoa SA. Bioactive secondary metabolites from a Thai collection of soil and marine-derived fungi of genera *Neosartorya* and *Aspergillus*. *Curr Drug Deliv* 2016;13:378–88.
- Redou V, Navarri M, Cladiere LM, Barbier G, Burgaud G. Species richness and adaptation of marine fungi from deep-subseafloor sediments. *Appl Environ Microbiol* 2015;81:3571–83.
- Zhou S, Wang M, Feng Q, Lin Y, Zhao H. A study on biological activity of marine fungi from different habitats in coastal regions. *Springer Plus* 2016;5:1966.
- Rateb ME, Ebel R. Secondary metabolites of fungi from marine habitats. *Nat Prod Rep* 2011;28:290–344.
- Yin Y, Cai M, Zhou X, Li Z, Zhang Y. Polyketides in *Aspergillus terreus*, biosynthesis pathway discovery and application. *Appl Microbiol Biotechnol* 2016;100:7787–98.
- Zhao C, Guo L, Wang L, Zhu G, Zhu W. Improving the yield of (+)-terrein from the salt-tolerant *Aspergillus terreus* PT06-2. *World J Microbiol Biotechnol* 2016;32:77.
- Raistrick H, Smith G. The metabolic Products of *Aspergillus terreus*. Two new chlorine-containing mold metabolic products are geodin and eridin. *Biochem J* 1936;30:1315–22.
- Bizukojc M, Ledakowicz S. Simultaneous biosynthesis of (+)-geodin by a lovastatin-producing fungus *Aspergillus terreus*. *J Biotechnol* 2007;132:453–60.
- Brase S, Encinas A, Keck J, Nising CF. Chemistry and biology of mycotoxins and related fungal metabolites. *Chem Rev* 2009;109: 3903–90.
- Abd Rahim MH, Hasan H, Montoya A, Abbas A. Lovastatin and (+)-geodin production by *Aspergillus terreus* from crude glycerol. *Eng Life Sci* 2015;15:220–8.
- Takatsuki A, Suzuki S, Tamura KG, Arima K. Antiviral and antitumor antibiotics X11 effect of geodin on virus multiplication. *Agric Biol Chem* 1969;33:1119–23.
- Rinderknecht H, Ward JL, Bergel F, Morrison AL. Studies on antibiotic II Bacteriological activity and possible mode of action of certain nonnitrogenous natural and synthetic antibiotics. *Biochem J* 1947;41:463–9.
- Sato S, Okusa N, Ogawa A, Ikenoue T, Seki T, Tsuji T. Identification and preliminary SAR studies of (+) geodin as a glucose uptake stimulator for rat adipocytes. *J Antibiot* 2005;58:583–9.
- Shinohara C, Chikanishi T, Nakashima S, Hashimoto A, Hamanaka A, Endo A, et al. Enhancement and fibrinolytic activity of vascular endothelial cells cheatoglobosin A. *J Antibiot* 2000;53:262–8.
- Ronnest MH, Raab MS, Anderhub S, Boesen S, Kramer A, Larsen TO, et al. Disparate SAR data of griseofulvin analogues for the dermatophytes trichophyton mentagrophytes, *T. rubrum*, and *MDA-MB-231* Cancer Cells. *J Med Chem* 2012;55:652–60.
- Chu M, Mierzwa R, Truumees I, King A, Sapidou E, Barrabee E, et al. A new fungal metabolite, sch 202596, with inhibitory activity in the galanin receptor GALR1 assay. *Tetrahedron Lett* 1997;38:6111–4.
- Wang S, Gustafson EL. Galanin receptor subtypes. *Drug News Perspect* 1998;11:458–68.
- Said G, Mou XF, Fang YW, Liang TM, Wei MY, Chen GY, et al. Secondary metabolites isolated from the soft coral-derived fungus *Aspergillus* sp. from the South China Sea. *Chem Nat Compd* 2018;54:547–9.
- Hargreaves J, Park JO, Ghisalberti EL, Sivasithamparan K, Skelton BW, White AH. New chlorinated diphenyl ethers from an *Aspergillus* species. *J Nat Prod* 2002;65:7–10.
- Barton DHR, Scott A. Constitutions of geodin and eridin. *J Chem Soc* 1958;0:1767–72.
- Bizukojc M, Pecyna M. Lovastatin and (+)-geodin formation by *Aspergillus terreus* ATCC 20542 in batch culture with the simultaneous use of lactose and glycerol as carbon sources. *Eng Life Sci* 2011;11:272–82.
- Rahim MHA, Hasan H, Harith HH, Abbas A. The effect of viscosity, friction, and sonication on the morphology and metabolite production from *Aspergillus terreus* ATCC 20542. *Bioproc Biosyst Eng* 2017;40:1753–61.
- Wucherpfennig T, Hestler T, Krull R. Morphology engineering- Osmolality and its effect on *Aspergillus Niger* morphology and productivity. *Microb Cell Factories* 2011;10:58.
- Bobowicz-Lassociska T, Grajek W. Changes in protein secretion of *Aspergillus Niger* caused by the reduction of water activity by potassium chloride. *Acta Biotechnol* 1995;15:277–87.
- Rukachaisirikul V, Satpradit S, Klaikey S, Phongpaichit S, Borwornwirivapan K, Sakavaroj J. Polyketide anthraquinone diphenyl ether and xanthone derivatives from the soil fungus *Penicillium* sp, PSU-RSPG99. *Tetrahedron* 2014;70:5148–52.