

Antimicrobial Activity of *E.coli*-expressed Defensin (Bj-AFP1) isolated from *Brassica juncea*.

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Summary

Antimicrobial activity of *E. coli*-expressed *Brassica juncea* defensin protein (Bj-AFP1) was evaluated against plant pathogens. The Bj-AFP1 was obtained by the *E. coli* gene expression system of Amersham using pGEX plasmid or the system of Invitrogen using pLEX plasmid with slight modification. Pathogens were cultured in 96 well microplates with Bj-AFP1 protein and I.C.50 values of Bj-AFP1 were calculated from O.D.595 using the Sunrise microplate reader (Tecan)

The Bj-AFP1 protein (expressed by pGEX system) inhibited the growth of *Pyricularia oryzae* and *Fusarium graminearum* with an I.C.50 value of 0.63 and 1.31 $\mu\text{g}/\text{ml}$ respectively. In the other pathogens, *Xanthomonas campestris*, *Burkholderia plantarii*, *B. glumae*, *Acidovorax avenae* subsp. *avenae*, *Erwinia carotovora* subsp. *carotovora*, *Pseudomonas syringae* pv. *maculicora* and *X. campestris* pv. *campestris*, the I.C.50 values of Bj-AFP1 (expressed by pLEX system) were presumed above 25 $\mu\text{g}/\text{ml}$.

The inhibitory effect of Bj-AFP1 against rice blast fungi, *P. oryzae*, was not less than those of Kasugamycin and Ferimzone. The I.C.50 values of Bj-AFP1 (expressed by pLEX system) against nine *P. oryzae* strains of different pathogenic races were 2.01 - 6.02 $\mu\text{g}/\text{ml}$.

These results suggested that Bj-AFP1 might be a fungicide against the rice blast fungus, *P. oryzae*, and the barley scab fungus, *F. graminearum*. It was also suggested that genetic transformation of a crop with the Bj-AFP1 gene could confer the broad disease resistance on them.

V 摘要

- (1) 大腸菌で発現させたカラシナ由来ディフェンシン (Bj-AFP1) はオオムギ赤かび病菌に対して抗菌活性を示した。イネ白葉枯病菌, イネ苗立枯細菌病菌, イネもみ枯細菌病菌, イネ褐条病菌, 蔬菜類軟腐病菌, ハクサイ黒斑細菌病菌およびキャベツ黒腐病菌に対しては, いもち病菌に効果を示した濃度の約10倍までの濃度においては抗菌活性を示さなかった。
- (2) カラシナ由来ディフェンシン (Bj-AFP1) の, いもち病菌に対する抗菌活性はフェリムゾン, カスガマイシンとほぼ同等かそれ以上であった。
- (3) カラシナ由来ディフェンシン (Bj-AFP1) は, イネいもち病菌のレースにかかわらず抗菌活性を示した。

謝 辞

本研究を行うに当たり, イネの病原細菌は中央農業総合研究センター細菌病害研究室(現:病害虫検出同定法研究チーム)の畔上耕兒博士より, フェリムゾンは住友武田農薬株式会社より御提供頂いた。

また, 農林水産省アグリ・ゲノム研究の総合的な推進プロジェクト(GB-1002)に御支援頂いた。ここに厚く御礼を申し上げる。

引用文献

- Alves, A. L. V., De Samblanx, G. W., Terras, F. R. G., Cammue, B. P. A. and Broekaert, W. F. (1994) Expression of functional *Raphanus sativus* antifungal protein in yeast. FEBS. Lett., 348, 228-232.
- Broekaert, W. F., Terras, F.R.G., Cammue, B.P.A., and Osborn, R.W. (1995) Plant Defensins: Novel antimicrobial peptides as components of the host defence system. Plant Physiol., 108, 1353-1358.
- Broekaert, W. F., Terras, F.R.G., Cammue, B.P.A. and Vanderleyden, J. (1990) An automated quantitative assay for fungal growth inhibition. FEMS. Microbiol. Lett., 69, 55-60.
- Castro, M.S. and Fontes, W. (2005) Plant defence and antimicrobial peptides. Protein and Peptide Lett., 12, 13-18.
- Kanzaki, H., Nirasawa, S., Saitoh, H., Ito, M., Nishihara, M., Terauchi, R. and Nakamura, I. (2002) Overexpression of the wasabi defensin gene confers enhanced resistance to blast fungus (*Magnaporthe grisea*) in transgenic rice. Theor. Appl. Genet., 105, 809-814.
- 川田元滋・黒田 秧・田中宥司 (2005) 抗菌蛋白質ディフェンシンの多様な機能特性. 化学と生物, 43, 229-234.
- 川田元滋・中島敏彦・松村葉子・及川鉄男・黒田 秧 (2003) アブラナ科野菜がもつ抗菌タンパク質ディフェンシン遺伝子群の解析. 農業および園芸, 78 (4), 470-476.
- Kawata, M., Nakajima, T., Yamamoto, T., Mori, K., Oikawa, T., Fukumoto, F. and Kuroda, S. (2003) Genetic Engineering for Disease Resistance in Rice (*Oryza sativa* L.) using antimicrobial peptides. JARQ, 37, 71-76.
- Moreno, M., Segura, A. and Garcia-Olmedo, F. (1994) Pseudothionin-St1, a potato peptide active against potato pathogens. Eur. J. Biochem., 223, 135-139.
- Osborn, R. W., De Samblanx, G. W., Thevissen, K., Goderis, I., Torrekens, S., Van Leuven, F., Attenborough, S., Rees, S. B. and Broekaert, W. F. (1995) Isolation and characterization of plant defensins from seeds of Asteraceae, Fabaceae,