

STRUCTURAL ANALYSIS OF POLYHYDROXYALKANOATES SYNTHASE GENES

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ABSTRACT

POLY(HYDROXYALKANOATES) (PHAS) ARE MICROBIAL POLYESTERS THAT CAN BE USED AS COMPLETELY BIODEGRADABLE POLYMERS. BECAUSE OF THE HIGH PRODUCTION COST, THE APPLICATION OF PHA IS LIMITED. THERE ARE MANY TYPES OF PHAS. ACCORDING TO THE CHAIN LENGTH OF CARBON ATOMS OF THE MONOMERS, THEY ARE CLASSIFIED TO THREE GROUPS: PHASCL, PHAMCL, AND PHALCL. PSEUDOMONAS RESINOVORANS HAS THE ABILITY TO PRODUCE PHAMCL COPOLYMER WHEN GROWN ON TALL-OW OR FATTY ACID. HOWEVER, THE PRODUCING STRAINS AS WELL AS THE CARBON SUBSTRATES EMPLOYED GIVE VARIETY TO THE FEATURES OF PHAMCL, INCREASING THEIR POTENTIALITY IN EXPLOITATION. IN THIS STUDY, THE PHA POLYMERS WERE CHARACTERIZED BY NILE RED COLONY STAINING, SOXTEC EXTRACTION, AND GAS CHROMATOGRAPHY. IT WAS SHOWN THAT C8 AND C10 WERE THE MAJOR MONOMER REPEAT-UNIT OF THE POLYMER. TO INVESTIGATE THE GENES FOR THE SYNTHESIS OF PHA IN P. RESINOVORANS, WE CONSTRUCTED A GENOMIC LIBRARY FROM THIS STRAIN. AFTER SEVERAL TIMES OF SCREENING, A PHAGE CLONE WITH A 15 KB ECORI FRAGMENT WAS FOUND TO CONTAIN PHA SYNTHASE (PHAC2). THE STRUCTURES OF THE GENES WILL BE REVEALED AND THEIR FUNCTIONS, SUCH AS EXPRESSION AND SUBSTRATE SPECIFICITY, WILL BE FURTHER INVESTIGATED.

Keywords : BIODEGRADABLE POLYMERS, POLY(HYDROXYALKANOATES), PHA, PHA SYNTHASE, P. RESINOVORANS

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REFERENCES

1. ABE C., Y. TAIMA, Y. NAKAMURA, AND Y. DOI (1990) NEW BACTERIAL COPOLYESTERS OF 3-HYDROXY-ALKANOATES AND 3-HYDROXY- ω -FLUORO-ALKANOATES PRODUCED BY PSEUDOMONAS OLEVORANS. POLYM. COMMUN., 31:404-406.
2. AKIYAMA M., Y. TAIMA, AND Y. DOI (1992) PRODUCTION OF POLY(3-HYDROXYALKANOATES) BY A BACTERIUM OF THE GENUS ALCALIGENES UTILIZING LONG-CHAIN FATTY ACIDS. APPL. MICROBIOL. BIOTECHNOL., 37:698-701.
3. ANDERSON A. J., AND E. A. DAWES (1990) OCCURRENCE, METABOLISM, METABOLIC ROLE, AND INDUSTRIAL USES OF BACTERIAL POLYHYDROXY-ALKANOATES. MICROBIOL. REV., 54:450-472.
4. ASHBY R. D., A.-M. CROMWICK, AND T. A. FOGLIA (1998) RADIATION CROSSLINKING OF A BACTERIAL MEDIUM-CHAIN-LENGTH POLY(HYDROXYALKANOATE) ELASTOMER FROM TALLOW. INT. J. BIOL. MACROMOL., 23:61-72.
5. BRANDL H., R.A. GROSS, R.W. LENZ, AND R.C. FULLER

(1988) PSEUDOMONAS OLEOVORANS AS A SOURCE OF POLY(β -HYDROXY-ALKANOATES) FOR POTENTIAL APPLICATIONS AS BIODEGRADABLE POLYESTERS. APPL. ENVIRON. MICROBIOL., 54:1977-1982. 6. BRANDL H., R.A. GROSS, R.W. LENZ, AND R.C. FULLER (1990) PLASTICS FROM BACTERIA AND FOR BACTERIA: POLY(β -HYDROXYBUTYRATES) AS NATURAL, BIOCOMPATIBLE, AND BIODEGRADABLE POLYESTERS. ADV. BIOCHEM. ENGIN. BIOTECHNOL., 41:77-93. 7. CHOI M. H., AND S. C. YOON (1994) POLYESTER BIOSYNTHESIS CHARACTERISTICS OF PSEUDOMONAS CITRONELLIS GROWN ON VARIOUS CARBON SOURCES, INCLUDING 3-METHYL-BRANCHED SUBSTRATES. APPL. ENVIRON. MICROBIOL., 60:3245-3254. 8. CROMWICK A.-M., T. FOGLIA, AND R. W. LENZ (1996) THE MICROBIAL PRODUCTION OF POLY(HYDROXYALKANOATES) FROM TALLOW. APPL. MICROBIOL. BIOTECHNOL., 46:464-469. 9. CURLEY J. M., B. HAZER, R. W. LENZ, AND R. C. FULLER (1996) PRODUCTION OF POLY(3-HYDROXYALKANOATES) CONTAINING AROMATIC SUBSTITUENTS BY PSEUDOMONAS OLEOVORANS. MACROMOLECULES, 29:1762-1766. 10. DAWES E. A., AND P. J. SENIOR (1973) THE ROLE AND REGULATION OF ENERGY RESERVE POLYMERS IN MICROORGANISMS. ADV. MICROB. PHYSIOL., 10:135-266. 11. DE SMET M. J., G. EGGINK, B. WITHOLT, J. KINGMA, AND H. WYNBERG (1983) CHARACTERIZATION OF INTRACELLULAR INCLUSIONS FORMED BY PSEUDOMONAS OLEOVORANS DURING GROWTH ON OCTANE. J. BACTERIOL., 154(2):870-878. 12. EGGINK G., P. DE WAARD, AND G. N. M. HUIJBERTS (1995) FORMATION OF NOVEL POLY(HYDROXYALKANOATES) FROM LONG-CHAIN FATTY ACIDS. CAN J. MICROBIOL., 1:14-21. 13. EGGINK G., H. WALTER VAN, G. N. M. HUIJBERTS, AND P. WAARD DE (1993) FATTY ACIDS AS A SUBSTRATE FOR POLY(3-HYDROXYALKANOATE) FORMATION IN ALCALIGENES EUTROPHUS AND PSEUDOMONAS PUTIDA. IND. CROPS PROD., 1:157-163. 14. FRITZSCHE K., R. W. LENZ, AND R. C. FULLER (1990) AN UNUSUAL BACTERIAL POLYESTER WITH A PHENYL PENDANT GROUP. MAKROMOL. CHEM., 191:1957-1965. 15. FUKUI T., AND Y. DOI (1997) CLONING AND ANALYSIS OF THE POLY(3-HYDROXYBUTYRATE-CO-3-HYDROXYHEXANOATE) BIOSYNTHESIS GENE OF AEROMONAS CAVIAE. J. BACTERIOL., 179:4821-4830. 16. FUKUI T., AND Y. DOI (1998A) EFFICIENT PRODUCTION OF POLYHYDROXYALKANOATES FROM PLANT OILS BY ALCALIGENES EUTROPHUS AND ITS RECOMBINANT STRAIN. APPL. MICROBIOL. BIOTECHNOL., 49:333-336. 17. FUKUI T., N. SHIOMI, AND Y. DOI (1998B) EXPRESSION AND CHARACTERIZATION OF (R)-SPECIFIC ENOYL COENZYME A HYDRATASE INVOLVED IN POLYHYDROXYALKANOATES BIOSYNTHESIS BY AEROMONAS CAVIAE. J. BACTERIOL., 180:667-673. 18. GAGNON K. D., R. W. LENZ, R. J. FARRIS, AND R. C. FULLER (1992) CRYSTALLIZATION BEHAVIOR AND ITS INFLUENCE ON THE MECHANICAL PROPERTIES OF A THERMOPLASTIC ELASTOMER PRODUCED BY PSEUDOMONAS OLEOVORANS. MACROMOLECULES, 25:3723-3728. 19. GORENFLO V., A. STEINBUCHER, S. MAROSE, M. RIESEBERG, AND T. SCHEPER (1999) QUANTIFICATION OF BACTERIAL POLYHYDROXYALKANOIC ACIDS BY NILE RED STAINING. APPL. MICROBIOL. BIOTECHNOL., 51: 765-772. 20. HAHN S. K., Y. K. CHANG, B. S. KIM, AND H. N. CHANG (1994) COMMUNICATION TO THE EDITOR OPTIMIZATION OF POLY(3-HYDROXYBUTYRATE) RECOVERY USING DISPERSIONS OF SODIUM HYPOCHLORITE SOLUTION AND CHLOROFORM. BIOTECHNOL. BIOENG., 44:256-261. 21. HANGII U. J. (1990) PILOT SCALE PRODUCTION OF PHB WITH ALCALIGENUS LATUS. IN: DAWES EA, EDITOR. NOVEL BIODEGRADABLE MICROBIAL POLYMERS. DORDRECHT: KLUWER. 60-65. 22. HAYWOOD G. W., A. J. ANDERSON, D. F. EWING, AND E. A. DAWES (1990) ACCUMULATION OF POLY(β -HYDROXYALKANOATE) CONTAINING PRIMARILY 3-HYDROXYDECANOATE FROM SIMPLE CARBOHYDRATE SUBSTRATES BY PSEUDOMONAS SP. STAIN NCIMB 40135. APPL. ENVIRON. MICROBIOL., 56:3354-3359. 23. HAZER B., R. W. LENZ, AND R. C. FULLER (1994) BIOSYNTHESIS OF METHYLBRANCHED POLY(β -HYDROXYALKANOATE)S BY PSEUDOMONAS OLEOVORANS. MACROMOLECULES, 27:45-49. 24. HEIN S., H. TRAN, AND A. STEINBUCHER (1998) SYNECHOCYSTIS SP. PCC6803 POSSESSES A TWO-COMPONENT POLYHYDROXYALKANOIC ACID SYNTHASE SIMILAR TO THAT OF ANOXYGENIC PURPLE SULFUR BACTERIA. ARCH. MICROBIOL., 170:162-170. 25. HOFFMANN N., A. STEINBUCHER, AND B. H. A. REHM (2000) HOMOLOGOUS FUNCTIONAL EXPRESSION OF CRYPTIC PHAGE FROM PSEUDOMONAS OLEOVORANS ESTABLISHES THE TRANSACYLASE-MEDIATED POLYHYDROXYALKANOATE BIOSYNTHETIC PATHWAY. APPL. MICROBIOL. BIOTECHNOL., 54:665-670. 26. HUIJBERTS G. N. M., G. EGGINK, P. DE WAARD, G. W. HUISMAN, AND B. WITHOLT (1992) PSEUDOMONAS PUTIDA KT2442 CULTIVATED ON GLUCOSE ACCUMULATES POLY(3-HYDROXYALKANOATES) CONSISTING OF SATURATED AND UNSATURATED MONOMERS. APPL. ENVIRON. MICROBIOL., 58:536-544. 27. HUIJBERTS G. N., T.C. DE RIJK, P. DE WAARD, AND G. EGGINK (1994) ¹³C NUCLEAR MAGNETIC RESONANCE STUDIES OF PSEUDOMONAS PUTIDA FATTY ACID METABOLIC ROUTES INVOLVED IN POLY(3-HYDROXYALKANOATE) SYNTHESIS. J. BACTERIOL., 176(6):1661-1666. 28. HUISMAN G. W., E. WONONK, R. MEIMA, B. KAZEMIER, P. TERPSTRA, AND B. WITHOLT (1991) METABOLISM OF POLY(3-HYDROXYALKANOATES) (PHAS) BY PSEUDOMONAS OLEOVORANS. IDENTIFICATION AND SEQUENCES OF GENES AND FUNCTION OF THE ENCODED PROTEINS IN THE SYNTHESIS AND DEGRADATION OF PHA. J. BIOL. CHEM., 266:2191-2198. 29. KIM Y. B., R. W. LENZ, AND R. C. FULLER (1992) POLY(β -HYDROXYALKANOATE) COPOLYMERS CONTAINING BROMINATED REPEATING UNITS PRODUCED BY PSEUDOMONAS OLEOVORANS. MACROMOLECULES, 25:1852-1857. 30. KIM Y. B., R. W. LENZ, AND R. C. FULLER (1991) PREPARATION AND CHARACTERIZATION OF POLY

(b-HYDROXYALKANOATE) OBTAINED FROM PSEUDOMONAS OLEOVORANS GROWN WITH MIXTURES OF 5-PHENYLVALERIC ACID AND N-ALKANOIC ACIDS. MACROMOLECULES, 24:5256-5360. 31.KING P. P. (1982) BIOTECHNOLOGY, AN INDUSTRIAL VIEW. J. CHEM. TECHNOL. BIOTECHNOL.,32:2-8. 32.LAGEVEEN R. G., G. W. HUISMAN, H. PREUSTING, P.KETELAAR, G. EGGINK,AND B. WITHOLT(1988) FORMATION OF POLYESTERS BY PSEUDOMONAS OLEOVORANS:EFFECT OF SUBSTRATES ON FORMATION AND COMPOSITION OF POLY(R)-3-HYDROXYALKANOATES AND POLY(R)-3-HYDROXYALKENOATES. APPL.ENVIR -ON. MICROBIOL., 54:2924-2932. 33.LAUZIER C. A., AND R. H. MARCHESSAULT (1994) POLYHYDROXYALKANOATES: MORPHOLOGY OF TENDRE MORSELS FOR MICROBES. IN: BRAUNEFF, G. (ED.), PROCEEDING OF THE SYMPOSIUM ON PHYSIO -LOGY, KINETICS, PRODUCTION AND USE OF BIOPOLYMERS, SEGGAU, AUSTRIA, MAY 13-15, TECHNIC -AL UNIVERSITY GRAZ, GRAZ, 59-69. 34.LEMOIGNE M. (1926) PRODUCTS OF DEHYDRATION AND OF POLYMERIZATION OF b-HYDROXYBUTYRIC AC -ID. BULL. SOC. CHEM. BIOL., 8:770-782. 35.LIEBERGESELL M., AND A. STEINBUCHER (1993) CLONING AND MOLECULAR ANALYSIS OF THE POLY (3-HYDROXYBUTYRIC ACID) BIOSYNTHETIC GENES OF THIOCYSTIS VIOLACEA. APPL. MICROBIOL. BIOTECHNOL., 38:493-501. 36.LIEBERGESELL M., AND A. STEINBUCHER (1992) CLONING AND NUCLEOTIDE SEQUENCES OF GENES RELEVANT FOR BIOSYNTHESIS OF POLY(3-HYDROXYBUTYRIC ACID) IN CHROMATIUM VINOSUM STAIN D. EUR. J. BIOCHEM., 209:135-150. 37.LINKO S., H. VAHERI, AND J. S. (1993) PRODUCTION POLY-b- HYDROXYBUTYRATE ON LACTIC ACID BY ALCALIGENES EUTROPHUS H16 IN A 3-1 BIOREACTOR. ENZYME MICROB. TECHNOL., 15:401-406. 38.MADISON L. L., AND G. W. HUISMAN (1999) METABOLIC ENGINEERING OF POLY(3-HYDROXYALKANOAT -ES): FROM DNA TO PLASTIC. MICROBIOL. MOL. BIOL., 63:21-53. 39.MCCOOL G. J., AND M. C. CANNON (2001) PHAC AND PHAR ARE REQUIRED FOR POLYHYDROXYALKANO -IC ACID SYNTHASE ACTIVITY IN BACILLUS MEGATERIUM. J. BACTERIOL., 183: 4235-4243. 40.NISHIMURA A.,M.MORITA, Y. NISHIMURA, AND Y. SUGINO (1990) A RAPID AND HIGHLY EFFICIENT METHOD FOR PREPARATION OF COMPETENT ESCHERICHIA COLI CELLS.NUCL.ACIDS RES.,18(20):6169 . 41.OSTLE A.G.,AND J.G.HOLT(1982) NILE BLUE A AS A FLUORESCENT STAIN FOR POLY-b-HYDROXYBUTY -RATE. APPL. ENVIRON. MICROBIOL., 44(1):238-241. 42.PEOPLES O. P., S. MASAMUNE, C. T. WALSH, AND A. J. SINSKEY (1987) BIOSYNTHETIC THIOLASE FROM ZOOGLOEA RAMIGERA. III. ISOLATION AND CHARACTERIZATION OF THE STRUCTURAL GENE. J. BIOL. CHEM., 262:97-102. 43.PEOPLES O.P.,AND A.J.SINSKEY (1989A) FINE STRUCTURAL ANALYSIS OF THE ZOOGLOEA RAMIGERA PHBA-PHBB LOCUS ENCODING b-KETOTHIOLASE AND ACETOACETYL-COA REDUCTASE: NUCLEOTIDE SEQUE -NCE OF PHBB. MOL. MICROBIOL., 3:349-357. 44.PEOPLES O. P., AND A. J. SINSKEY (1989B) POLY-b-HYDROXYBUTYRATE (PHB) BIOSYNTHESIS IN ALCALIGENES EUTROPHUS H16. IDENTIFICATION AND CHARACTERIZATION OF THE PHB POLYMERASE GE -NE (PHBC). J. BIOL. CHEM., 264:15298-15303. 45.PEOPLES O. P., AND A. J. SINSKEY (1989) POLY-b-HYDROXYBUTYRATE BIOSYNTHESIS IN ALCALIGE -NES EUTROPHUS H16. CHARACTERIZATION OF THE GENES ENCODING b-KETOTHIOLASE AND ACETOACET -YL-COA REDUCTASE. J. BIOL. CHEM., 264:15293-15307. 46.POIRIER Y., C. NAWRATH,, AND C. SOMERVILLE (1995) PRODUCTION OF POLY-b-HYDROXYALKANOAT -ES, A FAMILY OF BIODEGRADABLE PLASTICS AND ELASTOMERS, IN BACTERIA AND PLANTS. BIOTECH NOL., 13,142-150. 47.POIRIER Y., D. E. DENNIS, K. KLOMPARENS, AND C. SOMERVILLE (1992) POLYHYDROXYBUTYRATE, A BIODEGRADABLE THERMOPLASTIC, PRODUCED IN TRANSGENIC PLANTS. SCIENCE, 256:520-523. 48.POVOLO S., S. CASELLA, AND M. P. NUTI (1996) INVOLVEMENT OF AN ORF IN THE SYNTHESIS AND OR DEGRADATION OF POLYHYDROXYALKANOATE (PHA) IN RHIZOBIUM MELILOTI, P.4-01.IN ABSTRACTS OF 1996 INTERNATIONAL SYMPOSIUM ON BACTERIAL POLYHYDROXYALKANOATES, DAVOS, SWITZERLAND. 49.QINGSHENG Q.,BERND H.A. REHM,AND A. STEINBUCHER (1997) SYNTHESIS OF POLY(3-HYDROXYALKAN OATES) IN ESCHERICHIA COLI EXPRESSING THE PHA SYNTHASE GENE PHAC2 FROM PSEUDOMONAS AERU -GINOSA : COMPARISON OF PHAC1 AND PHAC2. FEMS MICROBIOL. LETT., 157,155-162. 50.RAMSAY B. A., I. SARACOVAN, J. A. RAMSAY, AND R. H. MARCHESSAULT (1992) EFFECT OF NITRO -GEN LIMITATION ON LONG-SIDE-CHAIN POLY- -HYDROXYALKANOATE SYNTHESIS BY PSEUDOMONAS RE -SINOVORANS. APPL. ENVIRON. MICROBIOL., 58(2):744-746. 51.REHM B. H. A., AND A. STEINBUCHER (1999) BIOCHEMICAL AND GENETIC ANALYSIS OF PHA SYNTHA -SES AND OTHER PROTEINS REQUIRED FOR PHA SYNTHESIS. INT. J. BIOL. MACROMOL., 25:3-19. 52.REHM B. H. A., N. KRUGER, AND A. STEINBUCHER (1998) THE NEW MORPHOLOGICAL AND 13C-NUCLE -AR METABOLIC LINK BETWEEN FATTY ACID DE NOVO SYNTHESIS AND POLYHYDROXYALKANOIC ACID SYNTHESIS. J. BIOL. CHEM., 273(7):579-584. 53.RODRIGUES M. F. A., L. F. DA SILVA, J. G. C. GOMEZ, H. E. VALENTIN, AND A. STEINBUCHER (1995) BIOSYNTHESIS OF POLY(3-HYDROXYBUTYRIC ACID-CO-3-HYDROXY-4-PENTENOIC ACID) FROM UNRELATED SUBSTANCES BY BURKHOLDERIA SP. APPL. MICROBIOL. BIOTECHNOL., 43:880-886. 54.SCHEMBRI M. A., R. C. BAYLY, AND J. K. DAVIES (1994) CLONING AND ANALYSIS OF THE POLYHY DROXYALKANOIC ACID SYNTHASE GENE FROM AN ACINETOBACTER SP. : EVIDENCE THAT THE GENE IS BOTH PLASMID AND CHROMOSOMALLY LOCATED. FEMS MICROBIOL. LETT., 118:145-152. 55.SCHUBERT P., A. STEINBUCHER, AND H. G. SCHLEGEL (1988) CLONING OF THE ALCALIGENES EUTR OPHUS GENES FOR SYNTHESIS OF POLY-b-HYDROXYBUTYRIC ACID (PHB) AND SYNTHESIS OF PHB

IN ESCHERICHIA COLI. J. BACTERIOL., 170(12):5837-5847. 56. SLATER S., K. L. HOUMIEL, M. TRAN, T. A. MITSKY, N. B. TAYLOR, S. R. PADGETTE, AND K. J. GRUYS (1998) MULTIPLE β -KETOTHIOLASES MEDIATE POLY(β -HYDROXYALKANOATE) COPOLYMER SYNTHESIS IN RALSTONIA EUTROPHA. J. BACTERIOL., 180(8):1979-1987.

57. SLATER S. C., W. H. VOIGE, AND D. E. DENNIS (1988) CLONING AND EXPRESSION IN ESCHERICHIA COLI OF THE ALCALIGENES EUTROPHUS H16 POLY- β -HYDROXYBUTYRATE BIOSYNTHETIC PATHWAY. J. BACTERIOL., 170(10):4431-4436. 58. SONG J. J., AND S. C. YOON (1996) BIOSYNTHESIS OF NOVEL AROMATIC COPOLYESTERS FROM INSOLUBLE 11-PHENOXYUNDECANOIC ACID BY PSEUDOMONAS PUTIDA BM01. APPL. ENVIRON. MICROBIOL., 62:536-544.

59. SOLAIMAN D. K. Y. (2000) PCR CLONING OF PSEUDOMONAS RESINOVORANS POLYHYDROXYALKANOATE BIOSYNTHESIS GENES AND EXPRESSION IN ESCHERICHIA COLI. BIOTECHNOL. LETT., 22:789-794. 60. SPIEKERMANN P., B. H. A. REHM, R. KALSCHUEER, D. BAUMEISTER, AND A. STEINBUCHEL (1999) A SENSITIVE, VIABLE-COLONY STAINING METHOD USING NILE RED FOR DIRECT SCREENING OF BACTERIA THAT ACCUMULATE POLYHYDROXYALKANOIC ACIDS AND OTHER LIPID STORAGE COMPOUNDS. ARCH. MICROBIOL., 171:73-80. 61. STEFAN K., G. D. ROO, B. WITHOLT, AND B. KESSLER (2000) ROLE OF PHAD IN ACCUMULATION OF MEDIUM-CHAIN-LENGTH POLY(β -HYDROXYALKANOATES) IN PSEUDOMONAS OLEOVORANS. APPL. ENVIRON. MICROBIOL., 66(9) 3705-3710.

62. STEINBUCHEL A., E. M. DEBSI, R. H. MARCHESSAULT, AND A. TIMM (1993) SYNTHESIS AND PRODUCTION OF POLY(β -HYDROXYVALERIC ACID) HOMOPOLYESTER BY CHROMOBACTERIUM VIOLACEUM. APPL. MICROBIOL. BIOTECHNOL., 39:443-449. 63. STEINBUCHEL A., AND H. E. VALENTIN (1995) DIVERSITY OF BACTERIAL POLYHYDROXYALKANOIC ACIDS. FEMS MICROBIOL. LETT., 128:219-228. 64. STEINBUCHEL A., B. FUCHTENBUSCH, V. GORENFLO, S. HEIN, R. JOSSEK, S. LANGENBACH, AND B. H. A. REHM (1997) BIOSYNTHESIS OF POLYESTERS IN BACTERIA AND RECOMBINANT ORGANISMS. POLYMER DEGRAD. STABIL., 59:177-182. 65. STEINBUCHEL A., E. HUSTEDE, U. PIEPER, A. TIMM, AND H. E. VALENTIN (1992) MOLECULAR BASIS FOR BIOSYNTHESIS AND ACCUMULATION OF POLYHYDROXYALKANOIC ACID IN BACTERIA. FEMS MICROBIOL. REV., 103:217-230. 66. STEINBUCHEL A., AND H. E. VALENTIN (1995) DIVERSITY OF BACTERIAL POLYHYDROXYALKANOIC ACIDS. FEMS MICROBIOL. LETT., 128:219-228.

67. SUDESH K., H. ABE, AND Y. DOI (2000) SYNTHESIS, STRUCTURE AND PROPERTIES OF POLYHYDROXYALKANOATES: BIOLOGICAL POLYESTERS. PROG. POLYM. SCI., 25:1503-1555. 68. TAGUCHI K., Y. AOYAGI, H. MATSUSAKI, T. FUKUI, AND Y. DOI (1999) CO-EXPRESSION OF 3-KETO-ACYL-COA REDUCTASE AND POLYHYDROXYALKANOATE SYNTHASE GENES INDUCES PHA PRODUCTION IN ESCHERICHIA COLI HB101 STAIN. FEMS MICROBIOL. LETT., 176:183-190. 69. TAGUCHI K., Y. AOYAGI, H. MATSUSAKI, T. FUKUI, AND Y. DOI (1999) OVER-EXPRESSION OF 3-KETO-ACYL-ACP SYNTHASE III OR MALONYL-COA-ACP TRANSACYLASE GENE INDUCES MONOMER SUPPLY FOR POLYHYDROXYALKANOATE PRODUCTION IN ESCHERICHIA COLI HB101. BIOTECHNOL. LETT., 21(7):579-584. 70. TAN I. K. P., K. KUMAR SUDESH, M. THEANMALAR, S. N. GAN, AND B. GORDON (1997) SAPONIFIED PLAM KERNEL OIL AND ITS MAJOR FREE FATTY ACIDS AS CARBON SUBSTRATES FOR THE PRODUCTION OF POLYHYDROXYALKANOATES IN PSEUDOMONAS PUTIDA PGA1. APPL. MICROBIOL. BIOTECHNOL., 47:207-211. 71. TIMM A., AND A. STEINBUCHEL (1990) FORMATION OF POLYESTERS CONSISTING OF MEDIUM-CHAIN-LENGTH β -HYDROXYALKANOIC ACIDS FROM GLUCONATE BY PSEUDOMONAS AERUGINOSA AND OTHER FLUORESCENT PSEUDOMONADS. APPL. ENVIRON. MICROBIOL. 56:3360-3367. 72. TIMM A., AND A. STEINBUCHEL (1992) CLONING AND MOLECULAR ANALYSIS OF THE POLY(β -HYDROXYALKANOIC ACID) GENE LOCUS OF PSEUDOMONAS AERUGINOSA PAO1. EUR. J. BIOCHEM., 209:15-30. 73. TOMBOLINI R., S. POVOLO, A. BUSON, A. SEQARTINI, AND M. P. NUTI (1995) POLY- β -HYDROXYBUTYRATE (PHB) BIOSYNTHETIC GENES IN RHIZOBIUM MELILOTI 41. MICROBIOLOGY, 141:2553-2559. 74. UEDA S., T. YABUTANI, A. MAEHARA, AND T. YAMANE (1996) MOLECULAR ANALYSIS OF THE POLY(β -HYDROXYALKANOATE) SYNTHASE GENE FROM A METHYLOTROPHIC BACTERIUM, PARACOCCLUS DENITRIFICANS. J. BACTERIOL., 178:774-779. 75. UEDA F., Y. KITANO, Y. MURAKAMI, K. YAGI, Y. MIIURA, AND T. MIZOGUCHI. (1998) CLONING AND SEQUENCE ANALYSIS OF THE POLY(β -HYDROXYALKANOIC ACID) SYNTHESIS GENES OF PSEUDOMONAS ACIDOPHILA. APPL. BIOCHEM. BIOTECHNOL., 70-72:341-352. 76. YABUTANI T., A. MAEHARA, S. UEDA, AND T. YAMANE (1995) ANALYSIS OF β -KETOTHIOLASE AND ACETOACETYL-COA REDUCTASE GENES OF A METHYLOTROPHIC BACTERIUM, PARACOCCLUS DENITRIFICANS, AND THEIR EXPRESSION IN ESCHERICHIA COLI. FEMS MICROBIOL. LETT., 133:85-90. 77. YORK G. M., B. H. JUNKER, J. STUBBE, AND A. J. SINSKEY (2001) ACCUMULATION OF THE PHA-P PHASIN OF RALSTONIA EUTROPHA IS DEPENDENT ON PRODUCTION OF POLYHYDROXYBUTYRATE IN CELL. J. BACTERIOL., 183:4217-4226