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Toxicon 48 (2006) 491–498

TOXICON

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Investigation of destruxin A and B from 80 *Metarhizium* strains in China, and the optimization of cultural conditions for the strain MaQ10 ☆

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Received 17 March 2006; received in revised form 18 June 2006; accepted 19 June 2006
Available online 7 July 2006

Abstract

The production levels of destruxin A and B (DA and DB) of 80 *Metarhizium* strains from China were investigated. The average yields of DA and DB were 14.87 ± 2.30 and 3.65 ± 0.58 μg/mL, respectively. The strains isolated from soil had significantly ($p < 0.05$) lower production of DA and DB than the strains isolated from insect cadavers. Also, there was a positive correlation in the yields of DA and DB, and the regressive equations were established. Of the 80 strains, MaQ10 had the highest yields of DA and DB, amounted to 106.78 ± 9.41 and 29.52 ± 2.63 μg/mL, respectively, at 10 days fermentation period. Followed by the strains MaQ05, MaQ07 and MaQ12, their yields of DA were 79.72 ± 5.36, 77.69 ± 8.54 and 70.04 ± 10.1 μg/mL, respectively, and DB were 16.81 ± 1.51, 13.38 ± 0.41 and 16.88 ± 1.15 μg/mL, respectively. Furthermore, the cultural conditions of MaQ10 were optimized to produce DA and DB. The optimal inoculum, initial pH, temperature and rotary speed were 8%, pH 9.0, 27 °C and 240 r/min for DA, and 8%, pH 9.0, 25 °C and 220 r/min for DB. Under these optimal culture conditions, the predicted production of DA and DB was 193.87 and 39.85 μg/mL, and the actual production was 189.13 and 43.35 μg/mL, respectively.

Keywords: *Metarhizium*; Destruxins; Investigation; Production; Optimization

1. Introduction

The entomopathogenic fungus *Metarhizium anisopliae* (Metchnicoff) Sorokin has been used as bio-control mycetes for long time. It produces a family of cyclic peptide toxins, destruxins (Pais et al., 1981; Pedras et al., 2002), which may play an important role in pathogenesis (Samuels et al., 1988; Kershaw et al., 1999; Milner et al., 2002; Rabie, 2002). These compounds are typically composed of five amino acids and a α-hydroxy acid forming a cyclic hexadepsipeptide. The general formula of destruxins is cyclo (–D-HA–L-Pro–L-Ile–L-MeVal–L-MeAla–β-Ala-), where HA represents a D-α-hydroxy acid residue. To date, 35 destruxins have been reported

☆ The research was supported by grants from National Tenth Five Research Plan (2004BA509B0604) and Natural Science Foundation of Guangdong Province(010312).

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0041-0101/$ - see front matter © 2006 Elsevier Ltd. All rights reserved.
doi:10.1016/j.toxicon.2006.06.018

(Pedras et al., 2002). They were found from different fungi, but the most extensively reported fungus was *M. anisopliae*. Some destruxins, especially destruxin A, E and B (DA, DE, DB) showed insecticidal activities (Hsieh et al., 1998; Cavelier et al., 1998; Amiri et al., 1999; Thomsen and Eilenberg, 2000). DB and desmethyl-DB were phytotoxic to the plants of *Brassica* (Buchwaldt and Jensen, 1991; Pedras et al., 2000; Saharan et al., 2003). DB also had suppressive effects on hepatitis B virus surface antigene gene expression in human hepatoma cells (Chen et al., 1997). In addition, destruxins showed erythropoietin-inducing and immunomodulating activities (Cai et al., 1998; Huxham et al., 1989; Vey et al., 2002), and anti-resorptive effect for osteoclasts (Nakagawa et al., 2003; Vazquez et al., 2005).

The production of destruxins is important for the insecticidal or phytotoxic effects, and the commercial registration of fungi strains (Hsieh et al., 1998; Starsser et al., 2000; Pedras et al., 2002). The first step for developing a new microbiological insecticide is to find good strains. In China, there are rich resources of entomopathogenic fungi with few reports on destruxins. The primary aim of this study was to investigate the production of destruxins in local *Metarhizium* strains and to screen high-yield strain and optimize its cultural conditions.

2. Materials and methods

2.1. Investigation of DA and DB from different strains

2.1.1. Preparation and collection of strains of Metarhizium

Eighty strains of the genus, *Metarhizium*, were tested. The *M. anisopliae* strains MaQ01-20, MaB01-04, MaY01-24, MaC01-02 were isolated from cadavers of different insects from different geographical locations (Supplementary materials, Table 1), while MaT01-25 were isolated from various soil (Supplementary materials, Table 2), they all were identified as *M. anisopliae*. Also, there were four *M. biformisporae* (Chen and Guo) Liu and Liang strains, Mb01-04, and one *M. flavoviride* strain Mf001 used in our experiments (Supplementary materials, Table 2). All slants of different strains were kept at 4 °C.

2.1.2. Culturing and HPLC determination

Pre-culture medium: Czapek-Dox broth plus 0.5% peptone: $NaNO_3$ 3 g, K_2HPO_4 1 g, $MgSO_4\cdots 7H_2O$ 0.5 g, KCl 0.5 g, $FeSO_4\cdots 7H_2O$ 0.01 g, cane sugar 30 g, peptone 5 g, and water 1000 mL.

Fermentation medium (Liu et al., 2000): Maltose 2.55%, peptone 0.75%, β-alanine 0.02%, and glucose 0.50%.

Slant or plate media were SDAY (peptone 10 g, glucose 10 g, yeast extracts 10 g, agar 16 g and water 1000 mL) and PDA (potato 200 g, glucose 20 g, agar 16 g and water 1000 mL).

All the apparati used namely Erlenmeyer flasks, Petri dishes, test tubes and cultured media were sterilized at 121 °C for 25–30 min in an autoclave (HVE-50 Hiclave, Hirayama, Japan).

The conidia spores from the slants of different strains were suspended into a concentration of 10^7 spores/mL. One mL of conidia suspension was poured into a 150-mL-flask with 49 mL pre-culture broth, and incubated for 3 days at 180 r/min and 26 °C. For fermentation culture, 10 mL of pre-cultured broth was inoculated into 90 mL of fermentation medium, and incubated for 10 d at 26 °C and 240 r/min. Each strain was replicated 3 times.

To detect DA and DB by HPLC, 1 mL fermented broth was absorbed from each flask into a centrifugal tube, and centrifuged at 7155*g* for 10 min to remove the mycelia and spores, then the supernatant was filtered through 0.45 μm membrane. HPLC was performed on an Agilent 1100 HPLC system equipped with a G1311A QuatPump, a G1314A VWD detector and an Agilent Hypersil ODS column (4.0 × 250 mm, 5 μm). The mobile phase was CH_3CN/H_2O 45/55 at 1.0 mL/min for 15 min, and 10 μl sample was injected, and the column temperature was about 25 °C. The concentrations of DA and DB were determined by the calibration curves of standards.

2.1.3. Calibration curves and data analysis

The DA standard was bought from Sigma. The in-house reference standards of DB was obtained from preparative HPLC and identified by MS (Pais et al., 1981). They were diluted with CH_3CN into a series of concentrations, and each concentration had three injections in HPLC. Their calibration curves were obtained by linear regression analysis of the average of peak areas (Y) against concentration (X) with the equation $Y = aX + b$.

The peak areas of DA and DB of each sample could be obtained by integration of the HPLC elution profiles. So the concentrations of DA and