

Pseudomonas avenae

Mannsにより産生される抗糸状菌物質の純化

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Purification of Anti-Fungal Substance Produced by *Pseudomonas avenae* Manns

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Key words: *Pseudomonas avenae* Manns, *Pyricularia oryzae* Cavara, antagonism, proteinic anti-fungal substance.

The production of antibiotics against phytopathogenic bacteria and fungi by pseudomonads has been well documented¹⁻¹⁴⁾. Recently the authors reported that *Pseudomonas avenae* Manns produces anti-fungal substance(s) which is especially antibiotic to the rice blast fungus *Pyricularia oryzae* Cavara. This substance was not dialyzable and not transferrable to organic solvents such as chloroform, petroleum ether, diethyl ether, ethylacetate and *n*-butanol. The activity of this substance was easily lost by a heat-treatment at 100 C for 10 min and protease K treatment. From these results, the substance was assumed as proteinic¹²⁻¹⁴⁾. In this report, the procedure for isolation and purification of this substance(s) will be contributed.

Pseudomonas avenae Manns, isolate No. H8201 was shake-cultured in potato semi-synthetic medium at 30 C for 7 days¹⁴⁾. The culture was centrifuged at 10,000 rpm for 20 min and the supernatants were collected. The substance was obtainable by precipitation with ethanol (90 %) or acetone (75~90 %) amendment. But this procedure needed large amount of the solvents and was time-consuming. Therefore, the adsorption chromatography with active carbon was conducted. Active carbon was added to the supernatant so as to be 2% w/v and stirred overnight at 4 C. The substance was adsorbed completely to the active carbon and eluted partly with methanol-water (1:1) and mainly with acetone-water (1:1). These eluates were combined and evaporated to dryness *in vacuo*. This sample was dissolved in 2 ml of distilled water and was subjected to Sephadex G-50 column chromatography (column: 1.6 cm diameter, 50 cm length) and eluted with distilled water at flow rate 36 ml/hr. The monitoring of the active substance was conducted by UV at 254 nm and bioassay with *Pyricularia oryzae* as previously reported¹³⁾. The active fraction which appeared just after the void volume were combined, evaporated to dryness *in vacuo* and dissolved in 2 ml of 0.01 M Tris-HCl buffer (pH 9.0). This sample was subjected to DEAE cellulose column chromatography (column: 1.7 cm diameter,

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1) Lindberg, G.D. (1981). Plant Disease 65: 680-683. 2) Nakano, T., Tsuyumu, S., Takikawa, Y. and Goto, M. (1985). Ann. Phytopath. Soc. Japan 51: 337. 3) Homma, Y., Kato, K., Shirahama, H., Hirayama, F., Konno, K. and Suzui, T. (1986). Ibid. 52: 543. 4) Adetuyi, F.C., Cartwright, D.W. and Aw, D. (1985). Ann. appl. Biol. 107: 25-32. 5) Adetuyi, F.C. and Cartwright, D.W. (1985). Ibid. 107: 33-43. 6) Homma, Y., Kato, K., Shirahama, H., Hirayama, F., Konno, K. and Suzui, T. (1986). Ann. Phytopath. Soc. Japan 52: 543. 7) Wakimoto, S., Hirayae, K., Tsuchiya, K., Kushima, Y., Furuya, N. and Matsuyama, N. (1986). Ibid. 52: 835-842. 8) Arie, T., Kijima, T., Namba, N., Yamashita, S. and Doi, Y. (1987). Ibid. 53: 73. 9) Kunitake, S., Matsuyama, N. and Wakimoto, S. (1987). Ibid. 53: 84. 10) Arie, T., Namba, N., Yamashita, S., Doi, Y. and Kijima, T. (1987). Ibid. 53: 117. 11) Arie, T., Namba, T., Yamashita, S., Doi, Y. and Kijima, T. (1987). Ibid. 53: 531-539. 12) Kunitake, S., Matsuyama, N., Iwai, H. and Wakimoto, S. (1988). Ibid. 54: 385-386. 13) Kunitake, S., Matsuyama, N. and Wakimoto, S. (1989). Ibid. 55: 79. 14) Kunitake, S., Matsuyama, N. and Wakimoto, S. (1988). Ibid. 54: 640-642.

20 cm length) and eluted stepwise with Tris-HCl buffer (0.01 M, pH 9.0~0.5 M, pH 6.5) at flow rate 36 ml/hr. The eluates at 0.1~0.2 M (pH 8.0~7.3) were combined and dialyzed against dist. water and freeze-dried. This sample was subjected to polyacrylamide gel electrophoresis (12.5% PAGE, slab gel, 25 mA). After electrophoresis, gel was cut to ten sections. Each piece was surface-disinfected with ethanol and confronted to the test organism on PS agar plate after evaporation of ethanol (Fig. 1). Corresponding area where active substance detected was cut from other gel sheets, homogenized with water and the substance was extracted. This sample was subjected to electrophoresis and stained with Coomassie Brilliant Blue R-250 and silver staining to test its purity. Single band was detected on the gel sheet and this band disappeared in the case of protease K-treated sample accompanying the loss of the activity.

Although the active substance was excellently purified by these procedures, these processes were time-consuming and caused a loss in yield. Therefore, another favorable method was tested. Fifty mg of the sample from DEAE cellulose column chromatography was dissolved in 50 ml of 2.5% ampholyte solution, subjected to preparative liquid isoelectric focussing (Roto-

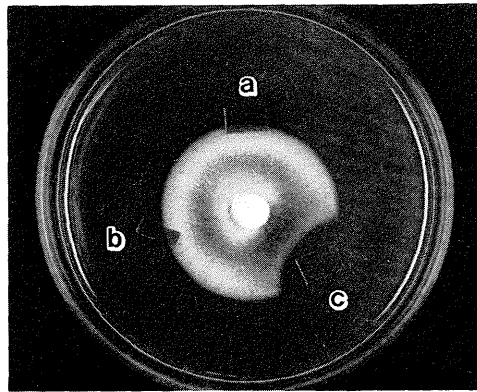


Fig. 1. Inhibition of mycelial growth of *Pyricularia oryzae* Cav. by the purified substance in PAGE gel piece. a: Check 1 (PAGE gel), b: Check 2 (PAGE gel cut from the neighbouring area of the substance, c: PAGE gel which contains the substance.

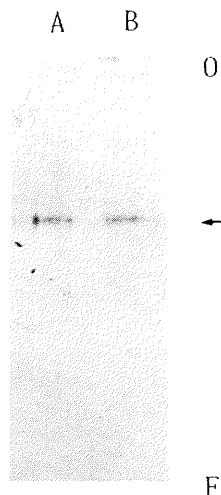


Fig. 2. Electrophoretic patterns (PAGE) of the substance purified by preparative liquid isoelectric focussing. A: Fraction at pH 3.5. B: Fraction at pH 4.0. Abbreviations: O: Origin, F: Front. Arrow indicates the stained band.

for, Bio-Rad Co. Ltd.) ranging from pH 2.0~9.0 at 12 W for 3 hr and fractionated to 20 tubes. These fractions were dialyzed against 1 N NaCl to eliminate ampholyte and freeze-dried. The fractions at pH 3.5~4.0 showed distinct anti-fungal activity. The purity of this sample was checked by PAGE (Fig. 2). From the results of isoelectric focussing and co-electrophoresis with standard proteins, it was assumed that the isoelectric point of this substance is pH 3.5~4.0 and molecular weight is *ca.* 100,000 daltons. Any of the anti-fungal substance was not detected in the extracts from not-inoculated PS medium.

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和 文 摘 要

國武幸子・松山宣明：*Pseudomonas avenae* Manns により産生される抗糸状菌物質の純化

Pseudomonas avenae は PS 培地中でタンパク性の抗糸状菌物質を産生する。*Ps. avenae*, H8201 株を 30 C で 7 日間振盪培養後、遠沈上清に活性炭を加え活性物質を吸着させた。メタノール-水 (1:1) およびアセトン-水 (1:1) により溶出した活性分画を濃縮後、Sephadex G-50 によりゲル濾過し、DEAE セルロースカラムクロマトグラフィーにより粗純化した。この試料をさらにポリアクリルアミドゲル電気泳動または調整用液体等電点電気泳動法により純化し、透析後、凍結乾燥保存した。Protease K 処理後の試料には活性はまったく認められず、電気泳動像からのバンドの消失が観察された。本物質の等電点は pH 3.5~4.0 であり、分子量は約 10 万ダルトンと推定される。

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