FIG. S1. (A) Isolated lytic plaques of *B. bacteriovorus* HD100 growing on *P. putida* as prey. (B) Alignment of the amino acid sequences of the extracellular mcl-PHA depolymerases from *B. bacteriovorus* HD100 ($PhaZ_{Bd}$) and *P. fluorescens* GK13 ($PhaZ_{GK13}$). Predicted processing site (between amino acids 20 to 21 of $PhaZ_{Bd}$ preprotein) is marked by vertical arrow. The lipase consensus sequence is boxed.

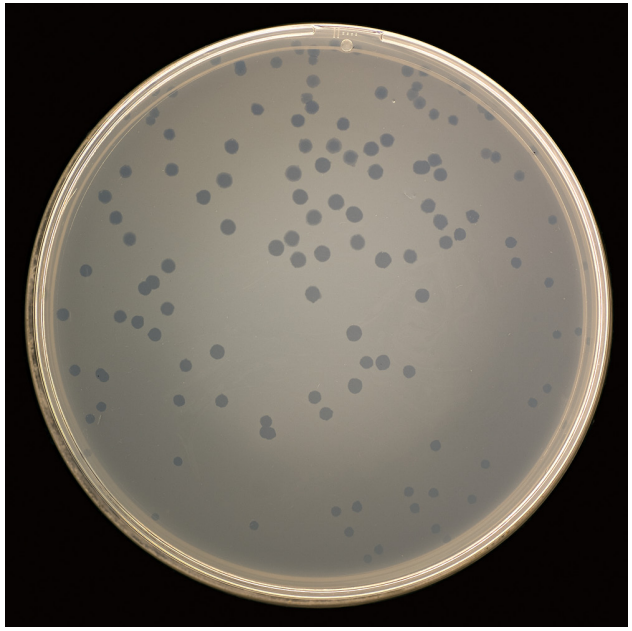
FIG. S2. Production of $PhaZ_{Bd}$ in *E. coli*. (A) SDS-PAGE analysis of the soluble crude extract fraction of *E. coli* strains grown in LB medium: lane 1, DH10B (pIZ1016); lane 2, DH10B (pIZBd1). (B) SDS-PAGE analysis of the concentrated culture supernatants of *E. coli* strains grown in minimal medium: lane 1, DH10B (pIZ1016); lane 2, DH10B (pIZBd1); lane 3, K1041 (pIZ1016); lane 4, K1041 (pIZBd1). Black arrow in panels (A) and (B) shows the position of $PhaZ_{Bd}$.

FIG. S3. Semi-purification of native $PhaZ_{Bd}$ produced by *P. putida* AΩ (pIZBd1). (A) SDS-PAGE analysis of *P. putida* AΩ: line 1, AΩ (pIZ1016) culture supernatant; lane 2, AΩ (pIZBd1) culture supernatant; lane 3, standard markers; lane 4, semi-purified $PhaZ_{Bd}$ (22 µg/ml) (B) Enzyme activity measured in mcl-PHA agar plates of 20 µl of the recovered fractions obtained after octyl-sepharose purification. (C) Assay of semi-purified $PhaZ_{Bd}$ activity in native polyacrylamide gels layered onto mcl-PHA agar plates: lane 1, 0.5 µg of semi-purified $PhaZ_{Bd}$; lane 2, 0.1 µg of semi-purified $PhaZ_{Bd}$. Depolymerase activity was detected after 2 h of incubation at 37ºC. Black arrows in panels (A) and (C) shows the position of the $PhaZ_{Bd}$.

FIG. S4. Identification by HPLC-MS of the mcl-PHA hydrolysis products catalyzed by the *B. bacteriovorus* HD100 depolymerase at 1 h of enzymatic hydrolysis. (A) HPLC

analysis of reaction products. (B) MS analysis of peaks depicted in the HPLC chromatogram. The analysis revealed the existence of five chromatographic peaks (A) with retention times of 11.0 min (*peak 1*), 15.7 min (*peak 2*), 16.6 min (*peak 3*), 17.3 min (*peak* 4) and 17.8 min (*peak 5*). The ESI(–) analysis of *peak 1* (B) showed a main single charged negative ion corresponding to the molecular mass of the deprotonated HO monomer (*m*/*z* 159). The analysis of *peak 2* provided two single charged negative ions that matched the molecular masses of the deprotonated HX-HO diester (*m*/*z* 273) and of the dimer adduct of HX-HO diester (*m*/*z* 547). The analysis of *peak 3* provided two single charged negative ions that matched the molecular masses of the deprotonated HO diester (*m*/*z* 301) and of the dimer adduct of HO diester (*m*/*z* 603). The *peak 4* showed two single charged negative ions corresponding to the molecular masses of the deprotonated HO-HX-HO triester (*m*/*z* 415) and of the dimer adduct of HO-HX-HO triester (*m*/*z* 830). Finally, the *peak 5* showed two single charged negative ions corresponding to the molecular masses of the deprotonated HO triester (*m*/*z* 443) and of the dimer adduct of HO triester (*m*/*z* 836).

(A)



(B)

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GK13              1 MPLRTLLCGLLLAVCLGQHALAASRCSERPRTLLRPAEVSCSYQSTWLDS     50
                    ::.||.|:...|.:...|.||.:.|....|.| ...::|.|... |.|
HD100             1 --MKKLLAGVFGVVAMSLSAQAAKKASNCEVTGL-VDRMTCPYLEK-LVS     46
GK13             51 GLVGQRKIIYQTPLGTPPAGGWPVVLIYQGSFFPLNDFSYHSNLPFGGYY    100
                    |....|.:.|..|.|..|..|||.|::||||.||: :||..|.:..|||.
HD100            47 GPHLTRHVKYSLPKGKTPKAGWPTVILYQGSLFPV-EFSRSSLMIAGGYN     95
GK13            101 EGKLVQNLLDHGYAVIAPSAPADLFWQTNIPGLAQAYELSTDYDFLGNVL    150
                    |.:|:|.|||.|:||||||.|...:.|.|||.|:  .|:.|.|:.|:..:|
HD100            96 EIRLIQTLLDSGFAVIAPPAIEGVAWMTNIVGI--DYDTSEDFYFVEELL    143
GK13            151 AAIASGHFGPLNAQRQYATGISSGGYNTSRMAVSFPGKFRALAVQSGSYA    200
                    .|:.:|.||.||..|.||||||||||::||||:|||.|:||||.|.|||
HD100           144 VAMGNGEFGKLNMDRLYATGISSGGYHSSRMAVAFPGVFKALAVHSASYA    193
GK13            201 TCSGPLCVVPDQLPADHPPTLFLHGFVDAVVPWWSMDLYYDRLLHQGIET    250
                    .|.||:|.||.|:|.:||||:||||.:|.|.|||..:|..|::.|.:|||:||
HD100           194 DCGGPMCFVPAQVPENHPPTIFLHGRLDPVVPVRTMYPYHETLKNQGVET    243
GK13            251 ARYTEPLGGHEWFAASPGKVLAWFNAHP    278
                    ..:..|...|||...:|..:..||..|.
HD100           244 EMFVSPWARHEWLEEAPELITNWFINHK    271
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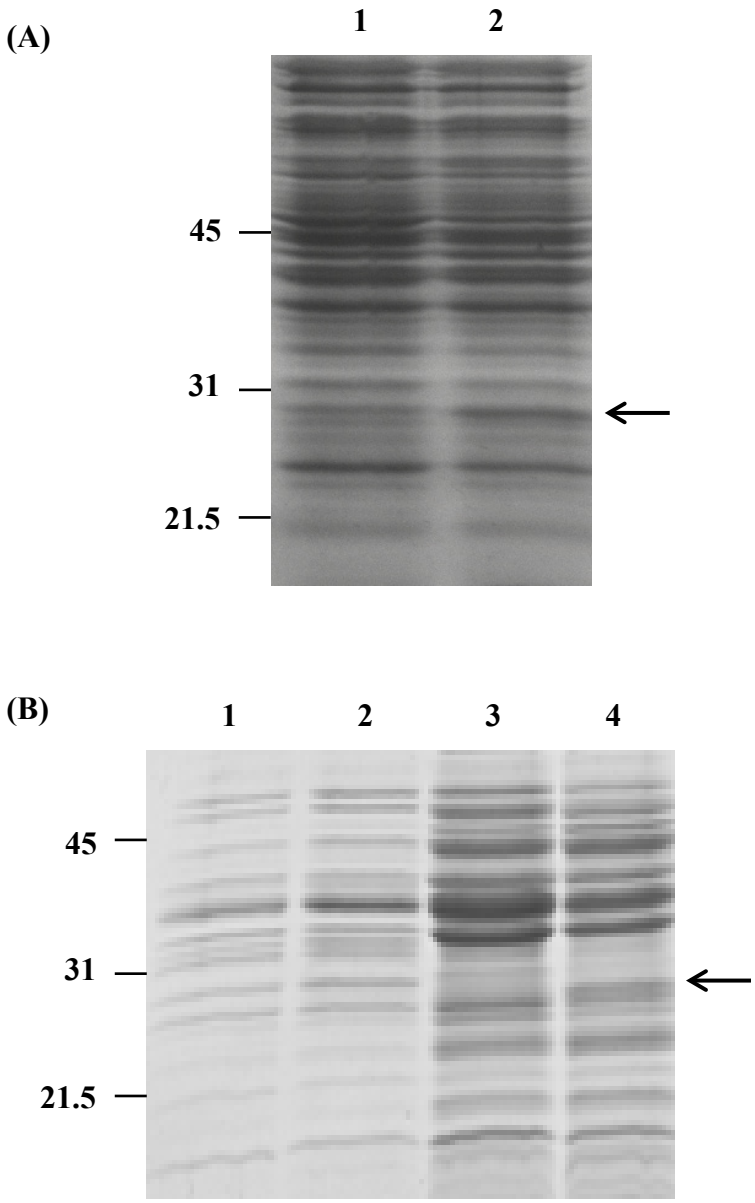
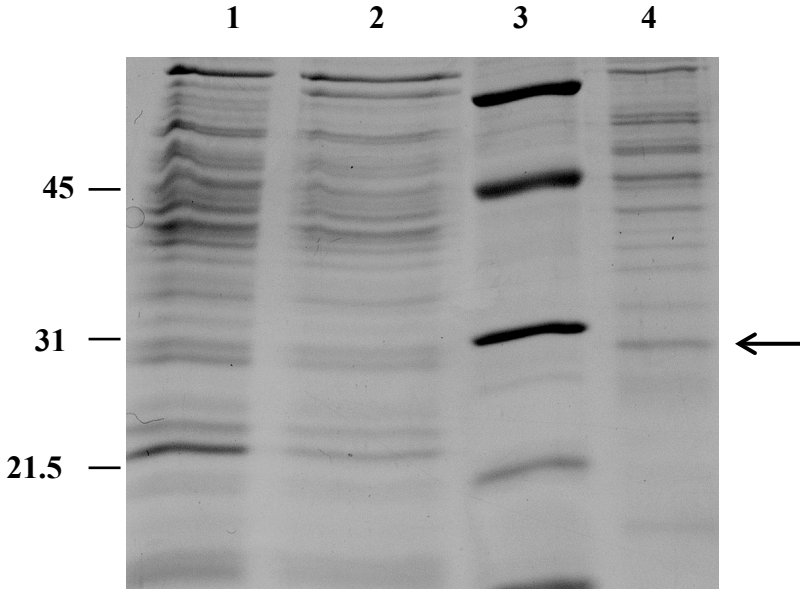
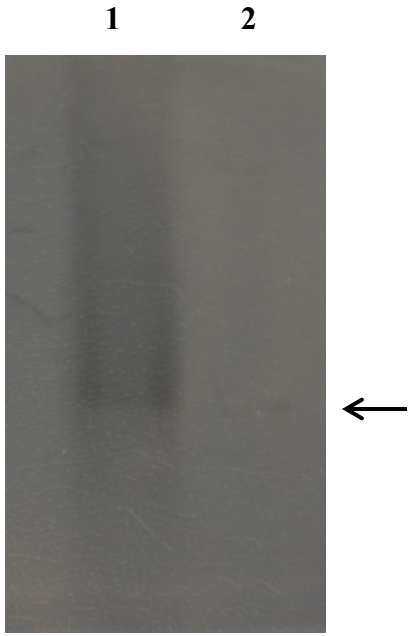

(A)
1
2
45
31
21.5
(B)
1
2
3
4
45
31
21.5

(A)

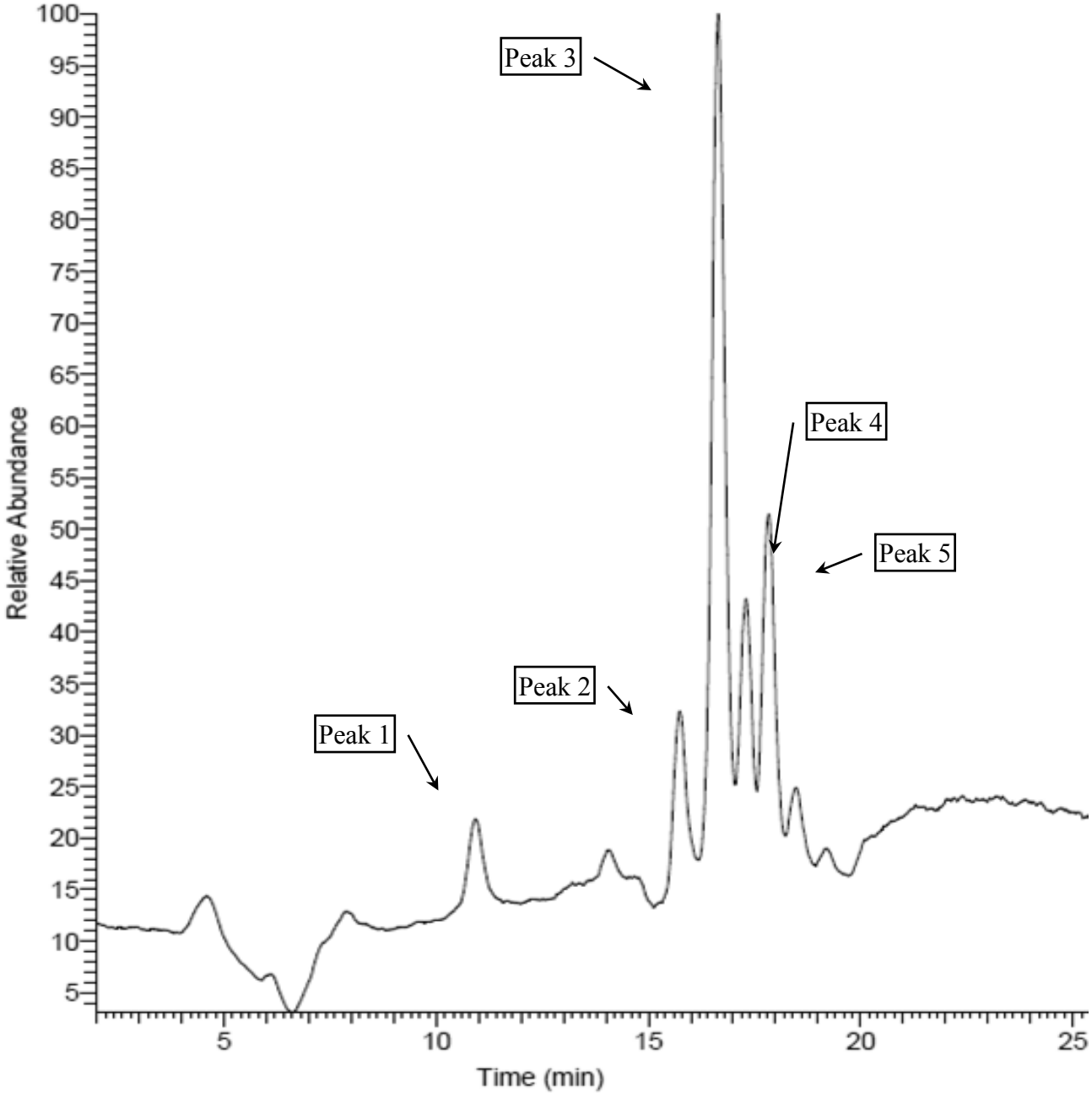


(B)

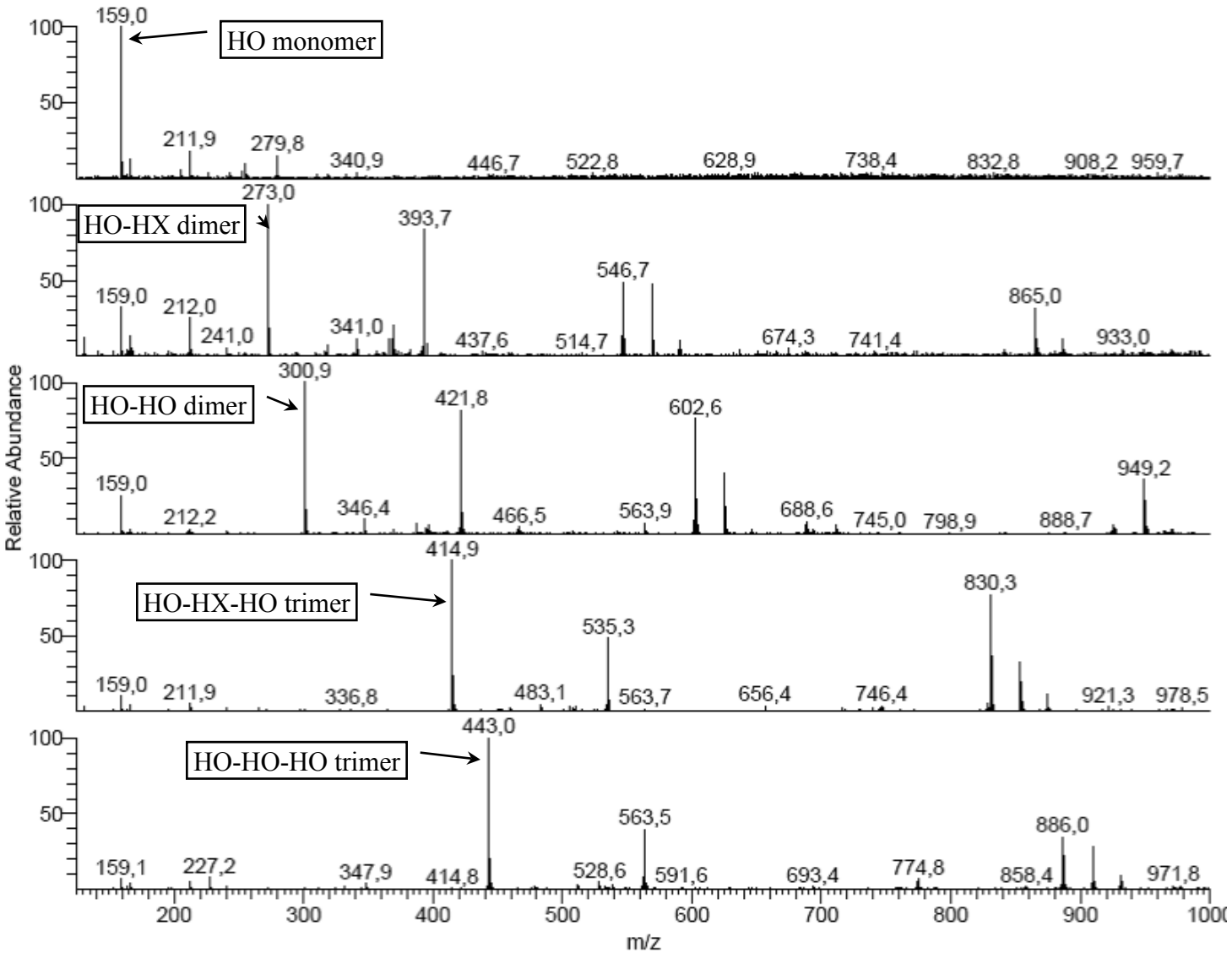
(C)



(A)


Peak 3
Peak 4
Peak 5
Peak 2
Peak 1
Relative Abundance
Time (min)
100
95
90
85
80
75
70
65
60
55
50
45
40
35
30
25
20
15
10
5
5
10
15
20
25

(B)


HO monomer
HO-HX dimer
HO-HO dimer
HO-HX-HO trimer
HO-HO-HO trimer
Relative Abundance
m/z
159,0
211,9
279,8
340,9
446,7
522,8
628,9
738,4
832,8
908,2
959,7
273,0
393,7
546,7
159,0
212,0
241,0
341,0
437,6
514,7
674,3
741,4
865,0
933,0
300,9
421,8
602,6
159,0
212,2
346,4
466,5
563,9
688,6
745,0
798,9
888,7
949,2
414,9
830,3
535,3
159,0
211,9
336,8
483,1
563,7
656,4
746,4
921,3
978,5
443,0
563,5
886,0
159,1
227,2
347,9
414,8
528,6
591,6
693,4
774,8
858,4
971,8