

Study on the production of prodigiosin by isolated *Serratia marcescens* DYU

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ABSTRACT

The structure of prodigiosin is a linear tripyrrole which is a typical secondary metabolite produced by *Serratia marcescens*, *Vibrio psychroerythrus*, *Pseudomonas magnesiorubra*, and *Streptomyces* spp. The characteristics of prodigiosin families were reported characteristics of having not only having antibiotics and immunosuppressant activities, but also given anticancer activity recently. A bacterial strain which had higher prodigiosin producing was isolated, and designated as *Serratia marcescens* DYU according to its 16S rDNA sequences. The optimization of submerged culture conditions was studied for the production of prodigiosin by *Serratia marcescens* DYU in a shake culture. Maximum biomass and prodigiosin production of *Serratia marcescens* DYU were both observed at 30 °C and pH range of 5.5-7. Experimental results suggest that the effect of pH may be related to the amount of prodigiosin. The maximum prodigiosin productivity by *Serratia marcescens* DYU was about 580 mg/L when medium containing sucrose (5 g/L) and peptone (15 g/L) as carbon and nitrogen source, respectively. Fourier transform infrared (FTIR) analysis indicated that the red product may be a prodigiosin derivative. Nuclear magnetic resonance (NMR) analysis indicated that the red product was prodigiosin. All the stability analysis of prodigiosin indicated that Prodigiosin needs preservation at -20 ~-25 °C, it's sensitizing to light, so the production of prodigiosin needs prevent the light; at the same time, prodigiosin was sensitizing to pH, too, the prodigiosin will change it's color by various pH value, but won't affect the concentration of the prodigiosin.

Keywords : Pigment、Prodigiosin、*Serratia marcescens*

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REFERENCES

- 1.姚蕙芳。2000。由*Serratia marcescens* FC-R1生產紅色色素之研究。國立台灣大學農業化學研究所碩士論文。台北。
- 2.林芊。2005。由*Serratia marcescens*生產prodigiosin之研究。國立台灣農業化學研究所碩士論文。台北。
- 3.陳盈安。2005。以*Serratia marcescens* BCRC 11576 生產prodigiosin之研究。國立台灣農業化學研究所碩士論文。台北。
- 4.余宛儒。2006。以*Serratia marcescens* C3生產天然抗癌藥物-靈菌紅素之發酵製程開發。元智大學生物科技暨生物資訊研究所碩士論文。桃園。
- 5.魏毓宏。2009。抗癌新利器-靈菌紅素的發酵製程開發與應用。經濟部工業局。第30-37頁。
- 6.Akimenko, VK., Trutko SM. 1991. Physiological role of the oversynthesis of intermediates and the biosynthesis of metabolites for microorganism-producers. . Prikl Biokhim Mikbiol 27: 17-35.
- 7.Alexander, RH., Reichenbach, DD., Merendino, KA. 1969. Sermtia marcescens endocarditis: a review of the literature and report of a case involving a homograft replacement of the aortic valve. Arch Surg; 98: 287-291.
- 8.Allen, EG. 1967. Conditions of the colour change of prodigiosin. Nature; 216:929-931.
- 9.Altieri, D.C. 2003. Validating survivin as a cancer therapeutic target. Nat Rev Cancer 3: 46 – 54.
- 10.Altieri, D.C. 2006. The case for survivin as a regulator or microtubule dynamics and celldeath decisions. Curr Opin Cell Biol 18: 609 – 615.
- 11.Altieri, D.C. 2008. Survivin, cancer networks and pathway-directed drug discovery. Nat Rev Cancer 8: 61 – 70.
- 12.Azuma, T., Watanabe, N., Yagisawa, H., Hirata, H., Iwamura, M. and Kobayashi, Y. 2000. Induction of apoptosis of activated murine splenic T cells by cycloprodigiosin hydrochloride, a novel immunosuppressant. Immunopharmacology 46: 29-37.
- 13.Bennett, J. W. and Bentley, R. 2000. Seeing red: The story of prodigiosin. Adv Appl Microbiol 47: 1-32.
- 14.Bosi, C., Davin-Regli, A., Charrel, R., Rocca By Monnet D, Bollet C. 1996. Sermtia marcescens nosocomial outbreak due to contamination of hexetidine solution. J Hosp Infect 33: 217-224.
- 15.Bunting, M. I., Robinow, C. F., and Bunting, H. 1949. Factors affecting the elaboration of pigment and polysaccharide by *Serratia marcescens*. J Bacteriol 58:114.
- 16.Campas, C., Dalmau, M., Montaner, B., Barragan, M., Bellosillo, B. Colomer, D., Pons, G., Perez-Tomas, R. and Gil, J. 2003. Prodigiosin induces apoptosis of B and T cells from B-cell chronic lymphocytic leukemia. Leukemia 17: 746-750.
- 17.Castro, A. J. 1967. Antimalarial activity of prodigiosin. Nature 213: 903-904.
- 18.Chang, S., Sanada, M., Johdo, O., Ohta, S., Nagamatsu, Y. and Yoshimoto, A. 2000. High production of prodigiosin by *Serratia marcescens* grown on ethanol. Biotechnol Lett 22: 1761 – 1765.
- 19.Cho, L. K., Lowe, J. A., Maguire, R. B., Tsang, J. C. 1987. Relationship of prodigiosin condensing enzyme active to the biosynthesis of prodigiosin and its precursors in *Serratia marcescens*. Experientia 43: 397-399.
- 20.Clements-Jewery, S. 1976. The reversal of glucose repressed prodigiosin production in *Serratia marcescens* by the cyclic3' 5'-adenosine monophosphate inhibitor theophylline. Experientia 32: 421-422.
- 21.Cohen, PS., Maguire, JH.,

Weinstein, L., Infective endocarditis caused by gram-negative bacteria: a review of the literature, 1945-1977. 1980. *Pmg Cardiovasc Dis* 22: 205-242. 22.Cox, CE. 1985. Aztreonam therapy for complicated urinary tract infections caused by multidrug-resistant bacteria. *Rev Infect Dis* 7 Suppl 4: S767-S770. 23.Deol, B. S., Alden, J. R., Still, J. L., Robertson, A. V. and Winkler. 1974. Isolation and structure confirmation of norprodigiosin from a *Serratia marcescens* mutant. *Aust J Chem* 27: 2657. 24.Dubois, M., Gilles, K. A., Hamilton, J. K., Rebers, P. A. and Smith, F. 1956. Colorimetric method for determination of sugars and related substances. *Anal Chem* 28:350 – 356. 25.Duran, N., Rosa, MA. D.,Annibale, A., Gianfreda, L. 2002. Applications of laccases and tyrosinases (phenoloxidases) immobilized on different supports: A review. *Enzyme Microb Technol* 31:907-931. 26.Feng, J. S., Webb, J. W. and Tsang, J. C. 1982. Enhancement by sodium dodecyl sulfate of pigment formation in *Serratia marcescens*. *Appl. Environ Microbiol:* 43:850-853. 27.Ferguson, T., Wilcken, N., Vagg, R., Ghersi, D., Nowak, A.K. 2007. Taxanes for adjuvant treatment of early breast cancer. *Cochrane Database Syst Rev* 17: CD004421. 28.Francisco, R., Perez-Tomas, R., Gimenez-Bonafe, P., Soto-Cerrato, V., Gimenez-Xavier, P.,Ambrosio, S. 2007. Mechanisms of prodigiosin cytotoxicity in human neuroblastoma cell lines. *Eur J Pharmacol* 572: 111 – 119. 29.Furstner, A. 2003. Chemistry and biology of roseophilin and the prodigiosin alkaloids: a survey of the last 2500 years. *Angew. Chem. Int. Ed.* 42: 3582-603. 30.Gaughran, ERL. 1968. Division of microbiology from superstition to science: the history of a bacterium. *Tmns NY Acad Sci. The meeting of the division. January 26.* 31.Gerber, N. N. 1975. Prodigiosin like pigment. *Crit Rev Microbiol* 3: 469-485. 32.Giri, A. V., Anandkumar, N., Muthukumaran, G. and Pennathur, G. 2004. A novel medium for the enhanced cell growth and production of prodigiosin from *Serratia marcescens* isolated from soil. *BMC Microbiol* 4: 1-10. 33.Goncalves, A., Braguer, D., Kamath, K., Martello, L., Briand, C., Horwitz, S., Wilson, L.,Jordan, M.A. 2001. Resistance to taxol in lung cancer cells associated with increased microtubule dynamics. *Proc Natl Acad Sci U. S. A.* 98: 11737 – 11741. 34.Gouin, F., Papazian, L., Martin, C .1993. A non-comparative study of the efficacy and tolerance of ceferpime in combination with amikacin in the treatment of severe infections in patients in intensive care. *J Antimicrob Chemother Suppl* B32:205-214. 35.Grimont, F. P. and Grimont,, A. D. 2006. The genus *Serratia*. *The Prokaryotes* 6: 219-244. 36.Grimont, P. A. D. and Grimont, F. 1978. *Serratia*. In *Bergey's Manual of Systematic Bacteriology* (N. R. Krieg. Ed.). Williams and Wilkins, Baltimore USA 1:477-484. 37.Grimont, PAD., Grimont, F., Genus, VIII. 1984. Sermtia. In: Krieg NR, Holt JG (eds) *Bergey 's Manual of systematic bacteriology*, vol 1. Baltimore, Williams and Wilkins.: 477-484. 38.Han, S. B., Park, S. H., Jeon, Y. J., Kim, H. M. and Yang K. H. 2001. Prodigiosin blocks T cell activation by inhibiting interleukin-2Ra expression and delays progression of autoimmune diabetes and collagen-induced arthritis. *J. Pharmacol. Exp Ther* 299: 415-42. 39.Harrison, F. C. 1924. The miraculous micro-organism. *Trans Roy Soc Canada Sec V Series III* 18: 1-17. 40.Hayes, D.F., Thor, A.D., Dressler, L.G.,Weaver, D., Edgerton, S., Cowan, D., Broadwater, G., Goldstein, L.J., Martino, S., Ingle, J.N., Henderson, I.C., Norton, L., Winer, E.P., Hudis, C. A., Ellis, M.J., Berry, D.A., Cancer and Leukemia Group B (CALGB) Investigators. 2007. HER2 and response to paclitaxel in node-positive breast cancer. *N Eng J Med* 357: 1496 – 1506. 41.Heinemann, B., Howard A.J. and Palocz H. J. 1970. Influence of dissolved oxygen levels on production of L-asparaginase and prodigiosin by *Serratia marcescens*. *Appl Microbiol* 19: 800-804. 42.Ho, T.-F., Ma, C.-J., Lu, C.-H., Tsai, Y.-T., Wei, Y.-H., Chang, J.-S., Lai, J.-K., Cheuh, P.-J., Yeh, C.-T., Tang, P.-C., Chang, J.T., Ko, J.-L., Liu, F.-S., Yen, H.E., Chang, C.-C. 2007. Undecylprodigiosin selectively induces apoptosis in human breast carcinoma cells independent of p53. *Toxicol Appl Pharmacol* 225: 318 – 328. 43.Hoffman, W.H., Biade, S., Zilfou, J.T., Chen, J., Murphy, M. 2002. Transcriptional repression of the anti-apoptotic survivin gene by wild type p53. *J Biol Chem* 277: 3247 – 3257. 44.Hubbard, R. and Rimington, C. 1950. The biosynthesis of prodigiosin, the tripyrrylmethene pigment from *Bacillus prodigiosin* (*Serratia marcescens*). *Biochem J* 46: 220-225. 45.Hung, M.-C. 2004. Cancer-specific activation of the survivin promoter and its potential use in gene therapy. *Cancer Gene Ther* 11: 740 – 747. 46.Jemal, A., Siegel, R.,Ward, E., Hao, Y., Xu, J., Murray, T., Thun, M.J. 2008. Cancer statistics. *CA Cancer J Clin* 58: 71 – 96. 47.Kalesperis, G. S., Prahlad, K. V. and Lynch, D. L. 1975. Toxicogenic studies with the antiniotic pigments from *Serratia marcescens*. *Can J Microbiol* 21: 213-220. 48.Kamate, K., Okamoto, S., Oka, Si. Kamate, H., Agisawa, H.Y. and Hirata, H. 2000. Cycloprodigiosin hydrochloride suppresses tumor necrosis factor (TNF) – induced transcriptional activation by NF- B. *FEBS Lett* 507: 74-80. 49.Kawauchi, K., Tobiume, K., Iwashita, K., Inagaki, H., Morikawa, T., Shibukawa, Y.,Moriyama, Y., Hirata, H., Kamata, H. 2008. Cycloprodigiosin hydrochloride activates the Ras-PI3K-Akt pathway and suppresses protein synthesis inhibitor-induced apoptosis in PC12 cells. *Biosci Biotechnol Biochem* 72: 1564 – 1570. 50.Kobayashi, N. and Ichikawa, Y. 1985. Decrease in respiration activity related to prodigiosin synthesis in *Serratia marcescens*. *Microbiol Immunol* 29:301-308. 51.Kobayashi, N., Ichikawa, Y. 1985. Decrease in respiration activity related to prodigiosin synthesis in *Serratia marcescens*. *Microbiol Immunol* 89: 301-308. 52.Komer, RJ., Nicol, A., Reeves, DS., MacGowan AP. 1994. Hows J.Ciprofloxacin resistant *Serratia marcescens* endocarditis as a complication of non-Hodgkin ' s lymphoma. *J Infect* 29:73-76 53.Lack, A. 1949. Prodigiosin. I. Antibiotic action on *Coccidioides immitis* in vitro. *Proc Soc Exp Biol Med* 72: 656-658. 54.Lewis, S. M. and Corpe, W. A. 1964. Prodigiosin-producing bacteria from marine sources. *Appl Environ Microbiol* 12: 13-17. 55.Mapari, S. A. S., Nielsen, K. F., Larsen, T. O., Frisvad, J. C., Meyer, A. S., and Thrane, U. 2005. Exploring fungal biodiversity for the production of water-soluble pigments as potential natural food colorants. *Curr. Opin. Biotechnol* 16 :231 – 238. 56.McGrogan, B.T., Gilmartin, B., Carney, D.N., McCann, A., 2008. Taxanes, microtubules and chemoresistant breast cancer. *Biochim Biophys Acta* 1785: 96 – 132. 57.Montaner, B. and Perez-Tomas, R. 2001. Prodigiosin-induced apoptosis in human colon cancer cells. *Life Sciences* 68: 2025-2036. 58.Montaner, B. and Perez-Tomas, R. 2002. The cytotoxic prodigiosin induces phosphorylation of p38-MAPK but not of SAPK/JNK. *Toxicol Lett* 12: 89-93 59.Montaner, B., Navarro, S., Pique, M., Vilaseca, M., Martinell, M., Giralt, E., Gil, J. and Perez-Tomas, R. 2000. Prodigiosin from the supernatant of *Serratia marcescens* induces apoptosis in haematopoietic cancer cell lines. *Br J Pharmacol* 131: 585-593. 60.Montaner, B.,Castillo-Avila, W. Martinell, M., Ollinger, R.,Aymami, J., Giralt, E.and Perez-Tomas, R. 2005. DNA interaction and dual topoisomerase I and

II inhibition properties of the anti-tumor drug prodigiosin. *J Toxicol Sci* 85: 870-879. 61.Morrison, D. A. 1966. Prodigiosin synthesis in mutants of *Serratia marcescens*. *Indian J Pathol Bacteriol* 91: 1599-1604. 62.Mortellaro, A., Songia, S., Gnocch, P., Ferrari, M., Fornasiero, C., Alessio, R., Isetta, A., Colotta, F. and Golay, J. 1999. New immunosuppressive drug PNU156804 blocks IL-2 dependent proliferation and NF- κ B and AP-1 activation. *Journal of Immunology* 162: 7102-7109. 63.Moser, A. and Steiner, W. 1975. The influence of the term kd for endogenous metabolism on the evaluation of Monod kinetics for biotechnological processes. *Appl Microbiol Biotechnol* 1: 281- 289. 64.Moss, M. O. 1983. A note on a prodigiosin-producing pseudomonad isolated from a owlend river. *J Appl Bacteriol* 55: 373-375. 65.Ngan, C.Y., Yamamoto, H., Takagi, A., Fujie, Y., Takemasa, I., Ikeda,M., Takahashi-Yanaga, F.,Sasaguri, T., Sekimoto,M.,Matsuura,N.,Monden,M. 2008. Oxaliplatin inducesmitotic catastrophe and apoptosis in esophageal cancer cells. *Cancer Sci* 99: 129 – 139. 66.O'Connor, D.S.,Wall, N.R., Porter, A.C.G., Altieri, D.C. 2002. A p34cdc2 survival checkpoint in cancer. *Cancer Cell* 2: 43 – 54. 67.Oller, A. R. 2005. Media effects of sugars on pigmentation and antibiotic susceptibility in *Serratia marcescens*. *Trans Mo Acad Sci*: 243-246. 68.Pennati, M., Folini, M., Zaffaroni, N. 2007. Targeting survivin in cancer therapy: fulfilled promises and open questions. *Carcinogenesis* 28: 1133 – 1139. 69.Perez-Tomas, R., Montaner, B., Llagostera, E., Soto-Cerrato, V. 2003. The prodigiosins,proapoptotic drugs with anticancer properties. *Biochem Pharmacol* 66:1447 – 1452. 70.Phillips, I., King, A. 1977. *Serratia marcescens* in hospital practice. *Lancet* 1: 538. 71.Pinches, A. and Pallent, L. J. 1986. Rate and yield relationship in the production of xanthan gum by batch fermentation using complex and chemically defined growth media. *Biotechnol Bioeng* 26: 1484- 1496. 72.Paruchuri, D. K. and Harshey, M. R. 1987. Flagellar variation in *Serratia marcescens* is associated with color variation. *J. Bacteriol.*, 169:61-65. 73.Qadri, S. H., M. and Willaims, R. P. 1974. Incorporation of amino acid carbon into prodigiosin synthesized by nonproliferating cells of *Serratia marcescens*. *Can J Microbiol* 20: 461-468. 74.Rapoport, H. and Holden, K. G. 1961. The synthesis of prodigiosin. *J Am Chem Soc* 84:635-642. 75.Reed, G. B. 1937. Independent changes of several characteristics in *S. marcescens*. *J Bacteriol.*, 34: 255-265. 76.Repoport, H. and Holden, K. G. 1961. The synthesis of prodigiosin. *J Am Chem Soc* 83: 635-642. 77.Robinson, D. K. and Wang, D. I. C. 1988. A transport controlled bioreactor for the simultaneous production and concentration of xanthan gum. *Biotechnol Progr* 4: 231- 241. 78.Simpson, D., Plosker, G.L. 2004. Paclitaxel: as adjuvant or neoadjuvant therapy in early breast cancer. *Drugs* 64: 1839 – 1847. 79.Sole, M., Rius, N. and Loren, J. G. 2000. Rapid extracellular acidification induced by glucose metabolism in non-proliferating cells of *Serratia marcescens*. *Int Microbiol* 3: 39-43. 80.Sole, M., Rius, N., Francia, A. and Loren, J. G. 1994. The effect of pH on prodigiosin production by non-proliferation cells on *Serratia marcescens*. *Lett Appl Microbiol* 19: 341-344. 81.Soto-Cerrato, V., Llagostera, E., Montaner, B., Scheffer, G.L., Perez-Tomas, R. 2004. Mitochondria-mediated apoptosis operating irrespective of multidrug resistance in breast cancer cells by the anticancer agent prodigiosin. *Biochem Pharmacol* 68:1345 – 1352. 82.Soto-Cerrato, V., Vinals, F., Lambert, J.R., Kelly, J.A., Perez-Tomas, R. 2007b. Prodigiosin induces the proapoptotic gene NAG-1 via glycogen synthase kinase-3 activity in human breast cancer cells. *Mol Cancer Ther* 6: 362 – 369. 83.Soto-Cerrato, V., Vinals, F., Lambert, J.R., Perez-Tomas, R. 2007a. The anticancer agent prodigiosin induces p21WAF1/CIP1 expression via transforming growth factor-beta receptor pathway. *Biochem Pharmacol* 74: 1340 – 1349. 84.Sundaramoorthy, N., Yogesh, P.,Dhandapani, R. 2005. Production of prodigiosin from *Serratia marcescens* isolated from soil. *Indian J Sci Technol* 2: 32-34. 85.Tsaos, W ., RJddb, A. M., HE, D.G., Changc, J., and Floss, H. G. 1985. Identification of a red pigment from *Streptomyces coelicolor* A3(2) as a mixture of prodigiosin derivatives. *J. Antibiotics* 38: 128-131. 86.Wagner, M., Schmelz, K., Dorken, B., Tamm, I. 2008. Transcriptional regulation of human survivin byearly growth response (Egr)-1 transcription factor. *Int J Cancer* 122:1278 – 1287. 87.Wall, N.R., O'Connor, D.S., Plescia, J., Pommier, Y., Altieri, D.C. 2003. Suppression of survivin phosphorylaion on Thr34 by flavopiridol enhances tumor cell apoptosis. *Cancer Res* 63: 230 – 235. 88.Wei, Y. H. and Chen, W. C. 2005. Enhanced production of prodigiosin-like pigment from *Serratia marcescens* SM R by medium improvement and oil-supplementation strategies. *J Biosci Bioeng* 99: 616-622. 89.Wei, Y. H., Yu, W. J. and Chen, W. C. 2005. Enhanced undecylprodigiosin production from *Serratia marcescens* SS-1 by medium formulation and amino-acid supplementation. *J Biosci Bioeng* 99: 616 – 622. 90.Weiss, R. and Ollis, D. 1980. Extracellular microbial polysaccharides. . Substrate, biomass, and product kinetic equations for batch xanthan gum fermentation. *Biotechnol Bioeng* 22: 859- 873. 91.Wheat, R.P., Zuckerman, A., Rank, L.A. 1951. Infection due to Chromobacteria: report of eleven cases. *Arch Intern Med* 88:461-466. 92.Williams, R. P., Gott, C. L., Hussian, Qadri, S. M. and Scott, R. H. 1971. Influence of temperature of incubation and type of growth medium on pigmentation in *Serratia marcescens*. *J Bacteriol* 106: 438-443. 93.Williams, R. P. 1973. Biosynthesis of prodigiosin, a secondary metabolite of *Serratia marcescens*. *Appl Microbiol* 25: 396-402. 94.Williams, R. P., Gott, C. L., Qadri, S. M. 1971. Induction of pigmentation in nonproliferating cells of *Serratia marcescens* by addition of single amino acids. *J Bacteriol* 106: 444-448. 95.Williams, R. P., Qadir, S. M. H. 1980. The pigment of *Serratia*. CRC Press Boca Raton Florida: 31-75. 96.Williamson, N. R., Simonsen, H. T., Ahmed, R. A., Goldet, G., Slater, H., Woodley, L., Leeper, F. J. and Salmond, G. P. 2005. Biosynthesis of the red antibiotic, prodigiosin, in *Serratia*: identification of a novel 2-methyl-3-n-amyl-pyrrole (MAP)assembly pathway, definition of the terminal condensing enzyme, and implications for undecylprodigiosin biosynthesis in *Streptomyces*. *Microbiol. Mol Biol Rev* 56: 971-989. 97.Williamson, N.R., Fineran, P.C., Leeper, F.J., Salmond, G.P. 2006. The biosynthesis and regulation of bacterial prodiginines. *Nat Rev Microbiol* 4: 887 – 899. 98.Williamson, N.R., Fineran, P.C., Leeper, F.J., Salmond, G.P. 2006. The biosynthesis and regulation of bacterial prodiginines. *Nat Rev Microbiol* 4: 887 – 899. 99.Woods, CRY., Versalovic, J, Koeuth, T., Lupski, JR. 1971. Analysis of relationships among isolates of Citmbacter diversus by using DNA fingerprints generated by repetitive sequence-based primers in the polymerase chain reaction. *J Clin Microbiol* 30:2921-2929 100.Wrede, F. and Rothhas, A. 1934. tYber das Prodigiosin, den roten Farbstoff des Bacillus prodigiosus. VI. Hoppe-Seyler's Z. physiol. Chem 226: 95-107. 101.Xu, R., Zhang, P., Huang, J., Ge, S., Lu, J., Qian, G. 2007. Sp1 and Sp3 regulate basal transcription of the survivin gene. *J. Am Chem Soc* 356: 286 – 292. 102.Yamamoto, D., Kiyozuka,Y., Uemura, Y., Yamamoto, C., Takemoto, H. and Hirata, H.

2000. Cycloprodigiosin hydrochloride, a H⁺/Cl⁻ Symporter, induces apoptosis in human breast cancer cell line. *J Cancer Res Clin Oncol* 126: 191-197.

103.Yamamoto, H., Ngan, C.Y., Monden, M. 2008. Cancer cells survive with survivin. *Cancer Sci* 99: 1709 – 1714.

104.Yamashita, M., Nakagawa, Y., Li, H. and Matsuyama, T. 2001. Silica gel-dependent production of prodigiosin and serrawettins by *Serratia marcescens* in a liquid culture. *Microb Environ* 16: 250-254.

105.Yasutaka, M., Kenji, K., Quamrul, H., Toshifumi, S., Tuji, M., Kenji, Y. and Eiichi, T. 1997. Purification and characterization of a cold-active protease from psychrotrophic *Serratia marcescens* AP3081. *J Am Oil Chem Soc* 74: 1377-1383.

106.Yu, V. L. 1979. *Serratia marcescens*. Historical perspective and clinical review. *N. Eng. J. Med* 300:887-893.

107.Zaffaroni, N., Pennati, M., Colella, G., Perego, P., Supino, R., Gatti, L., Pilotto, S., Zunino, F., Daidone, M.G. 2002. Expression of the anti-apoptotic gene survivin correlates with taxol resistance in human ovarian cancer. *Cell Mol Life Sci* 59: 1406 – 1412.

108.Zhang, J., Yaling Shen, Y., Liu, J., Wei, D. 2005. Antimetastatic effect of prodigiosin through inhibition of tumor invasion. *Biochem Pharmacol* 69: 407 – 414.

109.Zhou, J., O'Brate, A., Zelnak, A., Giannakakou1, P. 2004. Survivin deregulation in β -tubulin mutant ovarian cancer cells underlies their compromised mitotic response to taxol. *Cancer Res* 64: 8708 – 8714.