

Appendix A

Nucleotide sequence of *lip* gene of *P. fluorescens*

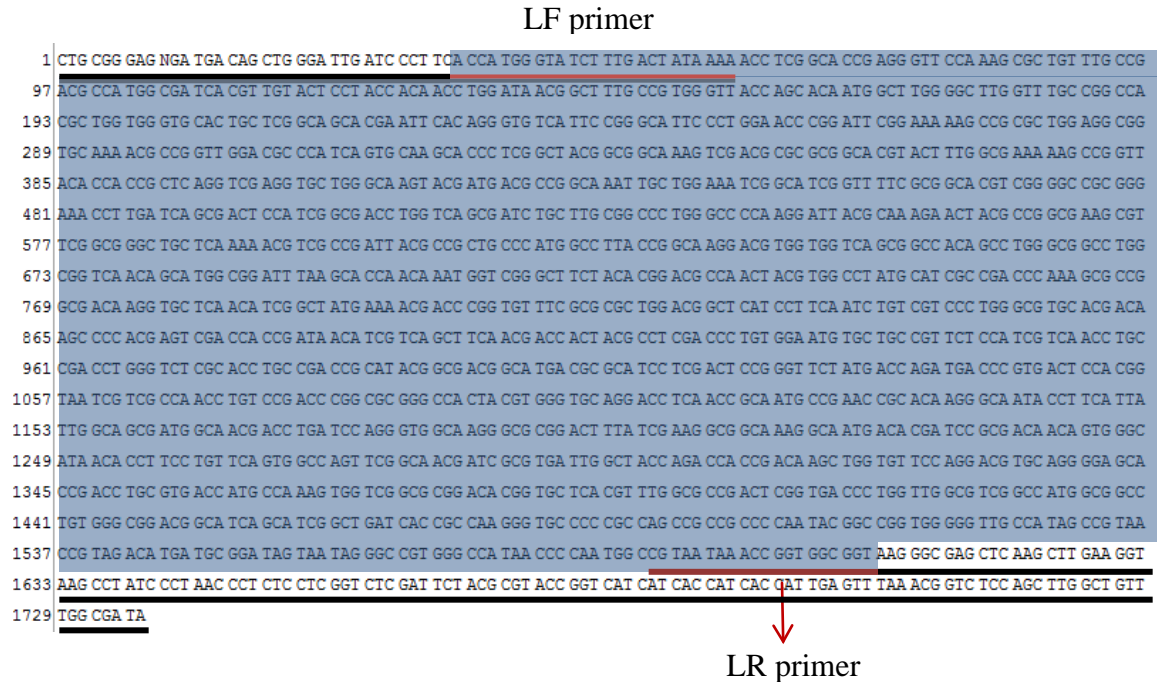


Figure I: Nucleotide sequence of *lip* gene in vector p2T-*lip* (shaded box). The LF and LR primers used in *lip* gene PCR amplification from *P. fluorescens* are underlined in red and the flanking nucleotide sequence of the pBAD-202 TOPO vector is underlined in black.

Appendix B

Multiple sequence alignment of *lip* gene

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|AAA25882|           MGIFDYKNLGTGEGSKTLFADAMAITLYSYHNLDNGFAVGYQHNLGLGLPATLVGALLGS 60
|lip_ATCC13525|      MGIFDYKNLGTGEGSKALFADAMAITLYSYHNLDNGFAVGYQHNLGLGLPATLVGALLGS 60
|BAB64913|           MGVFDYKNLGTGEGSKALFADAMAITLYTYHNLDNGFAVGYQHNLGLGLPATLVGALLGS 60
|CAY49369|           MGIFDYKNLGTGEGSKALFADAMAITLYTYHNLDNGFAVGYQHNLGLGLPATLVGALLGS 60
*:*****:*****:*****:*****:*****:*****:*****:*****:*****:*****:*****

|AAA25882|           TDSQGVIPGIPWNPDEKAALAVQKAGWTPISASALGYAGKVDARGTFFGEKAGYTAAQ 120
|lip_ATCC13525|      TNSQGVIPGIPWNPDEKAALAVQNAGWTPISASTLGYGGKVDARGTYFFGEKAGYTAAQ 120
|BAB64913|           TDSQGVIPGIPWNPDEKAALAVQQAGWTPIRASTLGYTKVDARGTFFGEKAGYTAAQ 120
|CAY49369|           TDSQGVIPGLPWNPDSEKAALDAVQKAGWTPISASTLGYGGKVDARGTFFGEKAGYTAAQ 120
*:*****:*****:*****:*****:*****:*****:*****:*****:*****:*****

|AAA25882|           VEVLGKYDDAGKLEIGIGFRGTSGPRETLISDSIGDLISDLLAALGPKDYAKNYAGEAF 180
|lip_ATCC13525|      VEVLGKYDDAGKLEIGIGFRGTSGPRETLISDSIGDLVSDLLAALGPKDYAKNYAGEAF 180
|BAB64913|           VEVLGKYDDAGKLEIGIGFRGTSGPRETLVSDSIGDLVSDLLAALGPKDYANNYAGEAF 180
|CAY49369|           VEVLGKYDDAGKLEIGIGFRGTSGPRETLISDSIGDLVSDLLAALGPKDYAKNYAGEAF 180
*****:*****:*****:*****:*****:*****:*****:*****:*****:*****

|AAA25882|           GLLKKNVADYAGAHGLTGKDVVVSGHSLGGLAVNSMADLSNYKWAGFYKDANYVAYASPT 240
|lip_ATCC13525|      GLLKKNVADYAAAHLTGKDVVVSGHSLGGLAVNSMADLSTNKWSGFYTDANYVAYASPT 240
|BAB64913|           GLLKKNVADYASAHGLSGHDVVVSGHSLGGLAVNSMADLSSSKWAGFYQDANYLAYASPT 240
|CAY49369|           GLLKKNVADYASAHGLSGKDVVVSGHSLGGLAVNSLADLSVKNWGGFYKDAHYVAYASPT 240
*****:*****:*****:*****:*****:*****:*****:*****:*****:*****

|AAA25882|           QSAGDKVLNIGYENDPVFRALDGSSFNLSLGVHDKPHESTTDNIVSFNDHYASTLWNVL 300
|lip_ATCC13525|      QSAGDKVLNIGYENDPVFRALDGSSFNLSLGVHDKPHESTTDNIVSFNDHYASTLWNVL 300
|BAB64913|           QSAGDKVLNIGYENDPVFRALDGSSFNWSSLGVHDKPHESTTDNIVSFNDHYASTLWNVL 300
|CAY49369|           QSAGDKVLNIGYENDPVFRALDGSSFNLSLGVHDKPHESTTDNIVSFNDHYASTLWNVL 300
*****:*****:*****:*****:*****:*****:*****:*****:*****:*****

|AAA25882|           PFSIVNLPWVSHLPTAYGDMTRILESGFYDQMRDSTVIVANLSDPARANTWVQDLNR 360
|lip_ATCC13525|      PFSIVNLPWVSHLPTAYGDMTRILDGSGFYDQMRDSTVIVANLSDPARATTWVQDLNR 360
|BAB64913|           PFSITNLPWVSHLPTGYGDMTRILESGFYQMSRDSTIIIVANLSDPARATTWVQDLNR 360
|CAY49369|           PFSIANLPWVSHLPTGYGDMTRIVESGFYEQMSRDATVIVANLSDPARANTWVQDLNR 360
****:*****:*****:*****:*****:*****:*****:*****:*****:*****

|AAA25882|           NAEPHKGNTFIIIGSDGNLDLIQGGMGADFIEGGKGNDFIRDNSGHNTFLFSGHFGNDRVIG 420
|lip_ATCC13525|      NAEPHKGNTFIIIGSDGNLDLIQGGMGADFIEGGKGNDFIRDNSGHNTFLFSGQFGNDRVIG 420
|BAB64913|           NAEPHKGNTFIIIGSDGNLDLIQGGMGADFIEGGKGNDFIRDNSGHNTFLFSGPFGQDRVIG 420
|CAY49369|           NAEPHKGNTFIIIGSDGDDLIQGGKGVDFIEGGKGNDFIRDNSGHNTFLFSGHFGQDRVIG 420
*****:*****:*****:*****:*****:*****:*****:*****:*****:*****

|AAA25882|           YQPTDKLVFKDVQGSTDLRDHAKVVGADTVLTFGADSVTLVGVGHGGLWTEGVVIG 476
|lip_ATCC13525|      YQTTDKLVFKDVQGSTDLRDHAKVVGADTVLTFGADSVTLVGVGHGGLWADGISIG 476
|BAB64913|           YQATDKLVFRDVGSDYRDKVVGADTVISFGADSVTLVGV--VLSGEGIVIG 474
|CAY49369|           YQLTDKLVFKDVQGSVDYREH---GGDTVISVGGDSVTLVGV--GGLG--EVVIG 469
** *****:*****:*****:*****:*****:*****:*****:*****:*****:*****

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Figure II: Lipase protein sequence alignment by ClustalW between four different *P. fluorescens* strains BW25 (accession no.: CAY49369.1), KB700A (accession no.: BAB64913.1), B52 (accession no.: AAA25882.1) and ATCC 13525 (in this study). Homology of substrate-binding domain among the *P. fluorescens* strains was showed in black box and glycine-rich consensus sequence showed in red box. “*” denoted nucleotides are identical, “.” denoted nucleotides are conserved substitutions, “.” denoted nucleotides are semi-conserved substitutions.

Appendix C

Nucleotide sequence of *phaC1* gene from *P. putida*

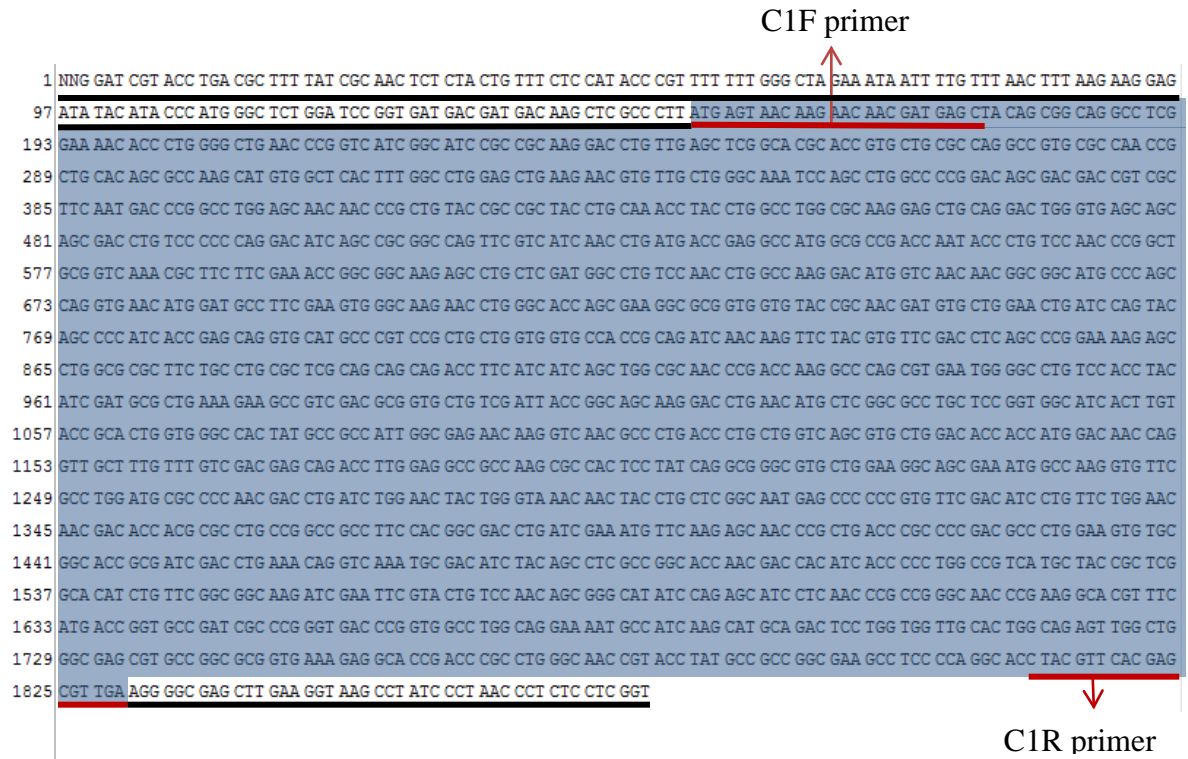


Figure III: Nucleotide sequence of *phaC1* gene in vector pT-*phaC1* (shaded box). The C1F and C1R primers used in *phaC1* gene PCR amplification from *P. putida* are underlined in red and the flanking nucleotide sequence of the pBAD-TOPO vector is underlined in black.

Appendix D

Multiple alignment of the *phaC1* synthase

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P.putidaKT2440      MSNKNNDELQRQASENTLGLNPVIGIRRKDLLSSARTVLRQAVRQPLHSAKHVAHFGLEL 60
P.putidaPGA1       MSNKNNDELQRQASENTLGLNPVIGIRRKDLLSSARTVLRQAVRQPLHSAKHVAHFGLEL 60
P.oleovorans      MSNKNNDELQRQASENTLGLNPVIGIRRKDLLSSARTVLRQAVRQPLHSAKHVAHFGLEL 60
P.aeruginosaPA01  MSQKNNNELPKQAAENTLNLNPVIGIRKDLLTSARMVLLQAVRQPLHSARHVAHFSLEL 60
                   **:***: * :*: *****:*****:*** ** *****:*****:***

P.putidaKT2440      KNVLLGKSSLAPDSDRRFNDPAWSNNPLYRRLQTYLAWRKELQDVVSSDLSPODISR 120
P.putidaPGA1       KNVLLGKSSLAPDSDRRFNDPAWSNNPLYRRLQTYLAWRKELQDVVSSDLSPODISR 120
P.oleovorans      KNVLLGKSSLAPESDRRFNDPAWSNNPLYRRLQTYLAWRKELQDWIGNSDLSPODISR 120
P.aeruginosaPA01  KNVLLGQSELRPGDSDRRFSDPAWSQNPLYKRYMQTYLAWRKELHSWISHSDLSPODISR 120
                   *****: * * . *****:*****:***:***:***:*****: * :. *****

P.putidaKT2440      GQFVINLMTAMAPTNTLSNPAAVKRFFETGGKSLLDGLSNLAKDMVNNGGMPQVNMDA 180
P.putidaPGA1       GQFVINLMTAMAPTNTLSNPAAVKRFFETGGKSLLDGLSNLAKDMVNNGGMPQVNMDA 180
P.oleovorans      GQFVINLMTAMAPTNTLSNPAAVKRFFETGGKSLLDGLSNLAKDMVNNGGMPQVNMDA 180
P.aeruginosaPA01  GQFVINLLTEAMSPNLSNPAAVKRFFETGGKSLLDGLHGLAKDMVNNGGMPQVMDA 180
                   *****:***:***:*****:*****:*****:*****:*****:***

P.putidaKT2440      FEVGKNLGTSEGAVVYRNDVLELIQYSPITEQVHARPLLVPVPPQINKFYVFDLSPEKSLA 240
P.putidaPGA1       FEVGKNLGTSEGAVVYRNDVLELIQYSPITEQVHARPLLVPVPPQINKFYVFDLSPEKSLA 240
P.oleovorans      FEVGKNLGTSEGAVVYRNDVLELIQYKPIEQVHARPLLVPVPPQINKFYVFDLSPEKSLA 240
P.aeruginosaPA01  FEVGKNLATTEGAVVYRNDVLELIQYRPITESVHERPLLVPVPPQINKFYVFDLSPDKSLA 240
                   *****: * :*****:*****:***** ** * *****:*****:***

P.putidaKT2440      RFCLRSQQQTFIISWRNPTKAQREWGLSTYIDALKEAVDAVLSITGSKDLNMLGACSGGI 300
P.putidaPGA1       RFCLRSQQQTFIISWRNPTKAQREWGLSTYIDALKEAVDAVLSITGSKDLNMLGACSGGI 300
P.oleovorans      RYCLRSQQQTFIISWRNPTKAQREWGLSTYIDALKEAVDAVLAITGSKDLNMLGACSGGI 300
P.aeruginosaPA01  RFCLRNGVQTFIVSWRNPTKSQREWGLTTYIEALKEAIEVVLSITGSKDLNLLGACSGGI 300
                   *:***. *****:*****:*****:***:*****: :. *:*****:*****:*****

P.putidaKT2440      TCTALVGHYAAIGENKVNALTLVSVLDTTMDNQVALFVDEQTLAAKRHSYQAGVLEGS 360
P.putidaPGA1       TCTALVGHYAAIGENKVNALTLVSVLDTTMDNQVALFVDEQTLAAKRHSYQAGVLEGS 360
P.oleovorans      TCTALVGHYAALGENKVNALTLVSVLDTTMDNQVALFVDEQTLAAKRHSYQAGVLEGS 360
P.aeruginosaPA01  TTAALVGHYVASGEKKNVAFQLVSVLDFELNTQVALFADEKTLEAAKRHSYQSGVLEGK 360
                   * :*****: * **:*****: * ***** : :. *****: ** :*****:***:*****.

P.putidaKT2440      EMAKVFAMWRPNDLIWNVWVNNYLLGNEPPVFDILFWNNDTTRLPAAFHGDLIEMFKSNP 420
P.putidaPGA1       EMAKVFAMWRPNDLIWNVWVNNYLLGNEPPVFDILFWNNDTTRLPAAFHGDLIEMFKSNP 420
P.oleovorans      EMAKVFAMWRPNDLIWNVWVNNYLLGNEPPVFDILFWNNDTTRLPAAFHGDLIEMFKSNP 420
P.aeruginosaPA01  DMAKVFAMWRPNDLIWNVWVNNYLLGNQPPAFDILYWNNDTTRLPAALHGEFVELFKSNP 420
                   : *****:*****:*****: * :. *****:*****:***: : : * :*****

P.putidaKT2440      LTRPDALVCGTAIDLKQVKCDIYSLAGTMDHITPWPSYRSAHLFGGKIEFVLSNSGHI 480
P.putidaPGA1       LTRPDALVCGTAIDLKQVKCDIYSLAGTMDHITPWPSYRSAHLFGGKIEFVLSNSGHI 480
P.oleovorans      LTRPDALVCGTPIIDLKQVKCDIYSLAGTMDHITPWQSCYRSAHLFGGKIEFVLSNSGHI 480
P.aeruginosaPA01  LNRPGALEVSGTPIIDLKQVTCDFYCVAGLMDHITPWESYK SARLLGGKCEFILSNHGHI 480
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P.putidaKT2440      QSILNPPGNPKARFMTGADRPDPAWQENAIKHADSWULHWQSWLGERAGALKKAPTRL 540
P.putidaPGA1       QSILNPPGNPKARFMTGADRPDPAWQENAIKHADSWULHWQSWLGERAGAVKEAPTRL 540
P.oleovorans       QSILNPPGNPKARFMTGADRPDPAWQENATKHADSWULHWQSWLGERAGELEKAPTRL 540
P.aeruginosaPAO1   QSILNPPGNPKARFMTNPELPAEPKAWLEQAGKHADSWULHWQSWLAERSGKTRKAPASL 540
*****:::*.:* ** *:* *****.**,**:* .:**: *

P.putidaKT2440      GNRTYAAGEASPGTYVHER 559
P.putidaPGA1       GNRTYAAGEAPPGPYVHER 559
P.oleovorans       GNRAYAAGEASPGTYVHER 559
P.aeruginosaPAO1   GNRKTYPAGEAAPGTYVHER 559
**::*.****.**,*****

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Figure IV: Multiple alignment of the *phaC1* synthase of *P. putida* PGA1 (in this study), *P. putida* KT2440 (accession no. : NP_747105), *P. aeruginosa* PAO1 (accession no. : NP_253743), *P. oleovorans* (accession no. : AAA25932) by ClustalW. The underlined sequences indicated the conserved α/β -hydrolases domain and the sequence in the box indicated the catalytic residue of α/β -hydrolases domain. “*” denoted nucleotides are identical, “.” denoted nucleotides are conserved substitutions, “:” denoted nucleotides are semi-conserved substitutions.