

# THE STUDIES ON THE ANTIMICROBIAL COMPOUNDS OF PSEUDOMONAS FLUORESCENS B-52

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## ABSTRACT

IN THIS STUDY, THE UTILIZATION OF SHRIMP AND CRAB SHELL POWER (SCSP) AND COFFEE GROUNDS WASTES BY MICROBES TO PRODUCE VARIOUS BIOAGENTS, SUCH AS FUNGICIDE, BIOFERTILIZER WAS DESCRIBED. IN THE FIRST PART, WE DESCRIBED THE ISOLATION AND IDENTIFICATION OF ONE STRAIN OF FUNGICIDE-PRODUCING MICROORGANISMS. THE STRAIN WAS NUMBERED AS B-52, IDENTIFIED AS STRAIN OF PSEUDOMONAS FLUORESCENS. IN THE SECOND PART, THE OPTIMUM CULTURAL MEDIA AND CONDITIONS WERE STUDIED. THE OPTIMUM CONDITION FOR B-52 WAS FOUND TO BE THE CULTURED WAS AT 35 °C FOR 3 DAYS IN 175 ML MEDIUM (PH4.0) CONTAINING 0.1%K<sub>2</sub>HPO<sub>4</sub>, 0.05% MGSO<sub>4</sub> · 7H<sub>2</sub>O, AND 0.5% SCSP. THE OPTIMUM CONDITION FOR B-52 WAS FOUND TO BE THE CULTURED AT 25 °C FOR 3 DAYS IN 50 ML MEDIUM (PH3.0) CONTAINING 0.1%K<sub>2</sub>HPO<sub>4</sub>, 0.05% MGSO<sub>4</sub> · 7H<sub>2</sub>O, AND 1% COFFEE GROUNDS. UNDER SUCH CONDITIONS, PSEUDOMONAS FLUORESCENS B-52 EXHIBITED THE MAXIMUM ANTIFUNGAL ACTIVITIES ON PATHOGENIC F. OXYSPOURUM. THE INHIBITORY ACTIVITIES ARE 97% AND 98%, RESPECTIVELY. IN THE THIRD PART, THE PROPERTIES OF FUNGICIDES PRODUCED BY B-52 WERE DESCRIBED. THE SCSP FUNGICIDE PRODUCED BY B-52 DISPLAYED THE MAXIMUM INHIBITORY ACTIVITY (55%-45%) ON PATHOGENIC F. OXYSPOURUM AT PH4.0-11.0. IT WAS REMARKABLY THERMOSTABLE AND RETAINED 56% OF ITS ACTIVITY EVEN AFTER BEING HEATED AT 100 °C FOR 60 MIN. THE COFFEE GROUNDS FUNGICIDE PRODUCED BY B-52 DISPLAYED THE INHIBITORY ACTIVITY (40%) AT PH 5.0. IT RETAINED 68% OF ITS ACTIVITY AFTER BEING HEATED AT 100 °C FOR 60MIN. THE MINIMUM INHIBITORY ACTIVITIES FOR SCSP AND COFFEE GROUNDS FUNGICIDES WERE 6% AND 10%, RESPECTIVELY. BOTH FUNGICIDES SHOWED NO CHITINASE ACTIVITY BUT CAUSED ABNORMAL HYPHAL SWELLING ON THE TIP OF F. OXYSPOURUM. IN THE FOURTH PART, THE FUNGICIDE OF PSEUDOMONAS FLUORESCENS B-52, PRODUCED UNDER THE OPTIMIZED CULTURE CONDITION, THE FIRST STEP WAS PRECIPITATED AND DIALYZED BY USING AMMONIUM SULFATE. THE FURTHER PURIFICATION AND SEPARATION PROCEDURES OF THE FUNGICIDE WERE PROCESSED BY THE USE OF DEAE-SEPHACEL IONIC EXCHANGE CHROMATOGRAPHY, SEPHACRYL S-200 GEL PERMEATION CHROMATOGRAPHY AND CHROMATOFOCUSING. THE MOLECULAR WEIGHT OF THE FUNGICIDE WAS IDENTIFIED AS 1.2 KDA. IN THE FIFTH SECTION, THE PROPERTIES OF COMPOSTS MADE BY INOCULATING CRAB AND SHELL WASTES WITH PSEUDOMONAS FLUORESCENS B-52 WERE MEASURED. THE EFFECTS OF THE COMPOSTS ON THE GROWTH OF WERE STUDIED. THE RESULTS SHOWED THE BEST RESULT.

Keywords : 無

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## REFERENCES

- 1.王三郎、李旭弘(1994) 中華生質能源會誌, 13:229.
- 2.王三郎(1996) 水產資源利用學, 高立圖書出版社.
- 3.王三郎(1997) 應用微生物學, 高立圖書出版社.
- 4.王啟浩、王三郎(1998) 林產資源有用成分回收再利用, 工業減廢暨永續發展研討會, 379.
- 5.王啟浩(1999) 利用細菌醱酵農水產廢棄物生產生物製劑之研究, 私立大葉大學食品工程研究所碩士論文.
- 6.王三郎(1999) 海洋未利用生物資源之回收再利用, 生物資源 生物技術, 1(1):1-8.
- 7.王三郎(2000) 生物技術, 高立圖書出版社.
- 8.邱少華(1997) 利用綠膿桿菌K-187醱酵蝦蟹殼廢棄物生產幾丁質酶之應用及量產條件之研究, 私立大葉大學食品工程研究所碩士論文.
- 9.阮進惠、林翰良、羅淑珍(1997)幾丁聚糖水解物之連續式生產及其抑菌作用, 中國農業化會誌35(6):596-611
- 10.林景和(1986) 利用廢棄菇類栽培介質製作堆肥之研究, 文翔圖書公司.
- 11.林天枝(1993) 香菇栽培之太空包廢渣在番茄生產利用之研究, 台中區農業改良場研究彙報, 40: 37-44
- 12.林景和(1993) 利用廢棄菇類栽培介質製作堆肥之研究, 台中區農業改良場研究彙報, 39: 17-27.
- 13.林志森(1995) 環境工程會刊, 2(4):29
- 14.李汪章(1999) 生物資源生物技術, 1:9.
- 15.施英隆(1999) 生物資源、生物技術1(1):23
- 16.徐華盛(1999) 有機農場使用資材特性, 農業世界雜誌194:70--74.
- 17.陳啟禎(1993) 食用菇類發展的近況與展望, 食品工業期刊25(4):23-27.
- 18.陳昱初(1996) 談作物病蟲害之生物防治, 高雄區農業專訊, 15.
- 19.陳能敏(1996) 永續農業過去、現在、未來, 農資中心資訊科學叢書.
- 20.張明暉、向為民、簡宜裕、黃維廷(1997) 廢棄菇類木屑堆肥應用於洋香瓜育苗介質之評估, 中華 農業研究, 46(1):60-69.
- 21.陳俊位(1999) 生物農藥枯草桿菌在植物病害防治上之應用台中區農業專訊, 26:19-21.
- 22.葉美雲(1991) 玉米穗軸堆肥三種作物生長之影響, 國立台灣大學農業化學研究所碩士論文
- 23.葉志超(1996) 利用綠膿桿菌醱酵蝦蟹殼廢棄物生產真菌抑制劑之研究, 私立大葉大學食品工程研究所碩士論文.
- 24.彭金騰(1996) 杏 菇稻草栽培之研究, 中華農業研究45(4):382-387.
- 25.彭金騰(1996) 杏 菇木屑塑膠包栽培之初步研究, 中華農業研究45(4):388-392.
- 26.彭武財(1997)廢棄木料及其纖維質類之污染堆肥化處理之介紹, 台灣農業33(4):209-229
- 27.黃秀華(1999) 生物技術在植物病害生物防治之應用, 台中區農業專訊, 26:22-25.
- 28.彭武財(2000)木材及其纖維質原料之有效再利用與再生, 生物資源 生物技術2(1):1-3
- 29.彭武財(2000)木材 / 塑膠復合材料之研發其重要性, 生物資源 生物技術2(1):4-7
- 30.楊秋忠(1991)土壤有機質的力地維持, 土壤管理手冊, 60-170.
- 31.楊光盛, 水耕栽培簡介, 1~17
- 32.楊政國、王三郎(1998) 工業減廢暨永續發展研討會論文集, 353-366.
- 33.楊政國(1999) 利用枯草菌進行蝦蟹殼去蛋白之研究, 私立大葉大學食品工程研究所碩士論文.
- 34.蔡永(1994)有機質添加物在防治作物線蟲病害之永續作為, 農藥世界, 126(2):56-65.
- 35.蔡宜峰、陳清文(1994) 牛糞堆肥化處理及應用酵液之研究, 台灣農業30(4):54-62.
- 36.蔡宜峰、陳清文、蔡精強(1995) 香菇太空包廢料堆肥之研究甲、台灣農業31(1):133-141.
- 37.蔡宜峰、陳清文、蔡精強(1995) 香菇太空包廢料堆肥對梨樹生產及土壤性質之影響, 台灣農業, 31(1):142-148.
- 38.簡宜裕、莊作權(1997) 中華農業研究46(1):70-81
- 39.羅朝村(1997)作物病害生物防治的應用與展望, 農業專論35(1):12-22
- 40.嚴式清(1989) 畜牧廢棄物在有機農業之利用, 臺中區農業改良場特刊42:229-2.
- 41.ANDREAS NATSCH, CHRISTOPH KEEL, NICOLE HEBECKER, EVE LAASIK, GENEVIEVE DEFAGO (1998) IM -PACT OF PSEUDOMONAS FLUORESCENS STRAIN CHA0 AND A DERIVATIVE WITH IMPROVED BIOCONTROL ACTIVITY ON THE CULTURABLE RESIDENT BACTERIAL COMMUNITY ON CUCUMBER, FEMS MICROBIOLOGY ECOLOGY, 27:365-380.
- 42.API 20NE INSTRUCTION MANUALS (1994). BIOM' ERIEUX VITEK INC., U.S.A.
- 43.Y. DURSUN, A. CALIK, Z. AKSU (1999) DEGRADATION OF FERROUS( )CYANIDE COMPLEX IONS BY PSEUDOMONAS FLUORESCENS, PROCESS BIOCHEMISTRY, 34:901-908.
- 44.DERGHAM, Y., LELLY, J., AND ERNST, A.A. (1991) WASTE PAPE AS A SUBSTITUTE FOR PEAT IN THE MUSHROOM (AGARICUS BISPORUS) CASING SOIL PRODUCTION. MUSHROOM SCIENCE 13(1):263-267.
- 45.EMANUELE SMACCHI AND MARCO GOBBETTI (1998) PEPTIDES FROM SEVERAL ITALIAN CHEESES INHIBI -TORY TO PROTEOLYTIC ENZYME OF LACTIC ACID BACTERIA, PSEUDOMONAS FLUORESCENS ATCC 948 AND TO THE ANTIOTENSIN I-CONVERTING ENZYME, ENZYME MICROB.

TECHNOL., 22:687-694- 46.E. P. SCHOKKER AND M. A. J. S. VAN BOEKEL (1997) PRODUCTION, PURIFICATION AND PARTIAL CHARACTERIZATION OF THE EXTRACELLULAR PROTEINASE FROM PSEUDOMONAS FLUORESCENS 22F, ELSE -VIER SCIENCE, 7:265-271. 47.E. P. SCHOKER, A. C. M. VAN WAGENBERG, AND M. A. J. S. VAN BOEKEL (1998) A NOTE ON THE USE OF UREA IN STUDYING THE MECHANISM OF THERMAL INACTIVATION OF EXTRACELLULAR PROTEINASE FROM PSEUDOMONAS FLUORESCENS 22F, ENZYME MICROB. TECHNOL, 22:695-698 48.FANG, S.W., LI, C.F., AND SHIN, D.Y.C. (1994) ANTIFUNGAL ACTIVITY OF CHITOSAN AND ITS PRESERVATIVE EFFECT ON LOW-SUGAR CANDIED KUMQUAT. J. FOOD PROT., 56:136-140. 49.FRAN M. SCHER. AND RALPH BAKER. (1982) EFFECT OF PSEUDOMONAS PUTIDA AND A SYNTHETIC IRON CHELATOR ON INDUCTION OF SOIL SUPPRESSIVENESS TO FUSARIUM WILT PATHOGENS. PHYTOPATHOLOGY. 72(12):1567-1573. 50.JONES, D. AND M.D. COLLINS (ED).(1993)SECTION 4:GRAM-NEGATIVE AEROBIC RODS AND COCCI'BERGEY'S MANUAL OF SYSTEMATIC BACTERIOLOGY', VOL. 1, PP. 140-402.THE WILLIAMS & WILKINS CO.,BALTIMORE. 51.LEEPER, S.A.,WARD, T.E.,AND ANDREWS, G.F.(1991) REPORT NO.EGG-2645; U.S. DEPARTMENT OF ENERGY;WASHINGTON D.C. 52.LIM, S. H., KIM, Y. S., AND KIM,S. D.(1991) PSEUDOMONAS STUTZERI YPL-1 GENETIC TRANSFORMATION AND ANTIFUNGAL MECHANISM AGAINST FUSARIUM SOLANI, AN AGENT OF PLANT ROOT ROT. APPL. ENVIR. MICROBIO. 57(2):510-516. 53.MATHUR, S.P., J.Y. DAILGLE, M. LEVESQUE, AND H. DINEL. (1986)THE FEASIBILITY OF PREPARING HIGH QUALITY COMPOSTS FROM FISH SCRAP AND PEAT WITH SEAWEEDES OR CRAB SCRAP. BIOLOGICAL AGRICULTURE AND HORTICULTURE 4:27-38. 54.MATHER, M. J. (1991) SPENT MUSHROOM COMPOST (SMC) AS A NUTRIENT SOURCE IN PEAT BASED POTTING SUBSTRATES. 13(2):645-650. 55.MCLOUGHLIN, T. J., QUINN, A. B., AND BOOKLAND, R. (1992) PSEUDOMONAS CEPACIA SUPPRESSION OF SUNFLOWER WILT FUNGUS AND ROLE OF ANTIFUNGAL COMPOUNDS IN CONTROLLING THE DISEASE. APPL. ENVIR. MICROBIO. 1760-1763. 56.MCGAN A. DICHL, JOHN S. CHAPMAN (1999) ASSOCIATION OF THE BIOCIDES 5-CHLORO-2-METHYL-ISOTHIAZOL-3-ONE WITH PSEUDOMONAS AERUGINOSA AND PSEUDOMONAS FLUORESCENS, INTERNATIONAL BIODETERIORATION &BIODEGRADATION, 44:191-199. 57.M GOBBETTI, A CORSETTI, E SMACCHI, J ROSSI (1997) PURIFICATION AND CHARACTERIZATION OF A PROTEINACEOUS COMPOUND FROM PSEUDOMONAS FLUORESCENS ATCC 948 WITH INHIBITORY ACTIVITY AGAINST SOME GRAM-POSITIVE AND GRAM-NEGATIVE BACTERIA OF DAIRY INTEREST, LAIT 77:267- 278. 58.MICHAEL CALLANAN, RAYMUND SEXTON, DAVID N. DOWLING, O'GARA (1996) REGULATION OF THE IRON UPTAKE GENES IN PSEUDOMONAS FLUORESCENS M114 BY PSEUDOBACTIN M114: THE PBRA SIGMA FACTOR GENE DOES NOT MEDIATE THE SIDEROPHORE REGULATORY RESPONSE, FEMS MICROBIOLOGY LETTERS, 144:61-66. 59.M.I. VAN DYKE, J.I. PROSSER (1998) EFFECT OF CELL DENSITY AND ATTACHMENT ON RESUSCITATION IN SOIL OF STARVED PSEUDOMONAS FLUORESCENS MON 787, FEMS MICROBIOLOGY ECOLOGY, 26: 63-70. 60.NORMAN LAYH, JULIAN PARRATT, ANDREW WILLETTS (1998)CHARACTERIZATION AND PARTIAL PURIFICATION OF AN ENANTIOSELECTIVE ARYLACETONITRILASE FROM PSEUDOMONAS FLUORESCENS DSM 7155, JOURNAL OF MOLECULAR CATALYSIS, 5:467-474. 61.P. BAGNASCO, L. DE LA FUENTE, G. GUALTIERI, F. NOYA AND A. ARIAS (1998) FLUORESCENT PSEUDOMONAS SPP. AS BIOCONTROL AGENTS AGAINST FORAGE LEGUME ROOT PATHOGENIC FUNGI, SOIL-BIOL. BIOCHEM., 30(10):1317-1322. 62.PHILIPPE, L., PETER, A. H. M., WILLEM J. D. K., CLAUDE, A. AND BOB, S. (1993) ANTAGONISTIC EFFECT OF NONPATHOGENIC FUSARIUM OXYSPORUM FO47 AND PSEUDOBACTIN 358 UPON PATHOGENIC FUSARIUM OXYSPORUM F. SP. DIANTHI. APPL. ENVIR. MICROBIOL. 74-82. 63.SIMON G. EDWARDS, J. PETER W. YOUNG, ALASTAIR H. FITTER (1998) INTERACTIONS BETWEEN PSEUDOMONAS FLUORESCENS BIOCONTROL AGENTS AND GLOMUS MOSSEAE, AN ARBUSCULAR MYCORRHIZAL FUNGUS, WITHIN THE RHIZOSPHERE, FEMS MICROBIOLOGY LETTERS, 166:297-303. 64.SUDARSHAN, N.R., HOOVER, D.G., AND KNORR, D. (1992) ANTIBACTERIAL ACTION OF CHITOSAN. FOOD BIOTECH., 6(3):257-272. 65.VASILKOV, P. B. (1965) ABRISSE DER GEOGRAPHISCHEN VERBREITUNG DER HUTPILZE IN DER SOWJETUNION. 66.VASU D. APPANNA, LAJOS G. GAZSO, MICHEAL ST. PIERRE (1996) MULTIPLE-METAL TOLERANCE IN PSEUDOMONAS FLUORESCENS AND ITS BIOTECHNOLOGICAL SIGNIFICANCE, JOURNAL OF BIOTECHNOLOGY, 52:75-80. 67.VITEK PROCEDURES MANUAL.(1995) BIOMERIEUX VITEK INC., U.S.A. 68.WANG. S. L., CHANG,W. T, AND LU, M. C.(1995)PROC. NATL. SCI. COUNC. ROC(B), 19:105. 69.WANG. S. L., CHIO,S. H, AND CHANG, W. T.(1997)PROC. NATL. SCI. COUNC. ROC(B), 21:71. 70.WANG. S. L., CHANG,W. T, (1997)APPL. ENVIR. MICROBIOL. 63-380. 71.WANG. S. L., CHIO,S. H,(1998)ENZYME. MICROB. TECHNOL, 22:629. 72.WANG. S. L., CHIO,S. H,(1998)ENZYME. MICROB. TECHNOL, 22:634. 73.WANG. S. L., YIEH, T. C. AND SHIH, I. L.(1999)ENZYME. MICROB. TECHNOL, 23:ACCEPTED. 74.Y. MOENNE-LOCCOZ, J. POWELL, P. HIGGINS, J. MCCARTHY, F. O'GARA (1998) AN INVESTIGATION OF THE IMPACT OF BIOCONTROL PSEUDOMONAS FLUORESCENS F113 ON THE GROWTH OF SUGARBEET AND THE PERFORMANCE OF SUBSEQUENT CLOVER-RHIZOBIUM SYMBIOSIS, APPLIED SOIL ECOLOGY, 7:225- 237. 75.ZADRAZIL, F. (1978) CULTIVATION OF PLEUROTUS. PP. 521-557.