

Synthesis of dipeptide derivatives of Monascus pigments

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Abstract

Monascus pigment-dipeptide derivatives were synthesized by *Monascus* in 48-well plates. Monascus pigment-dipeptides derivatives were identified as antibacterial agents. The antibacterial activities against 20strains were tested with ELISA Reader and 96-well plate.

Introduction

현재 시판되는 대다수의 향균, 함곰팡이, 항암제 등의 여러 생리활성물질들은 그들 특이적 반응물질 즉, starting material을 backbone으로 하고 가능한 여러물질들을 무작위적으로 결합 시킴으로써 다양한 조합물질을 만들어내는 이른 바 combinatorial synthesis에 의해 주로 생산되며 여러 가지 test를 거쳐서 vivo에서의 activity와 stability등이 뛰어난 물질들이 선별된다 아직까지의 세계적인 추세는 유기합성을 통한 combinatorial synthesis가 그 주류를 이루고 있으나 유기합성에 의한 combinatorial synthesis는 환경 친화적이지 못하고 동시에 isomer의 resolution등이 문제점으로 부각되면서 그 해결방안이 절실히 필요하게 되었다. 본 연구는 위에서 밝힌 유기합성에 의한 combinatorial synthesis가 갖는 문제점의 해결을 위하여 우선 수용성 모체인 monascus pigment를 이용, 화학반응이 아닌 조합형 생물반응공정으로 다양한 유도체를 만들며 그 유도체에 대하여 생리활성테스트를 수행하고 또한 이러한 조합형 생물반응공정의 개념을 정리, 안정화 하며 궁극적으로는 이를 산업적인 automation system을 확립 하는데 있다.

모나스쿠스 발효에 의해서 만들어지는 색소는 주로 monascin, ankaflavin, rubropunctatin, monascorubrin, rubropunctamine, monascorubramine등의 6가지구조를 가지고 있으며 이중 orange pigments인 rubropunctatin, monascorubrin은 어느정도의 anti-bacterial activity를 가지고 있다고 알려져 왔다. 이에 본 연구에서는 Rubropunctatin, Monascorubrin에 다양한 dipeptides를 combinatorial로 유도체화 하였고 HTS개념의 biological activity Test를 수행하였다

Material and methods

Microorganism

Monascus sp. J101, which is a high pigment producer obtained by mutation, was used.

Bacteria to anti-bacterial test

Total 20strains

Dipeptides and peptides

- Dipeptide 101sorts, tripeptide 3sorts, tetrapeptide 1sort

Media

N-source modified medium.

Cultivation methods

The stock culture was kept on Hiroi agar containing:

Mycelial blocks of *Monascus* sp. J101 were used for preparing seed cultures.

The inoculated seed culture were grown in 500-ml flask containing 75ml of Seed medium which contains: Glucose, 5g; Peptone, 2g; KH_2PO_4 , 0.8g; $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, 0.05g;

CH_3COOK , 0.2g; NaCl, 0.1g per 100ml distilled water at 30°C, 250rpm for 2days.

3.6ml seed culture was inoculated into 500-ml flask containing 75ml of N-source modified medium at 30°C, 250rpm for 2days. Then 400 μl fermentation culture was put in 48-well plate with dipeptide solutions and incubates at 30°C, 600rpm for 5days.

Results

대부분의 *monascus* pigment dipeptide derivative가 항균활성을 보였고 그 중 Ala-Gly, Asp-Phe, Thr-Gln, Gly-D-Ala, Val-Lys, His-Phe들은 여러 strain에 항균활성을 띄는 것으로 확인되었다.

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