## INVESTIGATION ON BIOFILM FORMATION STAGES IN SOME STRAINS OF *Pseudomonas fluorescens* AND THE INFLUENCE OF SOME NUTRITIONAL FACTORS ON BIOFILM FORMATION OF SELECTED STRAIN<sup>\*</sup>

## A. KAMALI\*\*, M. AHMADZADE and K. BEHBODI<sup>1</sup>

(Received: 29. 10 .2010; Accepted: 29. 7. 2011)

## Abstract

Biofilm formation on abiotic surfaces initiates in response to environmental cues, and the same is true for attachment to the plant surfaces. In this study the detection of biofilm formation of some *Pseudomonas fluorescens* strains was done indirectly by determining the extent of CV-stained cells attached to a surface. *Pseudomonas fluorescens* UTPF98 was selected because of its great ability of biofilm formation. And then the stages of biofilm formation (reversible and irreversible attachment, microcolony formation and macrocolony formation with exopolysacharid (EPS) production) in mentioned strain was investigated on glass slides. At last the effect of some nutritional factors like cations (Mg<sup>2+</sup>, B<sup>3+</sup>, Cu<sup>2+</sup>, Co<sup>2+</sup>, Mo<sup>6+</sup>, Zn<sup>2+</sup>, Mn<sup>2+</sup>, Ca<sup>2+</sup> and Fe<sup>2+</sup>), carbon sources (arabinose, rhamnose, glucose, mannose, galactose and xylose), aminoacids (aspartic acid, asparagine, phenylalanine, leucine, threonine, proline, glutamic acid, glutamine, arginine, tyrosine, histidine, alanine, lysine, isoleucine and glycine) and phosphorus on biofilm formation was investigated in this study. Cations had various effects on biofilm formation. All carbon sources and aminoacids tested promoted biofilm formation. In contrast, phosphorus reduced biofilm formation. These results clearly show that nutritional status of the medium can influence biofilm formation in vitro. For successful biocontrol by fluorescent pseudomonads, one needs to understand which and how nutritional status affects the biofilm formation in potential biocontrol products.

Keywords: Biofilm, Pseudomonas fluorescens, Nutritional factors.

See Persian text for figures and tables (Pages 497-4V•).

<sup>\*:</sup> A Part of MSc. Thesis, Submitted to College of Agriculture and Natural Resources, University of Tehran, Tehran, Iran.

<sup>\*\*:</sup> Corresponding Author, Email: arghavan.kamaly@gmail.com

<sup>1.</sup> Former MSc. Student, Assoc. Prof. and Assis. Prof. of Plant Pathology, Respectively, College of Agriculture and Natural Resources, University of Tehran, Tehran, Iran.

## References

- BANIN, E., VASIL, M. L. and GREENBERG, E. P. 2005. Iron and *Pseudomonas aeruginosa* biofilm formation. **Proc. Natlur. Acad. Sci. U S A.** 102: 11076–11081.
- COSTERTON, J.W., LEWANDOWSKE, Z., CALDWELL, D.E., KORBRE, D.R. and LAPPIN-SCOTT, H.M., 1995. Microbial biofilms. **Annu. Rev. Microbiol.** 49: 711–745.
- DAVEY, M. E. and O'TOOLE, G. A. 2000. Microbial biofilms: from ecology to molecular genetics. Microbiol. Mol. Biol. Rev. 64: 847-867.
- FLETCHER, M. 1977. The effects of culture concentration and age, time, and temperature on bacterial attachment to polystyrene. **Can. J. Microbiol.** 23: 1-6.
- FLETCHER, M. 1988. Attachment of *Pseudomonas fluorescens* to glass and influence of electrolytes on bacterium-substratum separation distance. J. Bacteriol. 170: 2027-2030.
- GEESEY, G. G., WIGGLESWORTH-COOKSEY, B. and COOKSEY, K. E. 2000. Influence of calcium and other cations on surface adhesion of bacteria and diatoms: A. review. **Biofouling** 15: 195–205.
- KNEE, E. M., GONG, F. C., GAO, M., TEPLITSKI, M., JONES, A. R., FOXWORTHY, A., MORT, A. J., and BAUER, W. D. 2001. Root mucilage from pea and its utilization by rhizosphere bacteria as a sole carbon source. Mol. Plant. Microbe. Intract. 14: 775–784.
- MADDULA, V. S. R. K., ZHANG, Z., PIERSON, E. A. and PIERSON III, L. S. 2006. Quorum sensing and phenazines are involved in biofilm formation by *Pseudomonas chlororaphis* (aureofaciens) Strain 30-84. Microb. Ecol. 52: 289–301.
- MCELDOWNEY, S. and FLETCHER, M. 1986. Variability of the influence of physicochemical factors affecting bacterial adhesion to polystyrene substrata. **App. Environ. Microbiol.** 52: 460–465.
- MOLINA, M. A., RAMUS, J. L. and EPINOSA-URGEL, M. 2003. Plant *Pseudomonas fluorescens* associated biofilms. Environ. Sci. Technol. 2: 99–108.
- O'TOOLE, G. A. and KOLTER, R. 1998. Initiation of biofilm formation in WCS365 proceeds via multiple, convergent signaling pathways: a genetic analysis. **Mol. Microbiol.** 28: 449-461.
- SHAKERI, Sh., KASRA KERMANSHAHI, R. and EMTIAZI, G. 2005. Study of *Micrococcus luteus* biofilm formation and evaluation of antimicrobial effects on its biofilm by microtiter plate test. Iran. J. Biol. 18: 5-14.
- SHIRZAD, A., SHARIFI-TEHRANI, A., AHMADZADEH, M., BEHBOUDI, K. and JAVAN-NIKKHAH, M. 2008. The expression of chitinase genes (nag1 and ech42) of Trichoderma atroviride in interaction with *Pseudomonas fluorescens*. Iran. J. Plant Pathol. 44: 330-346. (In Farsi With English Summary).
- SLININGER, P. J. and Jakson, M. A. 1992. Nutritional factors regulating growth and accumulation of phenazine 1-carboxylic acid by *Pseudomonas fluorescens* 2-79. Appl. Microbiol. Biotechnol. 37: 388-392.
- SONG. B. and LEFF, L. G. 2006. Influence of magnesium ions on biofilm formation by *Pseudomonas* fluorescens. Microbiol. Res. 161: 355-361.
- WIMPENNY, J. W. T. and COLASANTI, R. 1997. A unifying hypothesis for the structure of microbial biofilms based on cellular automaton models. FEMS Microbiol. Ecol. 22: 1-1