

Supplementary information

A marine fungus efficiently degrades polyethylene

Rongrong Gao^{a,b,c,d}, Rui Liu^{a,b,d}, Chaomin Sun^{a,b,c,d*}

^aCAS and Shandong Province Key Laboratory of Experimental Marine Biology & Center of Deep Sea Research, Institute of Oceanology, Chinese Academy of Sciences, Qingdao, China

^bLaboratory for Marine Biology and Biotechnology, Pilot National Laboratory for Marine Science and Technology, Qingdao, China

^cCollege of Earth Science, University of Chinese Academy of Sciences, Beijing, China

^dCenter of Ocean Mega-Science, Chinese Academy of Sciences, Qingdao, China

* Corresponding author

Chaomin Sun Tel.: +86 532 82898857; fax: +86 532 82898857.

E-mail address: sunchaomin@qdio.ac.cn

Supplementary Figures

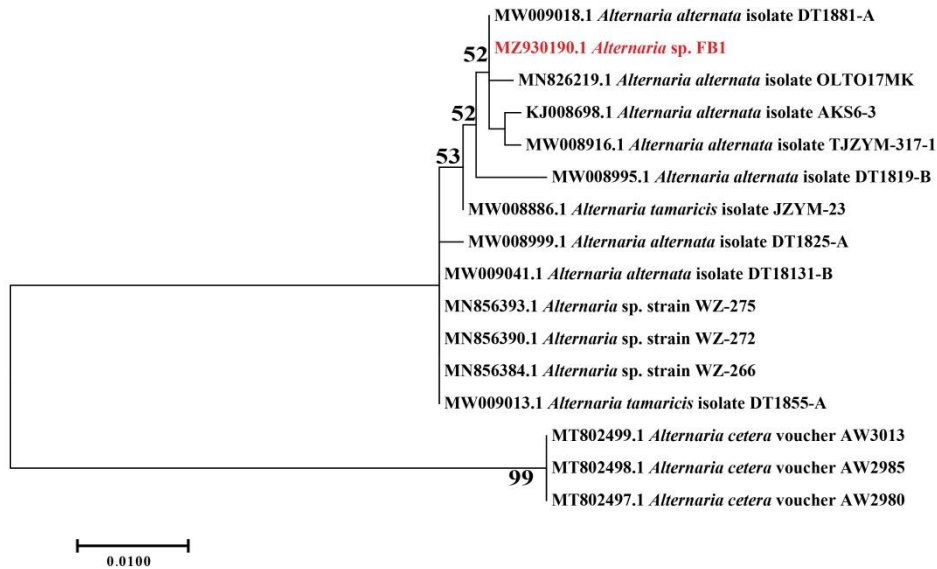


Figure S1. ITS-based phylogenetic tree of *Alternaria* sp. FB1 with other related fungal strains obtained from the GenBank. The accession number of each ITS is indicated after the species names. The phylogenetic tree was constructed by the neighbor-joining method and numbers above the branches are bootstrap values based on 1000 replicates. Bar, 0.01 substitutions per nucleotide position.

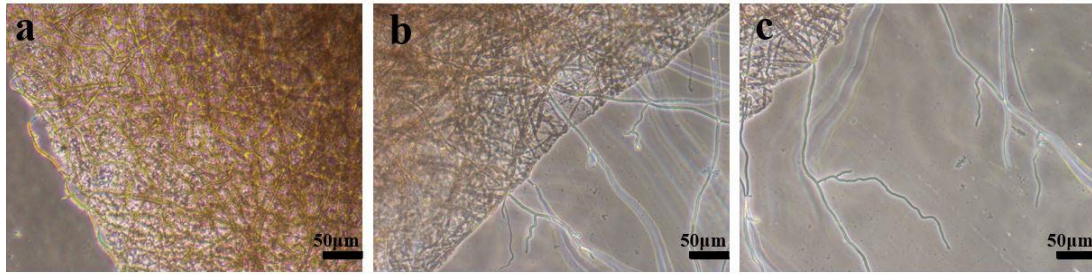


Figure S2. The light microscopy observation of the mycelium morphology of strain FB1 in the seawater supplemented with or without the PE film for 120 days. a, The mycelium morphology of strain FB1 incubated in the seawater for 120 days. **b, c,** The mycelium morphology of strain FB1 incubated in the seawater supplemented with the PE film for 120 days.

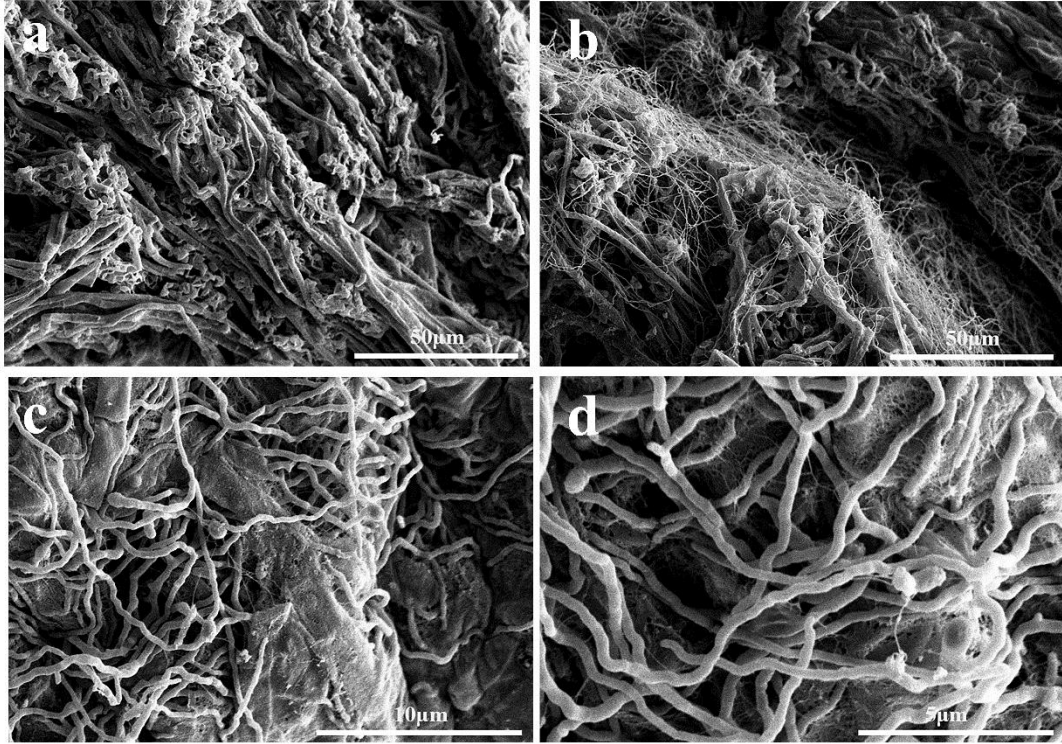


Figure S3. The SEM observation of the growth of strain FB1 in the seawater supplemented with or without the PE film for 120 days. **a**, The SEM observation of the mycelium morphology of strain FB1 in the seawater for 120 days. **b-d**, The SEM observation of mycelium morphology of strain FB1 in the seawater supplemented with the PE film for 120 days.

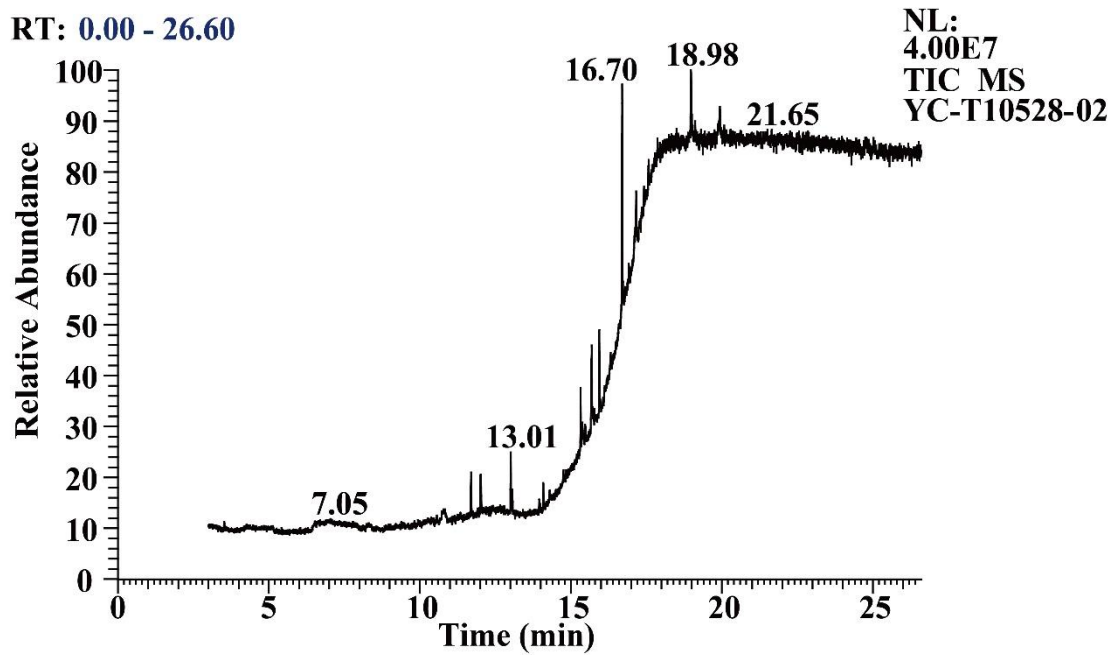


Figure S4. GC-MS chromatograms of the products extracted from PE biodegradation by *Alternaria* sp. FB1 for 60 days.

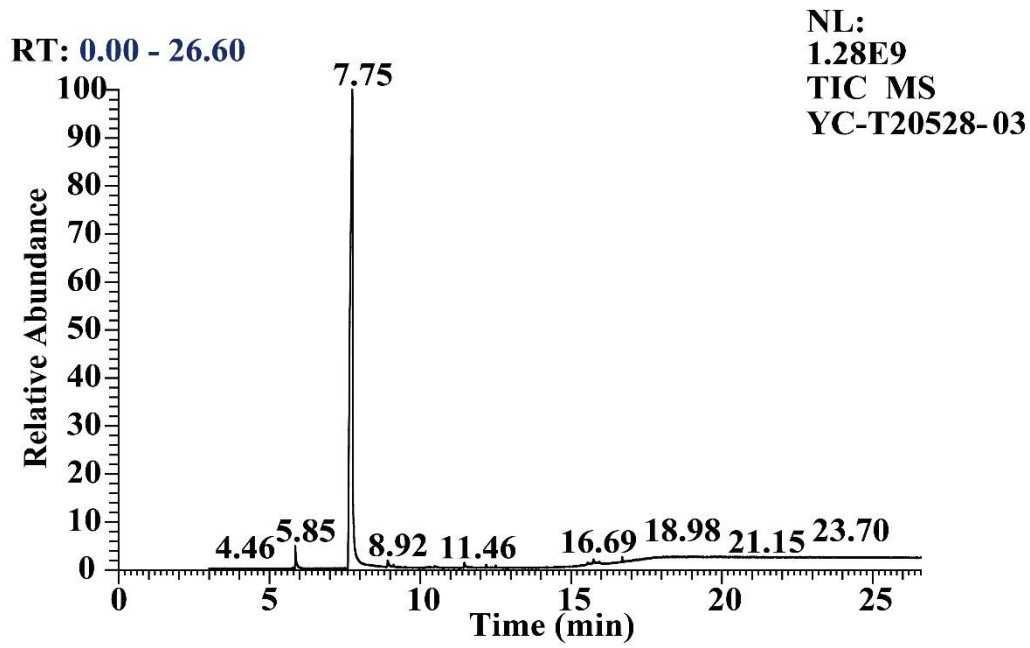


Figure S5. GC-MS chromatograms of the products extracted from PE biodegradation by *Alternaria* sp. FB1 for 120 days.

SI 888, RSI 932, replib, Entry# 20386, CAS# 101-83-7, Cyclohexanamine, N-cyclohexyl- Cyclohexanamine, N-cyclohexyl-
Formula C12H23N, MW 181, CAS# 101-83-7, Entry# 20386
Dicyclohexylamine

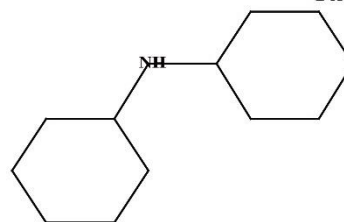
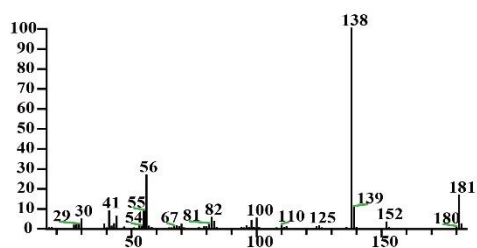


Figure S6. Mass spectrometry analysis of a component at the retention time of 11.70 min identified as Cyclohexanamine, N-cyclohexyl-.

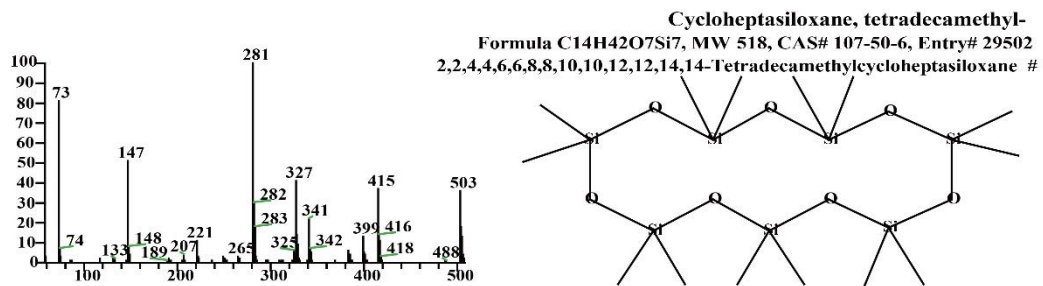


Figure S7. Mass spectrometry analysis of a component at the retention time of 12.01 min identified as Cycloheptasiloxane, tetradecamethyl-.

SI 901, RSI 935, replib, Entry# 14005, CAS# 126-73-8, Tributyl phosphate

Tributyl phosphate

Formula C₁₂H₂₇O₄P, MW 266, CAS# 126-73-8, Entry# 14005
TBP

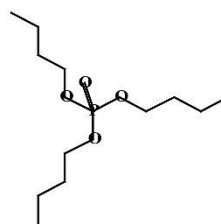
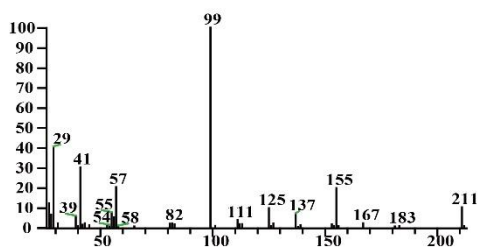


Figure S8. Mass spectrometry analysis of a component at the retention time of 13.01 min identified as Tributyl phosphate.

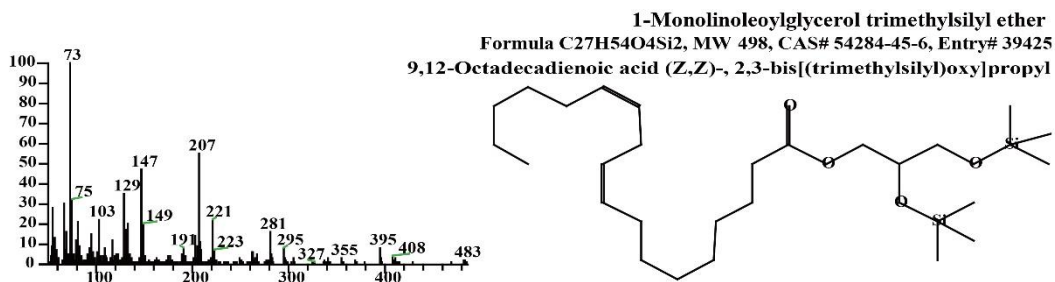


Figure S9. Mass spectrometry analysis of a component at retention times of 15.32 min, 15.69 min, 15.94 min, 17.58 min identified as 1-Monolinoleoylglycerol trimethylsilyl ether.

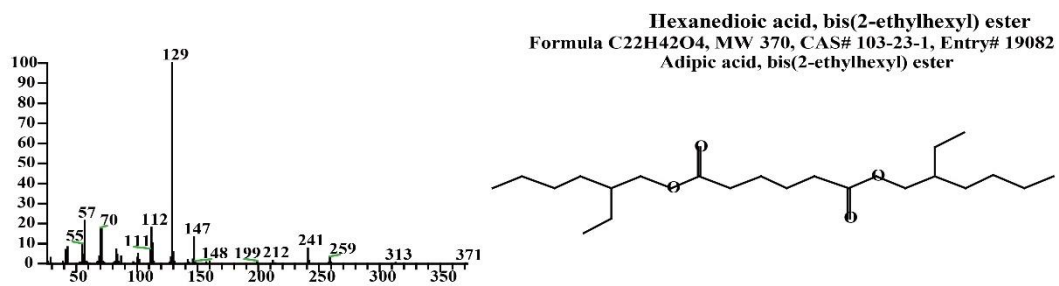


Figure S10. Mass spectrometry analysis of a component at the retention time of 16.70 min identified as Hexanedioic acid, bis(2-ethylhexyl) ester.

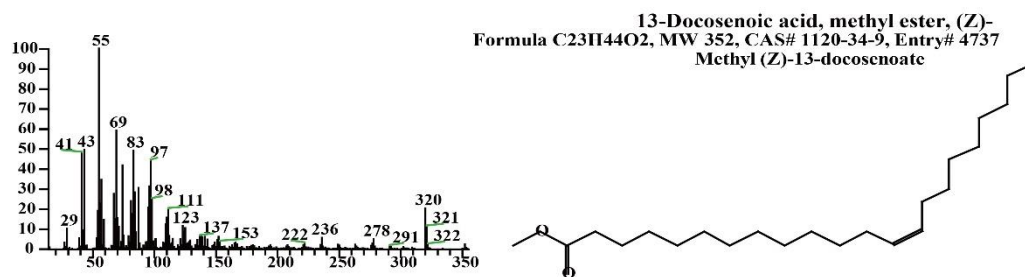


Figure S11. Mass spectrometry analysis of a component at retention time of 17.16 min identified as 13-Docosenoic acid, methyl ester, (Z)-.

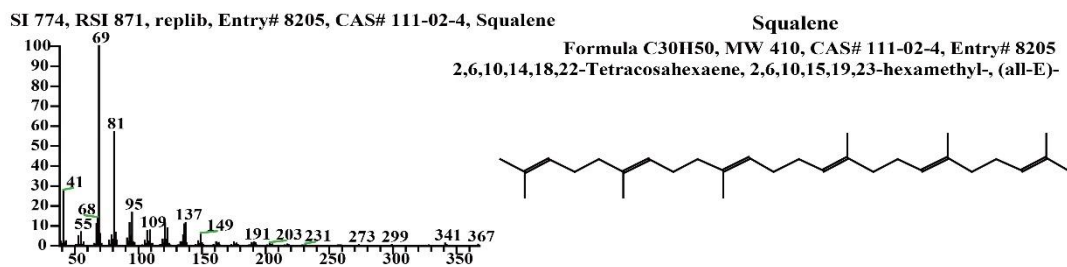
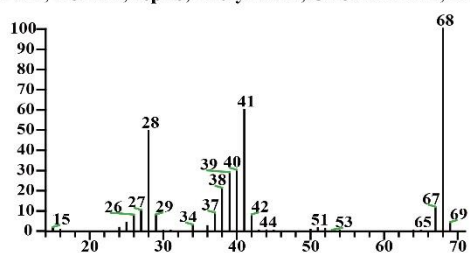


Figure S12. Mass spectrometry analysis of a component at the retention time of 18.98 min identified as Squalene.

SI 935, RSI 937, replib, Entry# 7805, CAS# 288-13-1, 1H-Pyrazole



1H-Pyrazole
Formula C3H4N2, MW 68, CAS# 288-13-1, Entry# 7805
Pyrazole

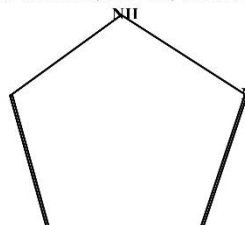
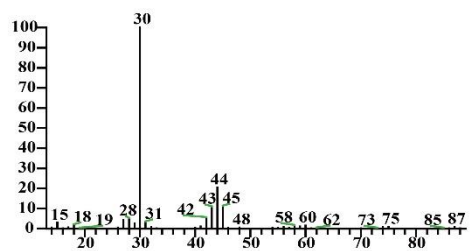


Figure S13. Mass spectrometry analysis of a component at the retention time of 5.85 min identified as 1H-Pyrazole.

SI 828, RSI 838, mainlib, Entry# 947, CAS# 929-06-6, Diglycolamine



Diglycolamine
Formula C₄H₁₁NO₂, MW 105, CAS# 929-06-6, Entry# 947
Ethanol, 2-(2-aminoethoxy)-



Figure S14. Mass spectrometry analysis of a component at the retention time of 7.75 min identified as Diglycolamine.

SI 788, RSI 830, mainlib, Entry# 25463, CAS# 4747-21-1, 2-Propanamine, N-methyl-

2-Propanamine, N-methyl-
Formula C₄H₁₁N, MW 73, CAS# 4747-21-1, Entry# 25463
Ethylamine, N,1-dimethyl-

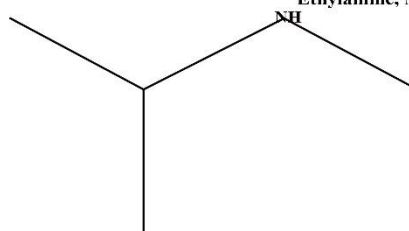
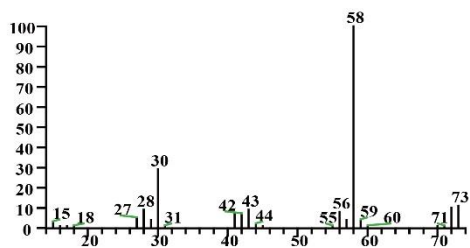


Figure S15. Mass spectrometry analysis of a component at the retention time of 8.92 min identified as 2-Propanamine, N-methyl-.

SI 879, RSI 881, replib, Entry# 14200, CAS# 622-40-2, 4-Morpholineethanol

4-Morpholineethanol
Formula C₆H₁₃NO₂, MW 131, CAS# 622-40-2, Entry# 14200
Morpholinoethanol

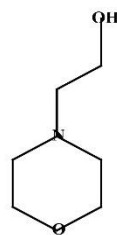
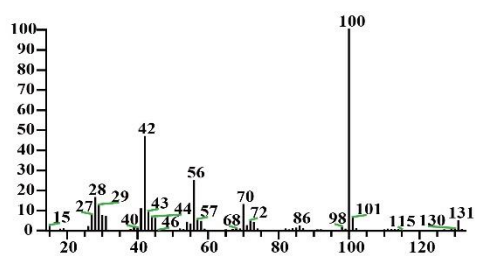


Figure S16. Mass spectrometry analysis of a component at the retention time of 9.12 min identified as 4-Morpholineethanol.

SI 781, RSI 815, mainlib, Entry# 51844, CAS# 26896-20-8, Neodecanoic acid

Neodecanoic acid

Formula C₁₀H₂₀O₂, MW 172, CAS# 26896-20-8, Entry# 51844

Wiltz-65

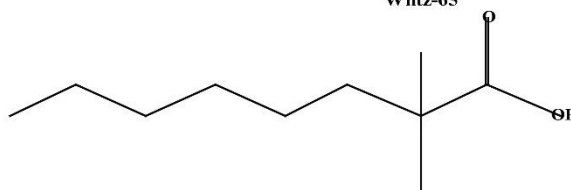
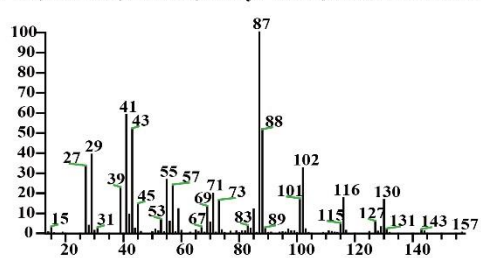


Figure S17. Mass spectrometry analysis of a component at the retention time of 10.47 min identified as Neodecanoic acid.

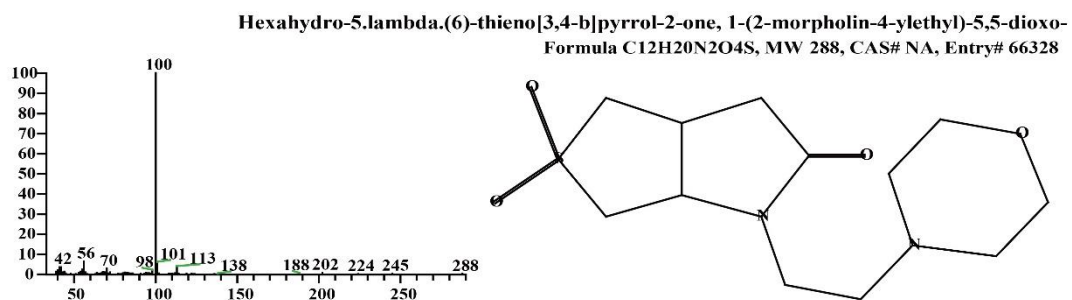


Figure S18. Mass spectrometry analysis of a component at the retention time of 11.46 min identified as Hexahydro-5.lambda.(6)-thieno[3,4-b]pyrrol-2-one, 1-(2-morpholin-4-ylethyl)-5,5-dioxo-.

SI 683, RSI 739, replib, Entry# 14195, CAS# 123-00-2, 4-Morpholinepropanamine

4-Morpholinepropanamine
Formula C7H16N2O, MW 144, CAS# 123-00-2, Entry# 14195
Morpholine, 4-(3-aminopropyl)-

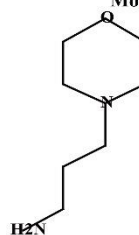
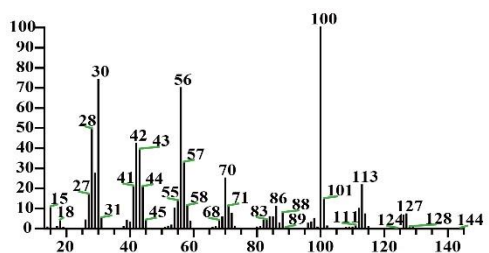


Figure S19. Mass spectrometry analysis of a component at the retention time of 12.18 min identified as 4-Morpholinepropanamine.

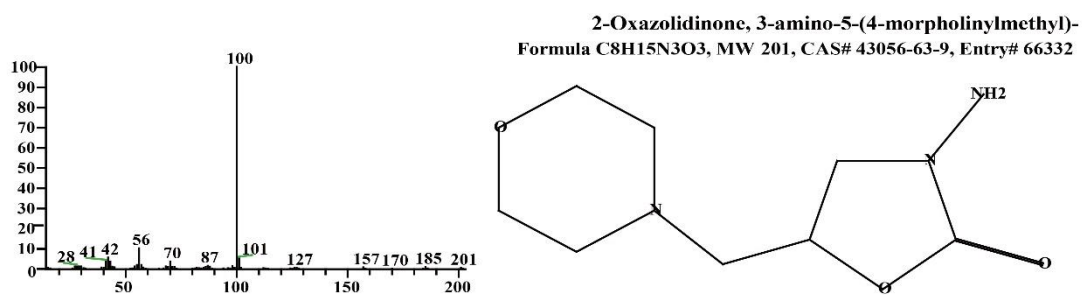


Figure S20. Mass spectrometry analysis of a component at the retention time of 12.50 min identified as 2-Oxazolidinone, 3-amino-5-(4-morpholinylmethyl)-.

SI 679, RSI 719, replib, Entry# 7652, CAS# 544-35-4, Linoleic acid ethyl ester

Linoleic acid ethyl ester

Formula C₂₀H₃₆O₂, MW 308, CAS# 544-35-4, Entry# 7652
Ethyl linoleate

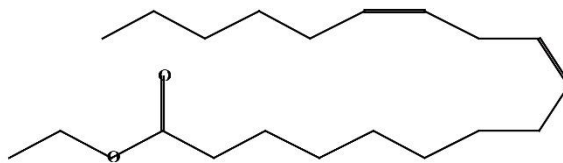
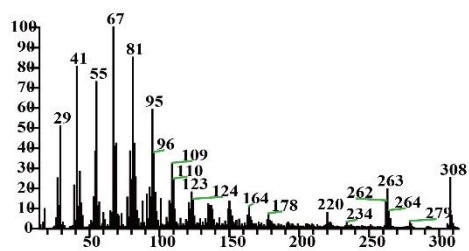


Figure S21. Mass spectrometry analysis of a component at the retention time of 15.56 min identified as Linoleic acid ethyl ester.

SI 855, RSI 901, replib, Entry# 7681, CAS# 60-33-3, 9,12-Octadecadienoic acid (Z,Z)- **9,12-Octadecadienoic acid (Z,Z)-**
Formula C18H32O2, MW 280, CAS# 60-33-3, Entry# 7681
cis-9,cis-12-Octadecadienoic acid

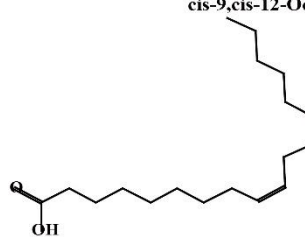
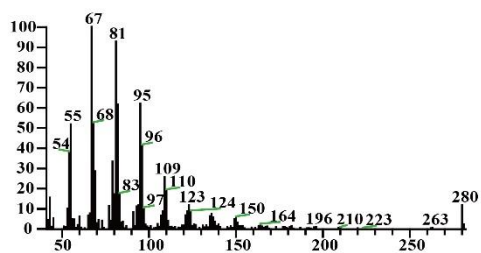


Figure S22. Mass spectrometry analysis of a component at the retention time of 15.74 min identified as 9,12-Octadecadienoic acid (Z,Z)-.

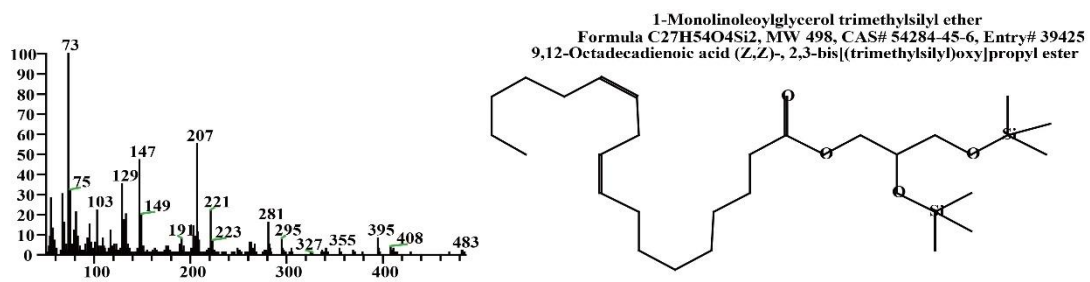


Figure S23. Mass spectrometry analysis of a component at the retention time of 15.86 min identified as 1-Monolinoleoylglycerol trimethylsilyl ether.

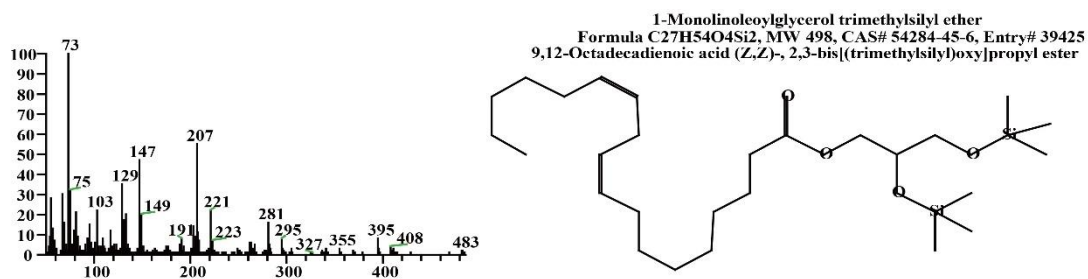
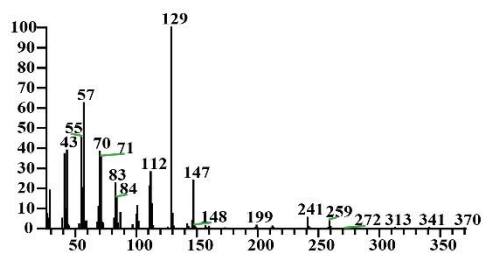


Figure S24. Mass spectrometry analysis of a component at the retention time of 15.94 min identified as 1,8-Dioxa-5-thiaoctane, 8-(9-borabicyclo[3.3.1]non-9-yl)-3-(9-borabicyclo[3.3.1]non-9-yloxy)-1-phenyl-.



Hexanedioic acid, bis(2-ethylhexyl) ester
Formula C₂₂H₄₂O₄, MW 370, CAS# 103-23-1, Entry# 19079
Adipic acid, bis(2-ethylhexyl) ester

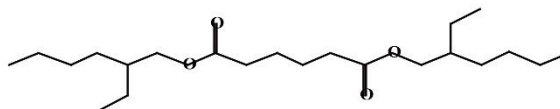


Figure S25. Mass spectrometry analysis of a component at the retention time of 16.69 min identified as Hexanedioic acid, bis(2-ethylhexyl) ester.

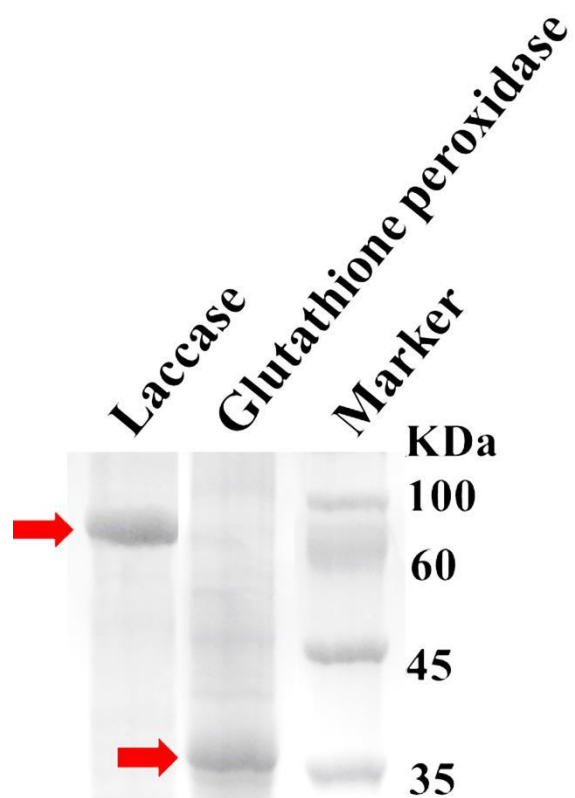


Figure S26. Coomassie blue-stained SDS gel of purified His-tagged Glutathione peroxidase and Laccase.

Supplementary Table S1. Mass spectrometric identification of compositions released from the PE film treated by *Alternaria* sp. FB1 for 60 days.

| Retention time | Peak Area | Area % | chemical formula | Compound Name | Cas # |
|----------------|-------------|--------|--|--|------------|
| 11.70 | 3803383.72 | 2.33 | C ₁₂ H ₂₃ N | Cyclohexanamine, N-cyclohexyl- | 101-83-7 |
| 12.01 | 5640482.26 | 3.45 | C ₁₄ H ₄₂ O ₇ Si ₇ | Cycloheptasiloxane, tetradecamethyl- | 107-50-6 |
| 13.01 | 7789352.35 | 4.77 | C ₁₂ H ₂₇ | Tributyl phosphate | 126-73-8 |
| 15.32 | 8879994.65 | 5.44 | C ₂₇ H ₅₄ O ₄ Si ₂ | 1-Monolinoleoylglycerol trimethylsilyl ether | 54284-45-6 |
| 15.69 | 14727808.96 | 9.02 | C ₂₇ H ₅₄ O ₄ Si ₂ | 1-Monolinoleoylglycerol trimethylsilyl ether | 54284-45-6 |
| 15.94 | 7910509.73 | 4.84 | C ₂₇ H ₅₄ O ₄ Si ₂ | 1-Monolinoleoylglycerol trimethylsilyl ether | 54284-45-6 |
| 16.70 | 26809165.70 | 16.42 | C ₂₂ H ₄₂ O ₄ | Hexanedioic acid, bis(2-ethylhexyl) ester | 103-23-1 |
| 17.16 | 12894410.05 | 7.90 | C ₂₃ H ₄₄ O ₂ | 13-Docosenoic acid, methyl ester, (Z)- | 1120-34-9 |
| 17.58 | 52152219.34 | 31.94 | C ₂₇ H ₅₄ O ₄ Si ₂ | 1-Monolinoleoylglycerol trimethylsilyl ether | 54284-45-6 |
| 18.98 | 22675990.39 | 13.89 | C ₃₀ H ₅₀ | Squalene | 111-02-4 |

Supplementary Table S2. Mass spectrometric identification of compositions released from the PE film treated by *Alternaria* sp. FB1 for 120 days.

| Retention time | Peak Area | Area % | chemical formula | Compound Name | Cas # |
|----------------|---------------|--------|---|--|------------|
| 5.85 | 160827301.91 | 1.98 | C ₃ H ₄ N ₂ | 1H-Pyrazole | 288-13-1 |
| 7.75 | 7586192268.64 | 93.28 | C ₄ H ₁₁ NO ₂ | Diglycolamine | 929-06-6 |
| 8.92 | 53491610.03 | 0.66 | C ₄ H ₁₁ N | 2-Propanamine, N-methyl- | 4747-21-1 |
| 9.12 | 8303541.90 | 0.10 | C ₆ H ₁₃ NO ₂ | 4-Morpholineethanol | 622-40-2 |
| 10.47 | 17177624.61 | 0.21 | C ₁₀ H ₂₀ O ₂ | Neodecanoic acid | 26896-20-8 |
| 11.46 | 40745877.94 | 0.50 | C ₁₂ H ₂₀ N ₂ O ₄ S | Hexahydro-5.lambda.(6)-thieno[3,4-b]pyrrol-2-one, 1-(2-morpholin-4-ylethyl)-5,5-dioxo- | NA |
| 12.18 | 10690688.81 | 0.13 | C ₇ H ₁₆ N ₂ O | 4-Morpholinepropanamine | 123-00-2 |
| 12.50 | 6114688.36 | 0.08 | C ₈ H ₁₅ N ₃ O ₃ | 2-Oxazolidinone, 3-amino-5-(4-morpholinylmethyl)- | 43056-63-9 |
| 15.56 | 23251808.47 | 0.29 | C ₂₀ H ₃₆ O ₂ | Linoleic acid ethyl ester | 544-35-4 |
| 15.74 | 54484769.63 | 0.67 | C ₁₈ H ₃₂ O ₂ | 9,12-Octadecadienoic acid (Z,Z)- | 60-33-3 |
| 15.86 | 9971750.86 | 0.12 | C ₂₇ H ₅₄ O ₄ Si ₂ | 1-Monolinoleoylglycerol trimethylsilyl ether | 54284-45-6 |
| 15.94 | 16603906.65 | 0.20 | C ₂₇ H ₅₄ O ₄ Si ₂ | 1-Monolinoleoylglycerol trimethylsilyl ether | 54284-45-6 |
| 16.69 | 15787407.31 | 0.19 | C ₂₂ H ₄₂ O ₄ | Hexanedioic acid, bis(2-ethylhexyl) ester | 103-23-1 |

Supplementary Table S3. Primers used for construction of vectors for expression of potential PE-degrading enzymes.

| Primer | Sequence |
|--------------------------|---------------------------------|
| Glutathione peroxidase F | GGATCCATGATTTCGATTGGGAGGGG |
| Glutathione peroxidase R | CTCGAGTTTGCCGCCAGCTCCTTC |
| Laccase F | GGATCCATGAGCGAGCACACTGATTCGC |
| Laccase R | CTCGAGGATTGTGTCCGTGTCTAGAATTCTT |