

Two roles of salicylic acid in the accumulation of polyhydroxyalkanoic acid in *Pseudomonas fluorescens* BM07 grown with mixtures of fructose and 11-phenoxyundecanoic acid: inhibition of PhaG-mediated aliphatic monomer-unit incorporation and shift of aromatic monomer-unit distribution

Ji Hoon Shim¹, Jong Kook Rho¹, Mun Hwan Choi², and Sung Chul Yoon^{1,2,3}

Biomaterials Science Laboratory,

¹Division of Applied Life Sciences (BK21), Graduate School

²Environmental Biotechnology National Core Research Center;

³Division of Life Science, College of Natural Sciences,

Gyeong sang National University, Chinju 660-701, Korea

TEL: +82-55-751-5942, FAX: +82-55-753-0765

Medium-chain-length-polyhydroxyalkanoic acid (MCL-PHA) formed in *Pseudomonas* spp. have a rather broad distribution of monomer-units whose precursors are supplied via ω -oxidation degradation of MCL fatty acids fed as the carbon source and/or via PhaG enzyme catalyzing the transformation of 3-hydroxyacyl-ACPs' derived from sugars to their coenzyme A forms. The material properties of MCL-PHA strongly depend on the length of side-chains of the constituent comonomer-units and their distribution. Thus, a monomer-unit ratio modulation may be necessary to improve their physical properties. We introduce a method to modulate the monomer-unit distribution of MCL-PHA to some extent using an unmetabolizable inhibitor. It was found that salicylic acid (SA), in a concentration dependent manner, inhibited the synthesis of PHA in *Pseudomonas fluorescens* BM07 from fructose as well as shifted the distribution of monomer-units derived from a MCL fatty acid co-added as carbon source (e.g., 11-phenoxyundecanoic acid (11-POU)). For the cells grown on medium supplemented with 50 mM fructose and 3 mM 11-POU in which monomer supplying through PhaG enzyme by fructose metabolism was partially open,

addition of SA resulted in the suppression of aliphatic monomer-units, probably due to the inhibition of PhaG, but the total content of aromatic monomer-units was rather enhanced compared to the control and a significant enhancement of the content of longer aromatic monomer-units was observed. Much more significant shift was observed in the cells grown with 50 mM fructose plus 5 mM 11-POU in which aliphatic PHA synthesis from fructose was maximally suppressed by 11-POU even in the absence of SA (the total amount of incorporated aliphatic monomer-units was only ~10 mol%): 3-hydroxy-5-phenoxyvalerate, from 66 (control) to 31 mol% at 1.5 mM SA 3-hydroxy-7-phenoxyheptanoate, from 30 to 48mol%; 3-hydroxy-9- phoxynonanoate, from 4 to 21 mol%, respectively. Thus the intervening role of SA in the accumulation of aromatic PHA in *P. fluorescens* BM07, probably resulted from the inhibition of -oxidation enzyme(s), was shifting of the aromatic monomer-unit distribution to longer units as well as significantly increasing the yield of conversion of 11-POU into PHA, which was possible only under the cometabolism of 11-POU and fructose.

References

1. Anderson, A.J. and E.A. Dawes. 1990. *Microbiol. Rev.* 54:450-472.
2. Madison, L.L. and G.W. Huisman. 1999. *Microbiol. Mol. Biol. Rev.* 63:21-53.
3. Choi, M.H., H.-J. Lee, J.K. Rho, S.C. Yoon, J.D. Nam, D. Lim, and R.W. Lenz, 2003, *Biomacromolecules*, 4:38-45.
4. Green, P.R., J. Kemper, L. Schechtman, L. Guo, M. Satkowski, S. Fiedler, A. Steinbchel, and B.H.A. Rehm. 2002. *Biomacromolecules*, 3:208-213.
5. Lee, H.-J. J.K. Rho, K.A. Noghabi, S.E. Lee, M.H. Choi, and S.C. Yoon, 2004. *Microbiol. Biotechnol.* 14:1256-1266.
6. Qi, Q., A. Steinbchel, and B.H.A. Rehm. 1998. *FEMS Microbiol. Lett.* 167:89-94.
7. Lee, H.-J. M.H. Choi, T.-U. Kim, and S.C. Yoon, 2001. *Appl. Environ. Microbiol.* 67:4963-4974.
8. Lee, S.Y.; Lee, Y.; Wang, F. *Biotechnol. Bioeng.* 1999. 65, 363-368.
9. Song, J.J. and S.C. Yoon. 1996. *Appl. Environ. Microbiol.* 62:536-544.
10. Price, C.D.T., I.R. Lee, and J.E. Gustafson. 2000. *J. Biochem. Cell Biol.* 32:1029-1043.
11. Choi, M.H. and S.C. Yoon. 1994. *Appl. Environ. Microbiol.* 60:3245-3254.

12. Hinz, B., V. Kraus, A. Pahl, and K. Brune. 2000. *Biochem. Biophys. Res. Commun.* 274:197-202.
13. Rehm, B. H. A., N. Krger, A. Steinbchel. 1998. *J. Biol. Chem.* 273: 24044-24051.
14. Fiedler, S., A. Steinbchel, and B. H. A. Rehm. 2000. *Appl. Environ. Microbiol.* 66: 2117-2124.
15. Hoffmann, N., A. Steinbchel, and B. H. A. Rehm. 2000. *Appl. Microbiol. Biotechnol.* 54: 665-670.
16. Song, J.J., M.H. Choi, S.C. Yoon and N.E. Huh. 2001. *J. Microbiol. Biotechnol.* 11:435-442.