

SUPPORTING INFORMATION FOR :

# Structural identification of antibacterial lipids from Amazonian palm tree endophytes through the Molecular Network approach

Morgane Barthélemy<sup>1</sup>, Nicolas Elie<sup>1</sup>, Léonie Pellissier<sup>2</sup>, Jean-Luc Wolfender<sup>2</sup>, Didier Stien<sup>3</sup>, David Touboul<sup>1</sup> and Véronique Eparvier<sup>1,\*</sup>

<sup>1</sup> CNRS-Institut de Chimie des Substances Naturelles, UPR2301, Université Paris-Saclay, 91198 Gif-sur-Yvette cedex, France; morgane.barthelemy@cnrs.fr, nicolas.elie@cnrs.fr

<sup>2</sup> School of Pharmaceutical Sciences, EPGL, University of Geneva, University of Lausanne, Rue Michel Servet 1, CH-1211, Geneva, Switzerland; leonie.pellissier@unige.ch, jean-luc.wolfender@unige.ch

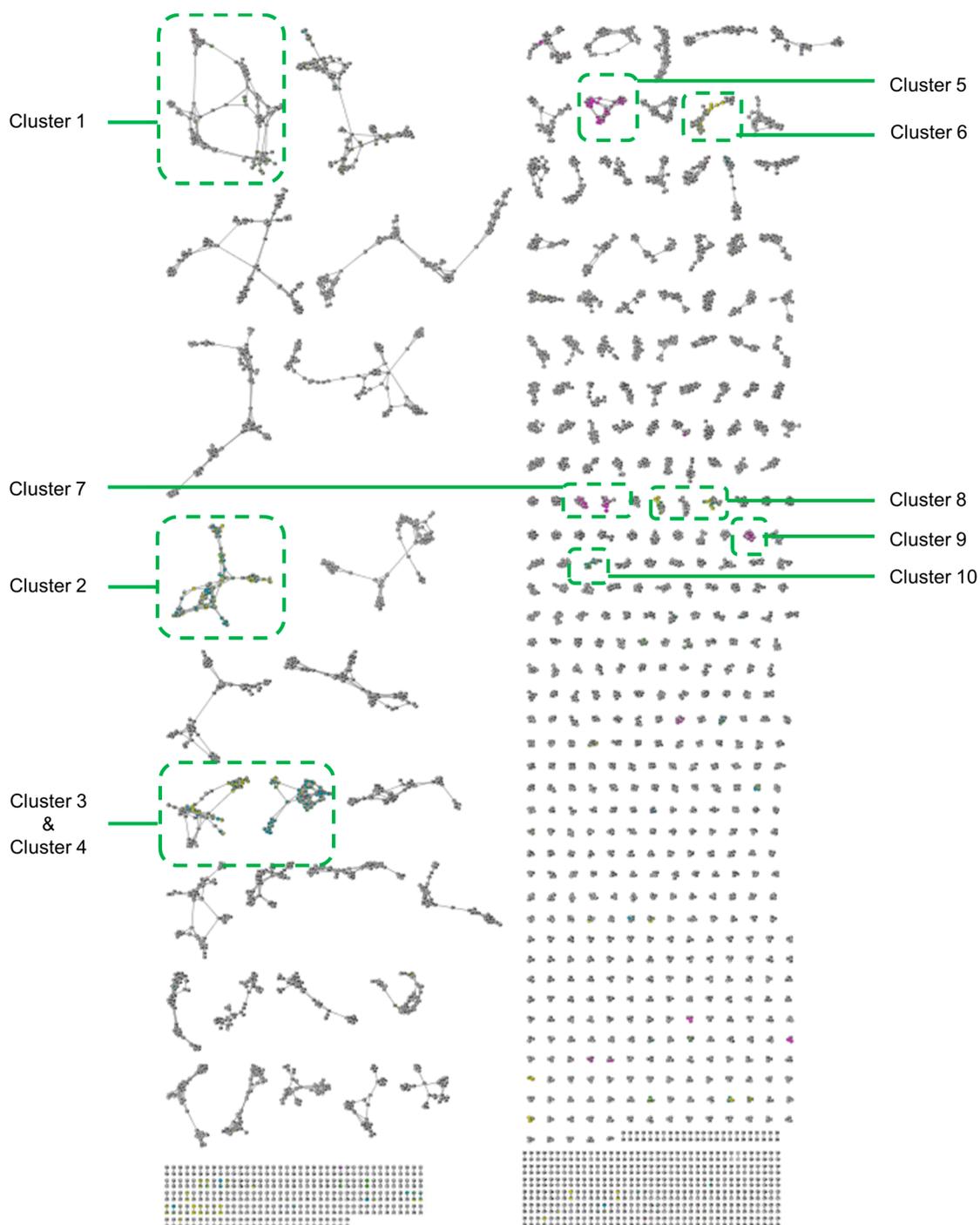
<sup>3</sup> Sorbonne Universités, UPMC Univ. Paris 06, CNRS, Laboratoire de Biodiversité et Biotechnologies Microbiennes (LBBM), Observatoire Océanologique, 66650 Banyuls-sur-Mer, France; didier.stien@cnrs.fr

\* Correspondence: veronique.eparvier@cnrs.fr; Tel.: +33-(0)16-982-3679, david.touboul@cnrs.fr; Tel: +33-(0)16-982-3032

## Table of content

Molecular network of the 131 extracts from fungal endophytes	S1
Dereplication process and MS <sup>2</sup> fragmentation spectra of nodes belonging to the clusters composed by ions from the four active fungal extracts.	S2
Molecular network of the 66 extracts from bacterial endophytes	S3
Dereplication process of nodes belonging to the clusters composed by ions from the <i>Luteibacter</i> sp. extract.	S4
Dereplication process of nodes belonging to the clusters composed by ions from the <i>Bacillus</i> sp. extract	S5
NMR spectra and HRMS of ( <i>R</i> )-2-hydroxy-13-methyltetradecanoic acid (1)	S6-S8
NMR spectra and HRMS of ( <i>R</i> )-3-hydroxy-14-methyl-pentadecanoic acid (2)	S9-S11
NMR spectra and HRMS of ( <i>S</i> )-β-hydroxypalmitic acid (3)	S12-S14
NMR spectra and HRMS of ( <i>R</i> )-3-hydroxy-15-methylhexadecanoic acid (4)	S15-17
NMR spectra and HRMS of ( <i>R</i> )-3-hydroxy-13-methyltetradecanoic acid (5)	S18-20
NMR spectra and HRMS of 13-methyltetradecanoic acid (6)	S21-23
NMR spectra and HRMS of 9 <i>Z</i> -hexadecenoic acid (7)	S24-26
NMR spectra and HRMS of 15-methyl-9 <i>Z</i> - hexadecenoic acid compound (8)	S27-29
Fragmentation of 9 <i>Z</i> -hexadecenoic acid (7)	S30
Fragmentation of 15-methyl-9 <i>Z</i> -hexadecenoic acid (8)	S31
Identification of endophytic microorganisms associated with <i>Astrocaryum sciophilum</i> and biological activities of their respective EtOAc extracts: antibacterial on methicillin-resistant <i>S. aureus</i> (MIC in µg/ml) and cytotoxicity on MRC-5 cells (viability percentage)	Table S1
Antibacterial activity of AcOEt extract of <i>Luteibacter</i> sp. and its fractions on MRSA (Minimum Inhibitory Concentration in µg/ml)	Table S2

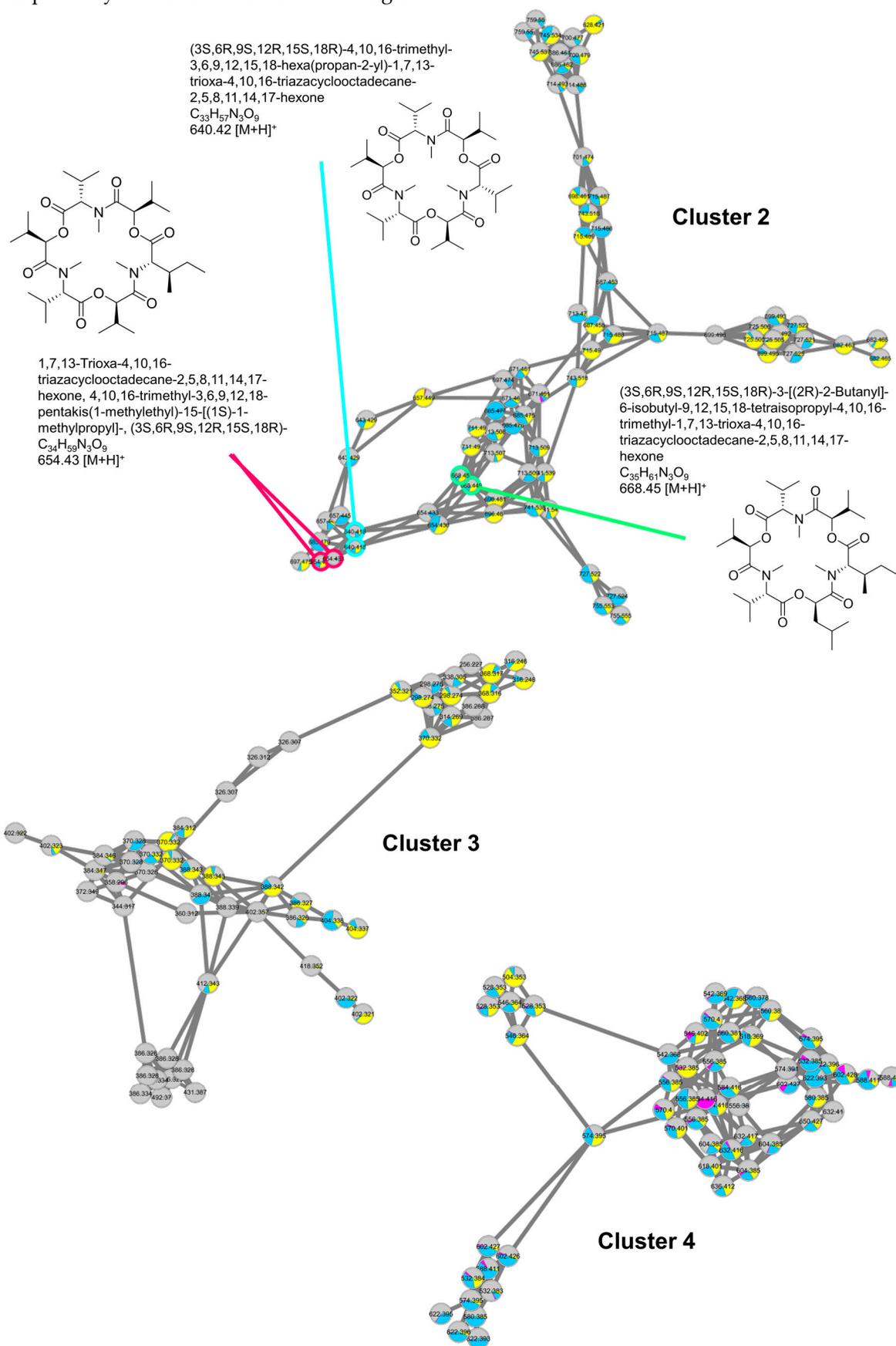
**Figure S1.** Molecular network of the 131 extracts from fungal endophytes. Relative quantification of each ion within the extracts are represented as a XIC area-dependent pie-chart drawing.

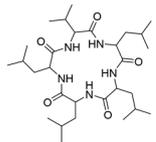


Relative quantification:

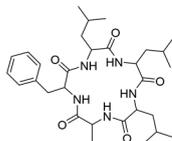
- Non active fungal extracts (MIC > 64 µg/mL)
- *Fusarium* sp. BSNB-0575 extract
- *Fusarium* sp. BSNB-0651 extract
- *Fusarium decemcellular* BSNB-0303 extract
- *Akanthomyces* sp. BSNB-0732 extract

**Figure S2.** Dereplication process and MS/MS fragmentation spectra of nodes belonging to the clusters composed by ions from the four active fungal extracts

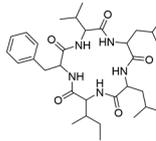




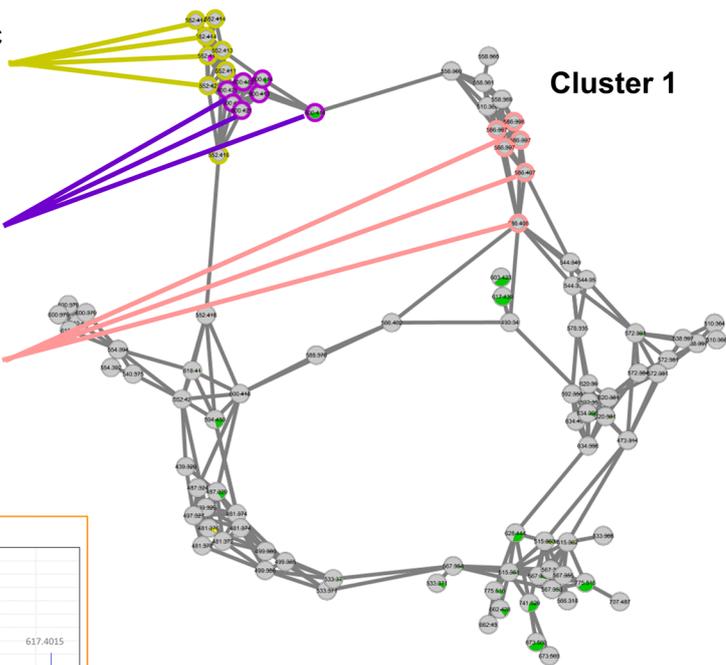
GameXPeptide C  
 $C_{29}H_{53}N_5O_5$   
 552.41 [M+H]<sup>+</sup>



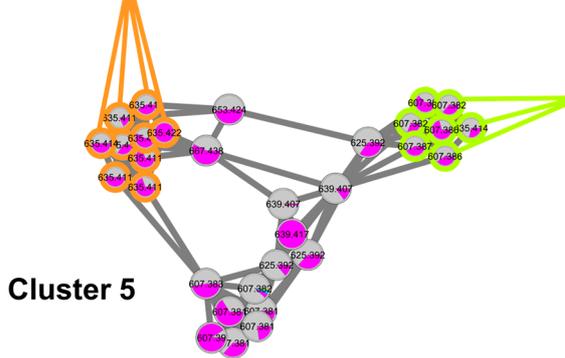
GameXPeptide B  
 $C_{33}H_{53}N_5O_5$   
 600.41 [M+H]<sup>+</sup>



GameXPeptide A  
 $C_{32}H_{51}N_5O_5$   
 586.39 [M+H]<sup>+</sup>



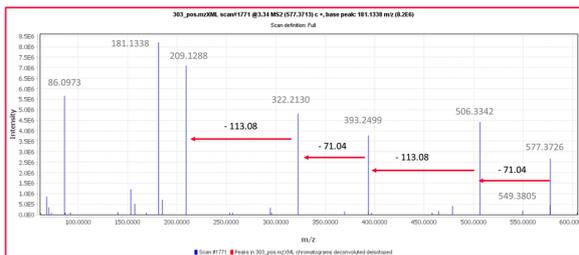
Cluster 1



Cluster 5



Cluster 7

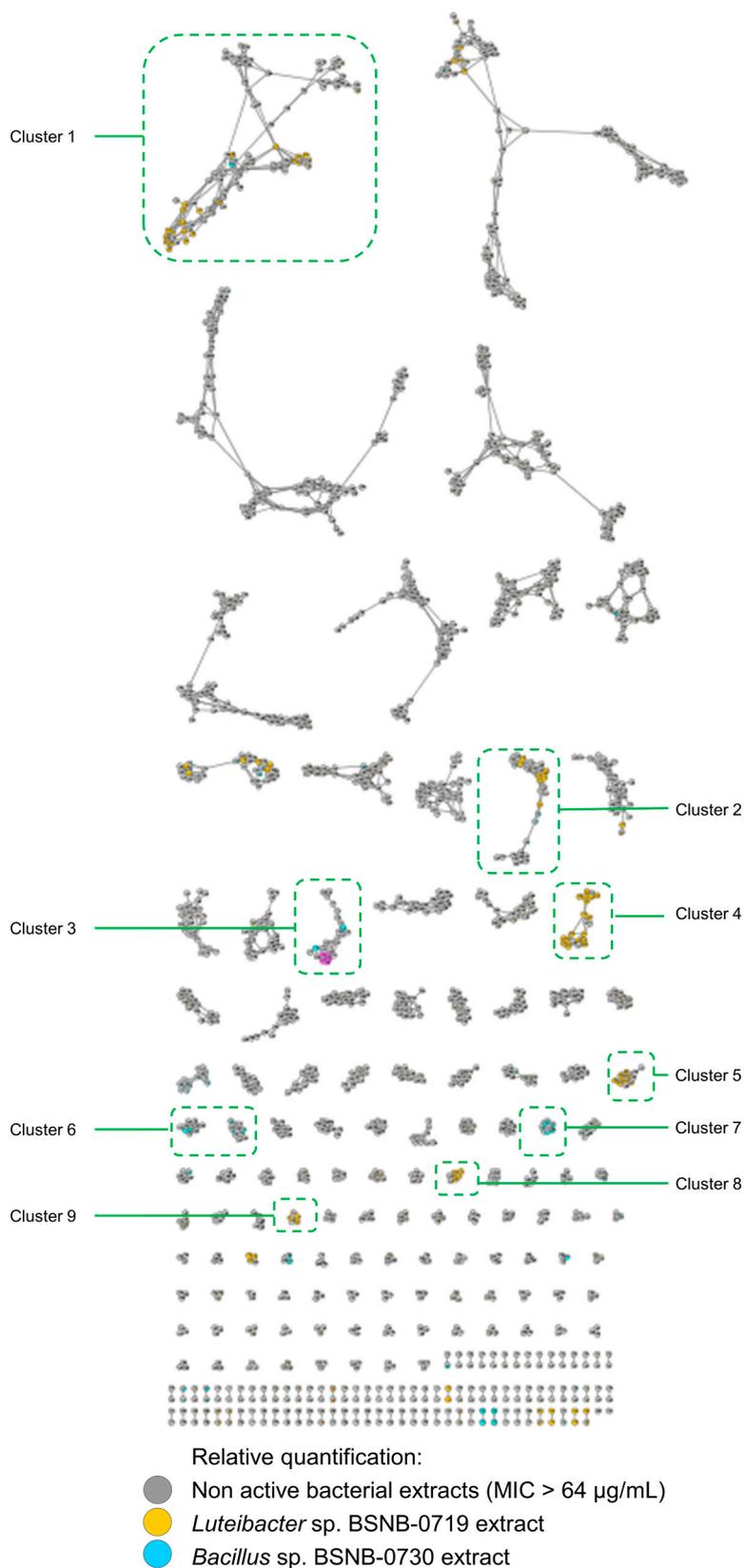


Cluster 7

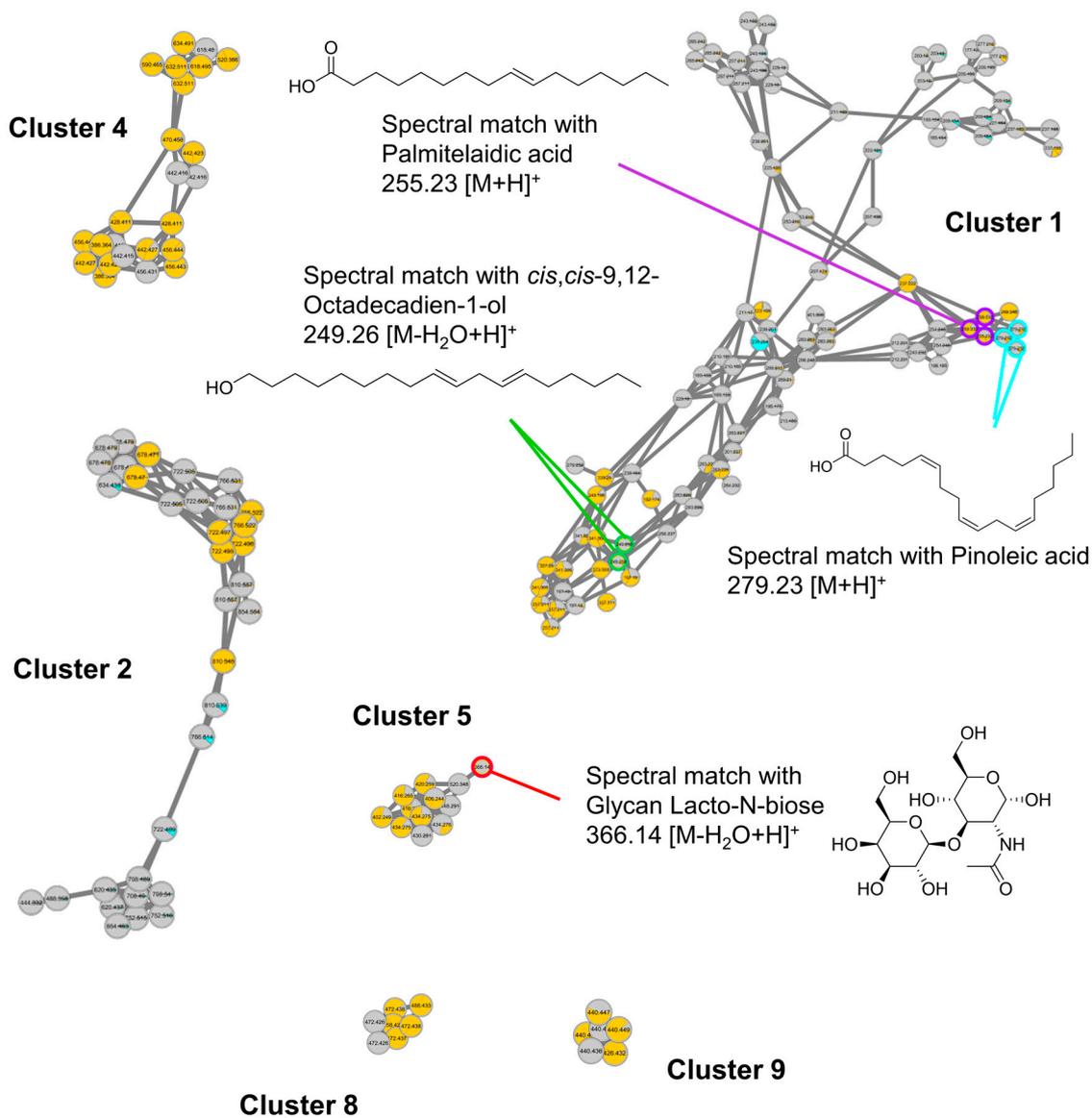




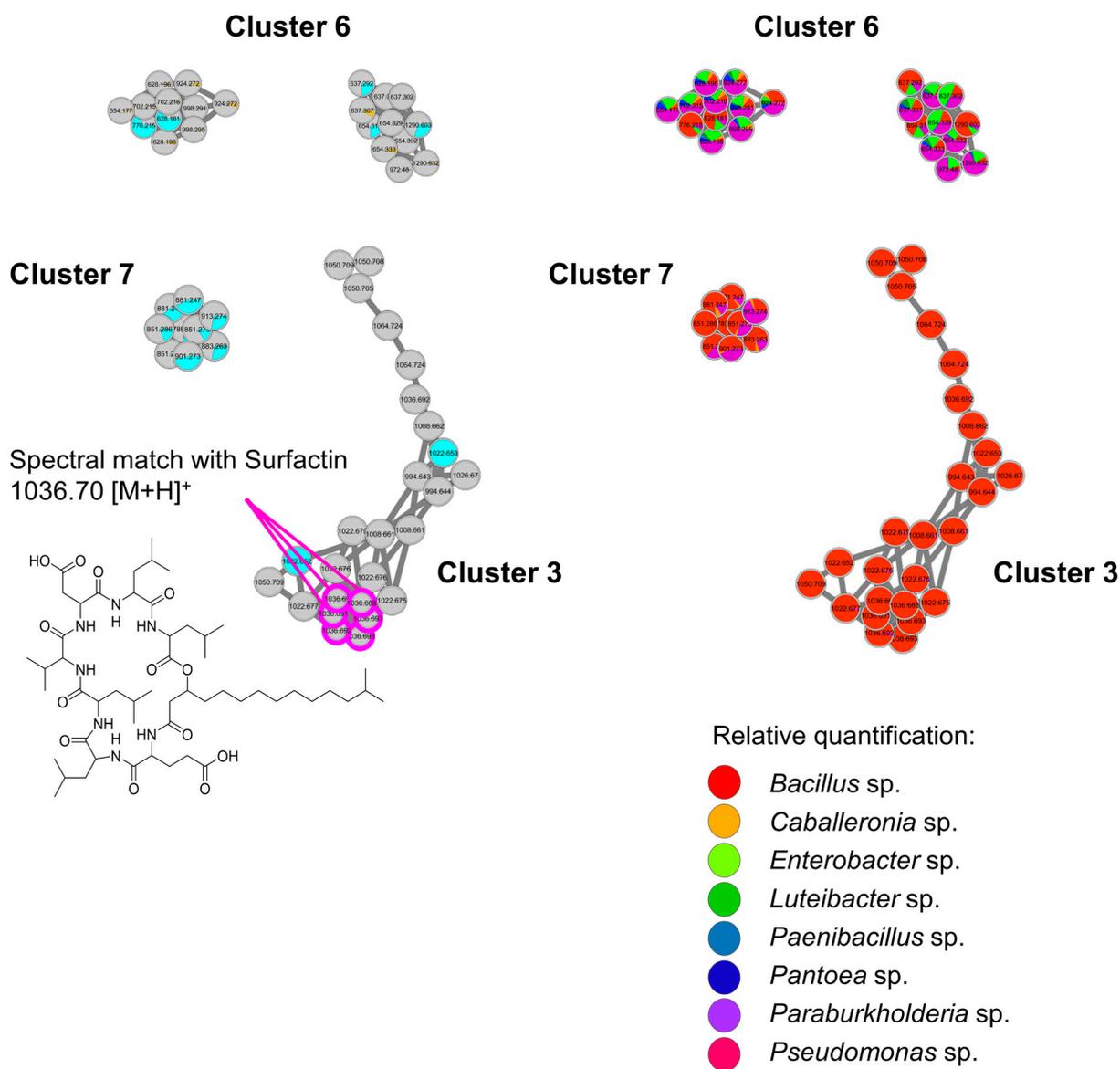
**Figure S3.** Molecular network of the 66 extracts from bacterial endophytes. Relative quantification of each ion within the extracts are represented as a XIC area-dependent pie chart drawing.



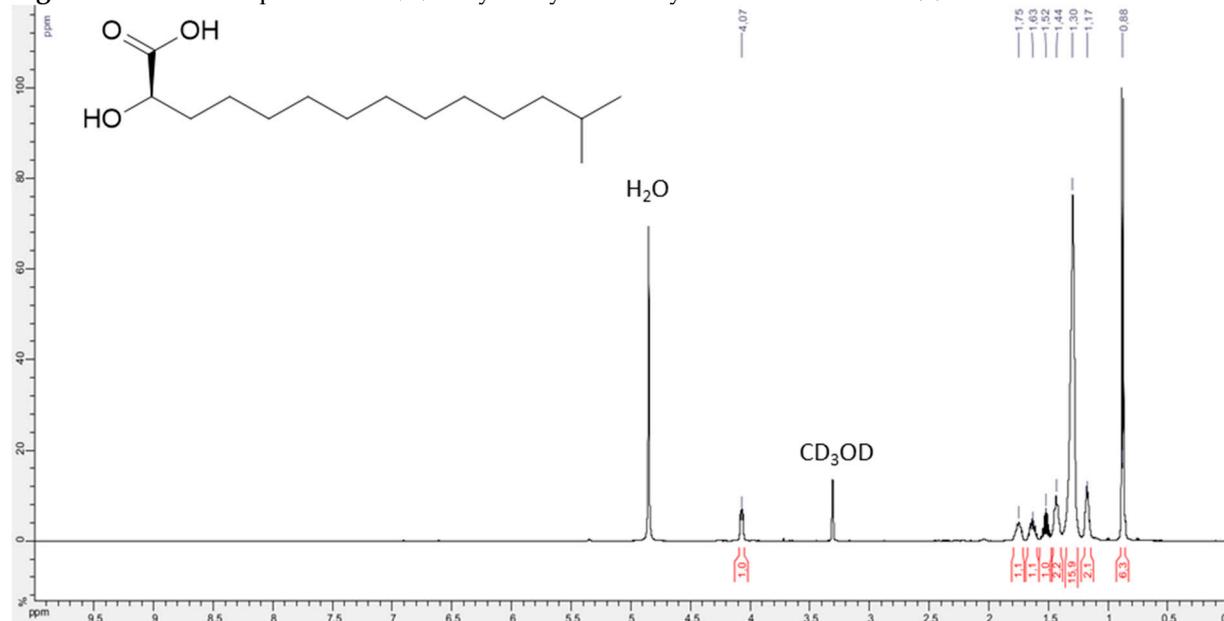
**Figure S4.** Dereplication process of nodes belonging to the clusters composed by ions from the *Luteibacter* sp. extract.



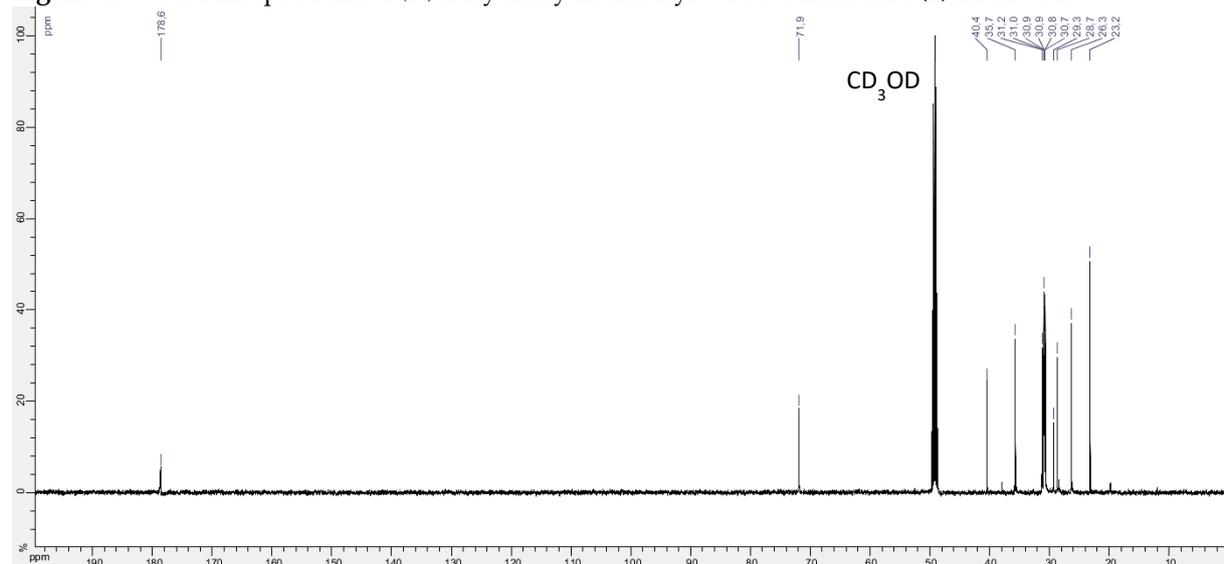
**Figure S5.** Dereplication process of nodes belonging to the clusters composed by ions from the *Bacillus* sp. extract (left). Relative quantification of those clusters depending on the bacterial genus (right).



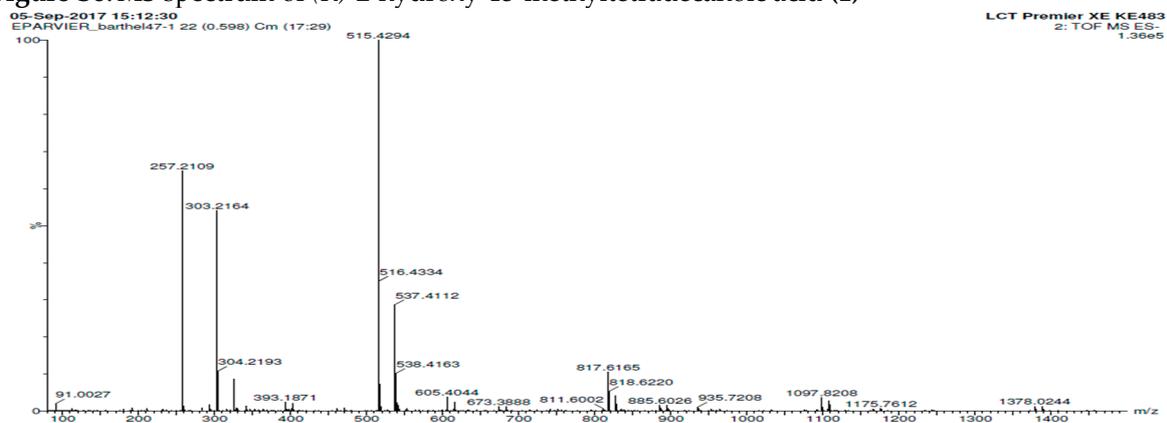
**Figure S6.**  $^1\text{H}$  NMR spectrum of (*R*)-2-hydroxy-13-methyltetradecanoic acid (**1**) in  $\text{CD}_3\text{OD}$



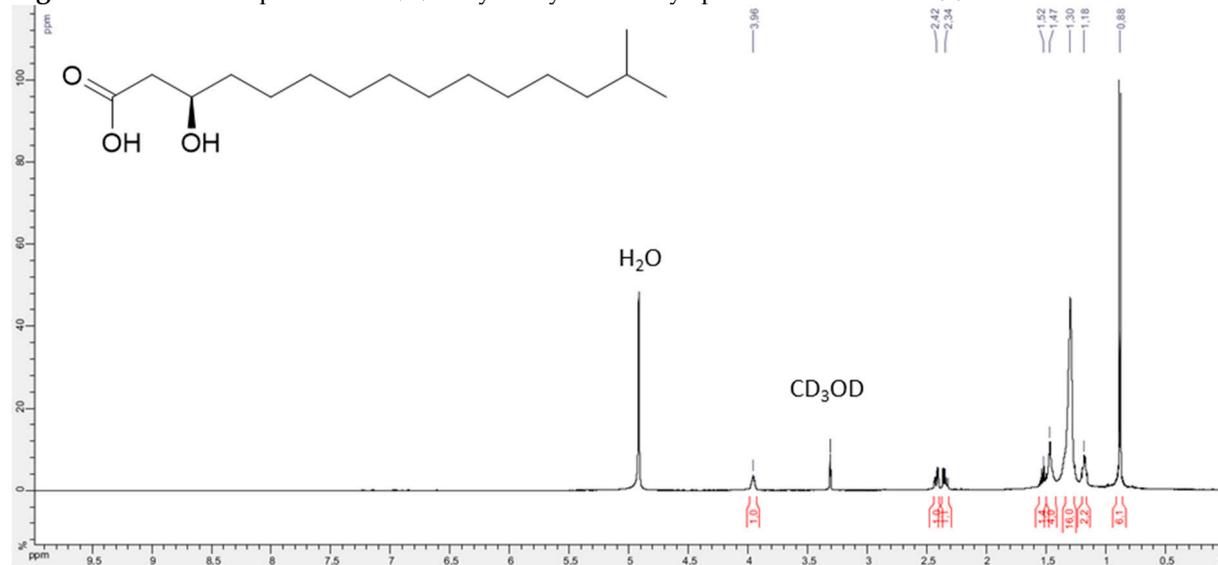
**Figure S7.**  $^{13}\text{C}$  NMR spectrum of (*R*)-2-hydroxy-13-methyltetradecanoic acid (**1**) in  $\text{CD}_3\text{OD}$



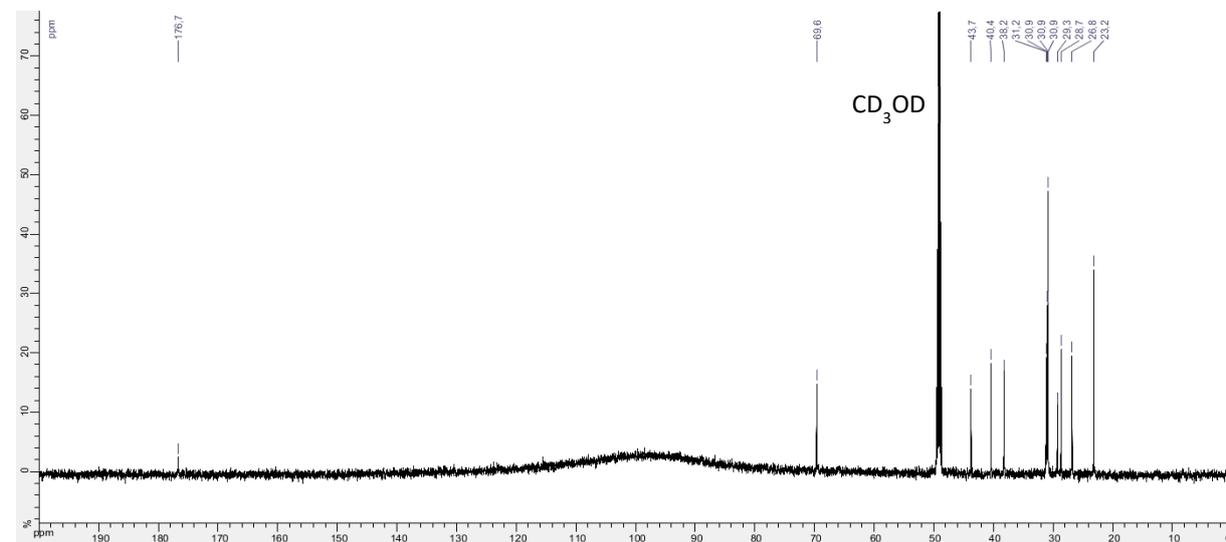
**Figure S8.** MS spectrum of (*R*)-2-hydroxy-13-methyltetradecanoic acid (**1**)



**Figure S9.**  $^1\text{H}$  NMR spectrum of (*R*)-3-hydroxy-14-methyl-pentadecanoic acid (**2**)



**Figure S10.**  $^{13}\text{C}$  NMR spectrum of (*R*)-3-hydroxy-14-methyl-pentadecanoic acid (**2**)



**Figure S11.** MS spectrum of (*R*)-3-hydroxy-14-methyl-pentadecanoic acid (**2**)

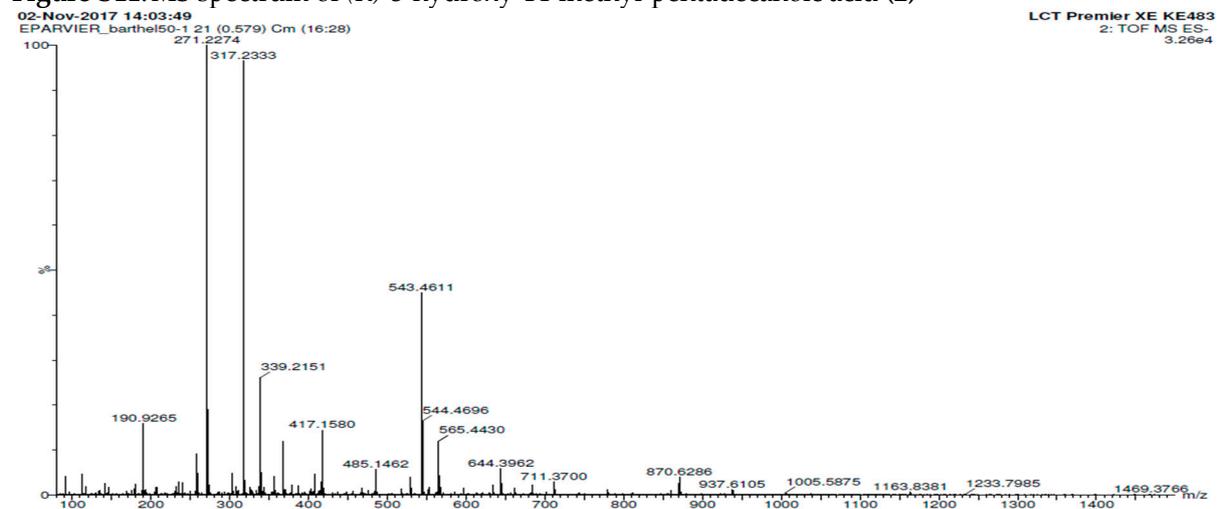


Figure S12. <sup>1</sup>H NMR spectrum of (*S*)-β-hydroxypalmitic acid (**3**)

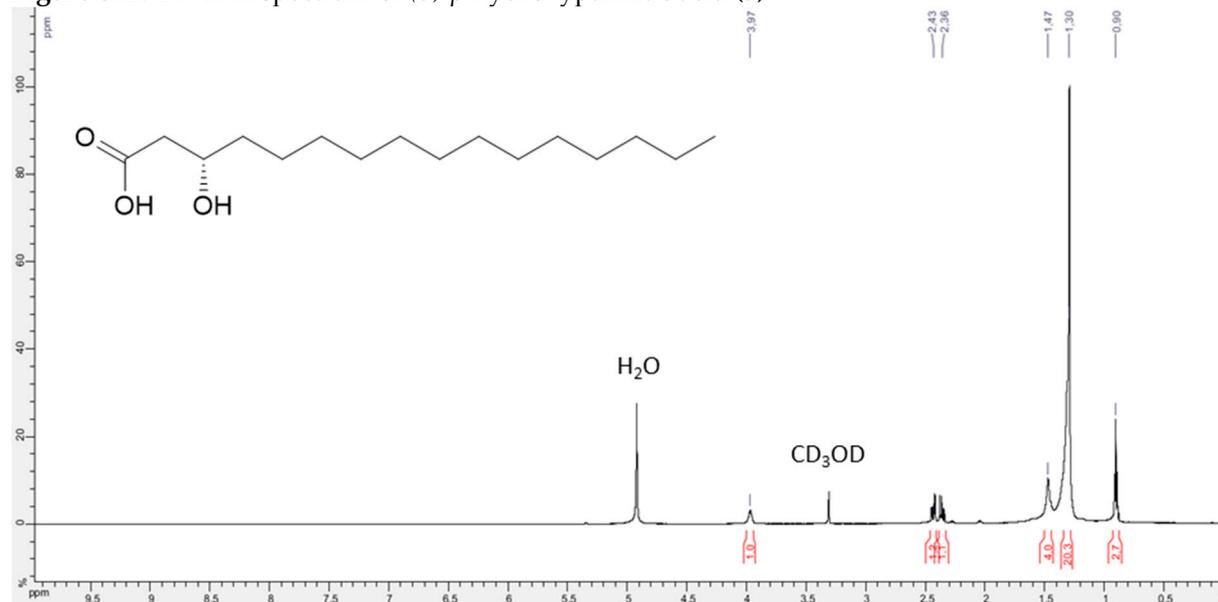


Figure S13. <sup>13</sup>C NMR spectrum of (*S*)-β-hydroxypalmitic acid (**3**)

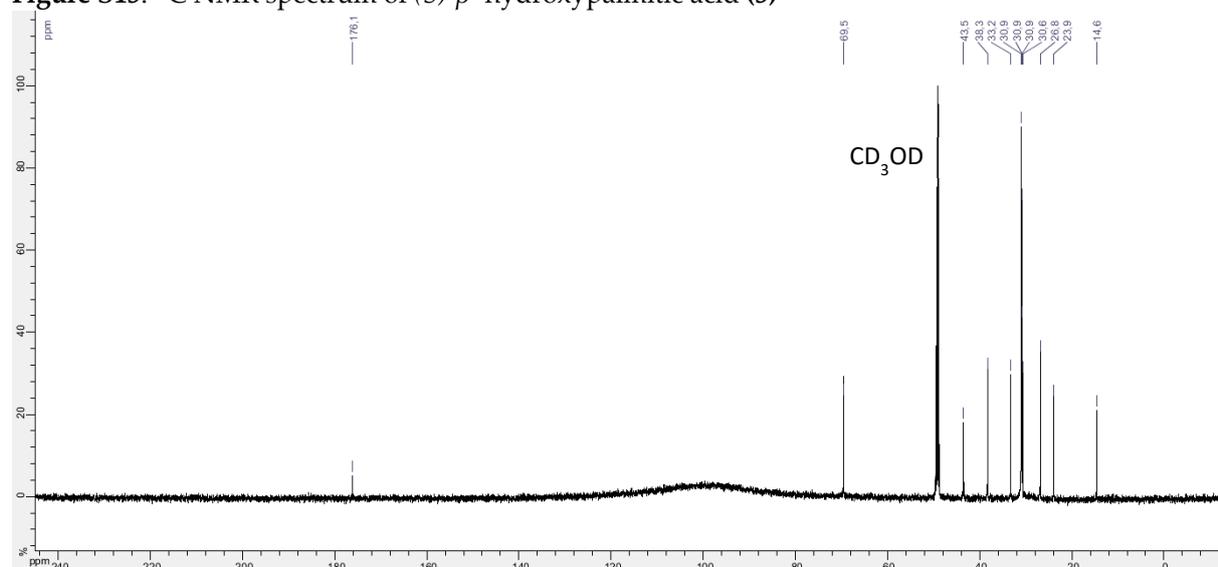
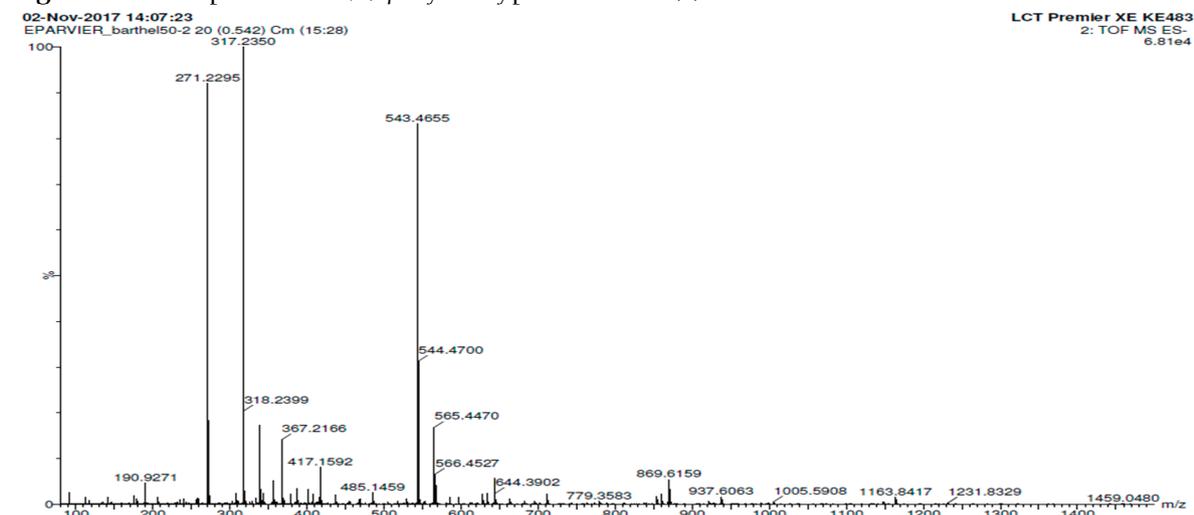
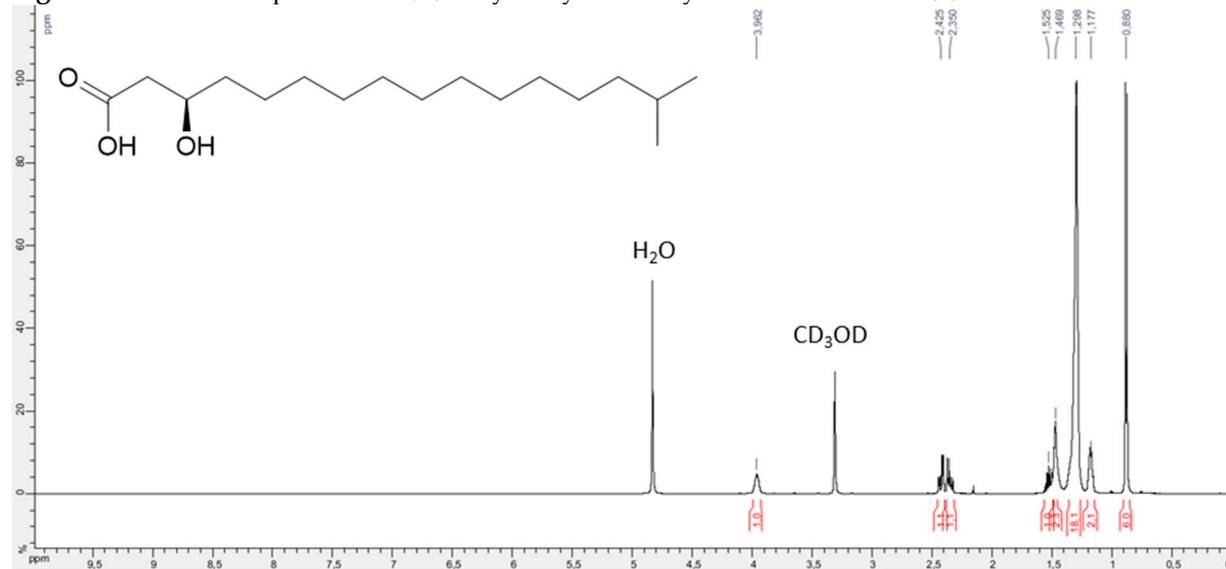


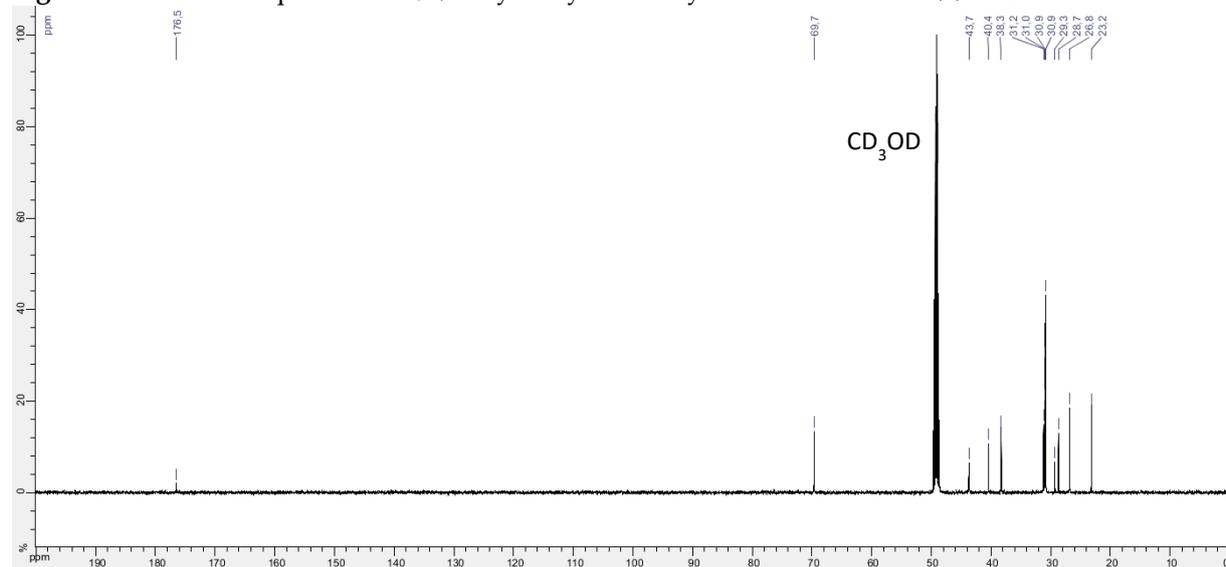
Figure S14. MS spectrum of (*S*)-β-hydroxypalmitic acid (**3**)



**Figure S15.**  $^1\text{H}$  NMR spectrum of (*R*)-3-hydroxy-15-methylhexadecanoic acid (**4**) in  $\text{CD}_3\text{OD}$



**Figure S16.**  $^{13}\text{C}$  NMR spectrum of (*R*)-3-hydroxy-15-methylhexadecanoic acid (**4**) in  $\text{CD}_3\text{OD}$



**Figure S17.** MS spectrum of (*R*)-3-hydroxy-15-methylhexadecanoic acid (**4**)

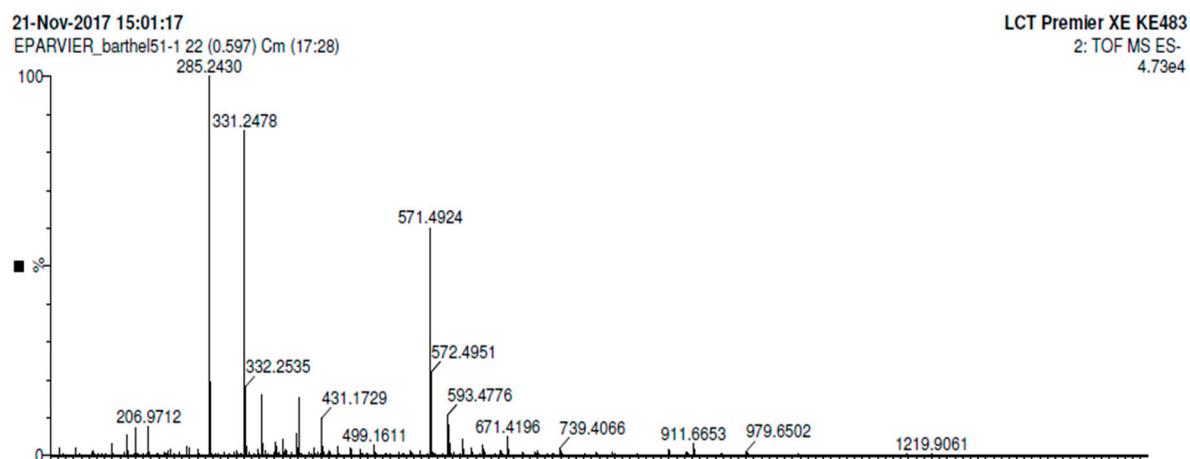




Figure S21. <sup>1</sup>H NMR spectrum of 13-methyltetradecanoic acid (**6**) in CD<sub>3</sub>OD

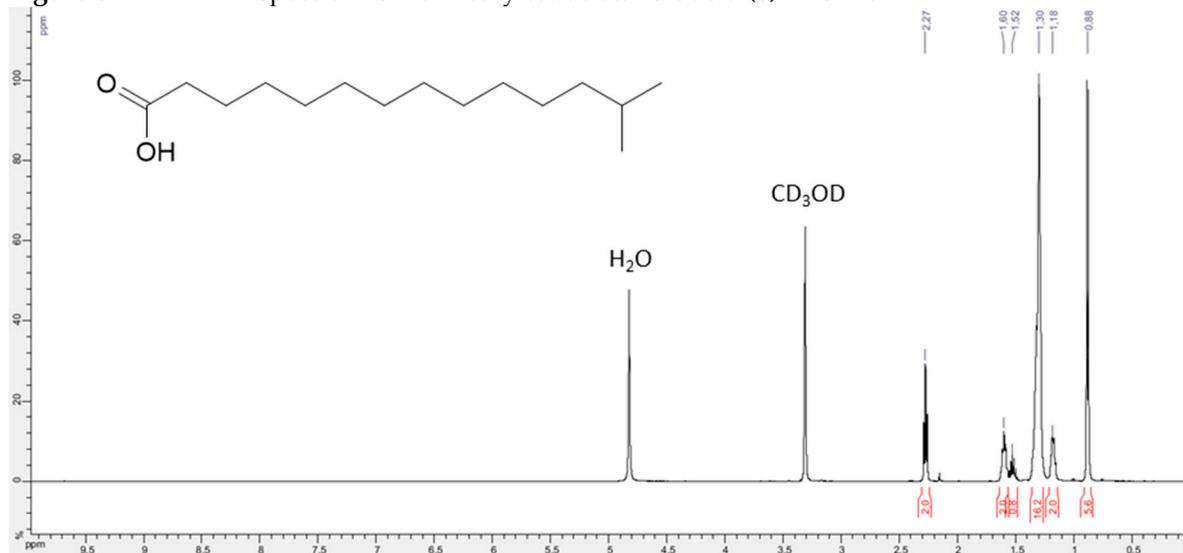


Figure S22. <sup>13</sup>C NMR spectrum of 13-methyltetradecanoic acid (**6**) in CD<sub>3</sub>OD

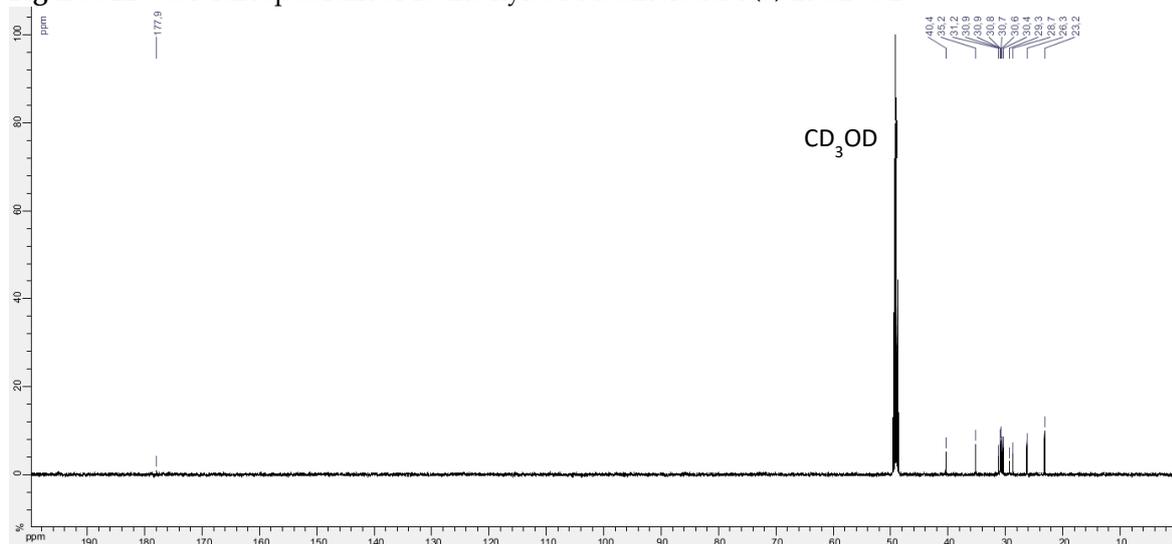
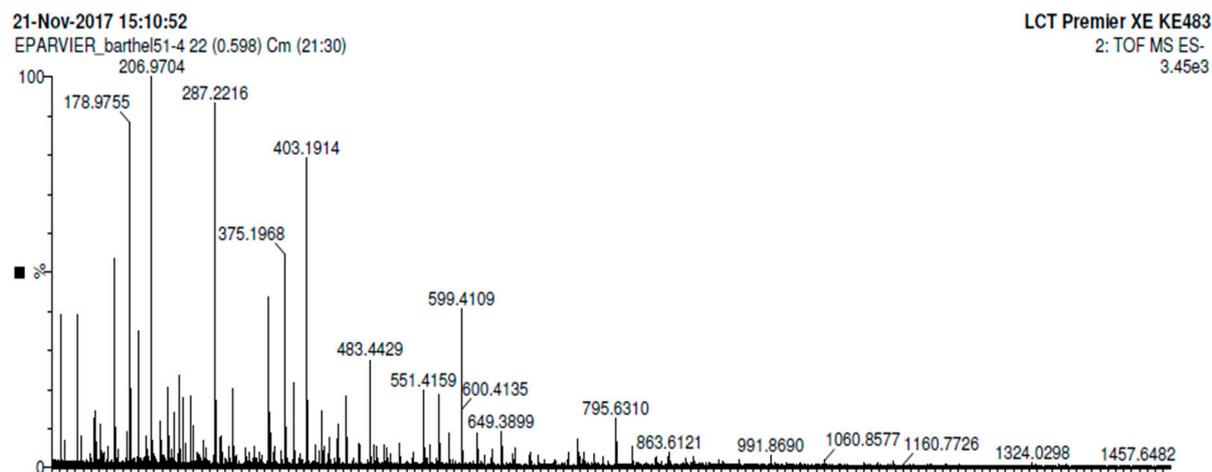
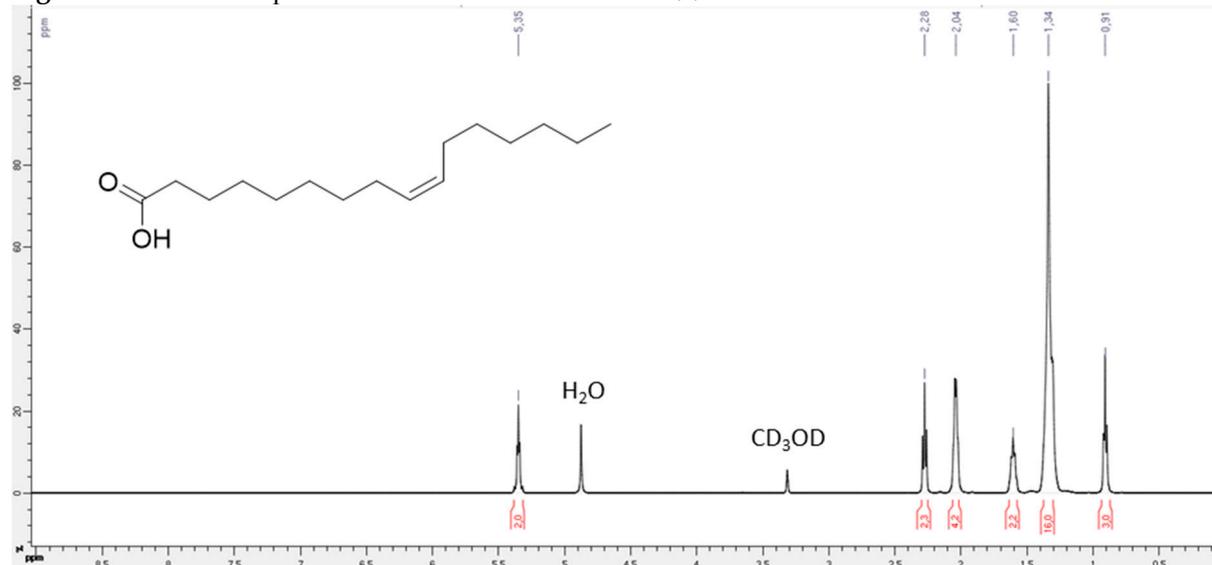


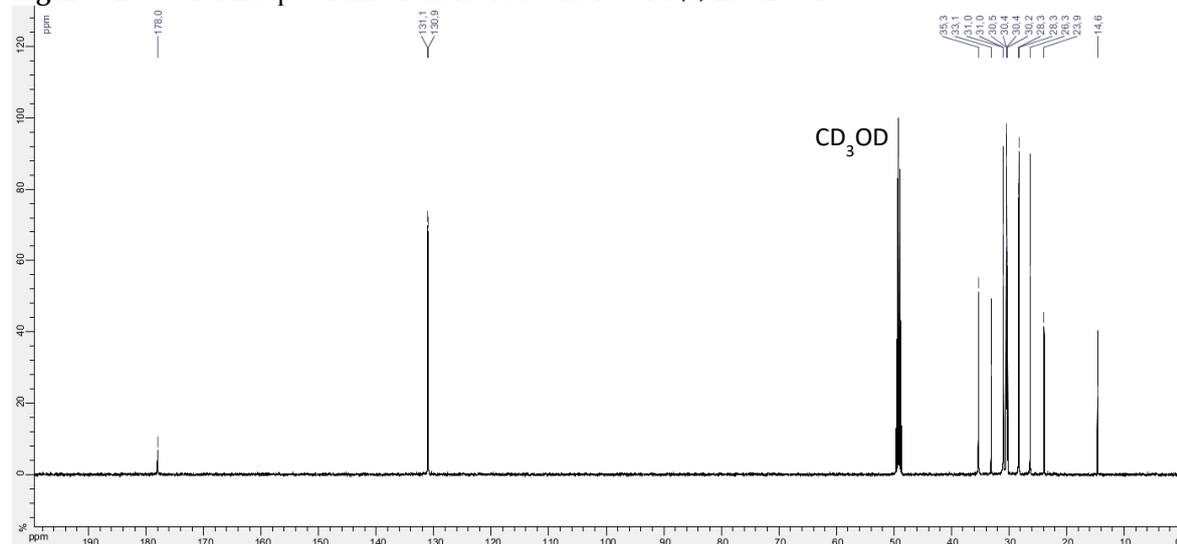
Figure S23. MS spectrum of 13-methyltetradecanoic acid (**6**)



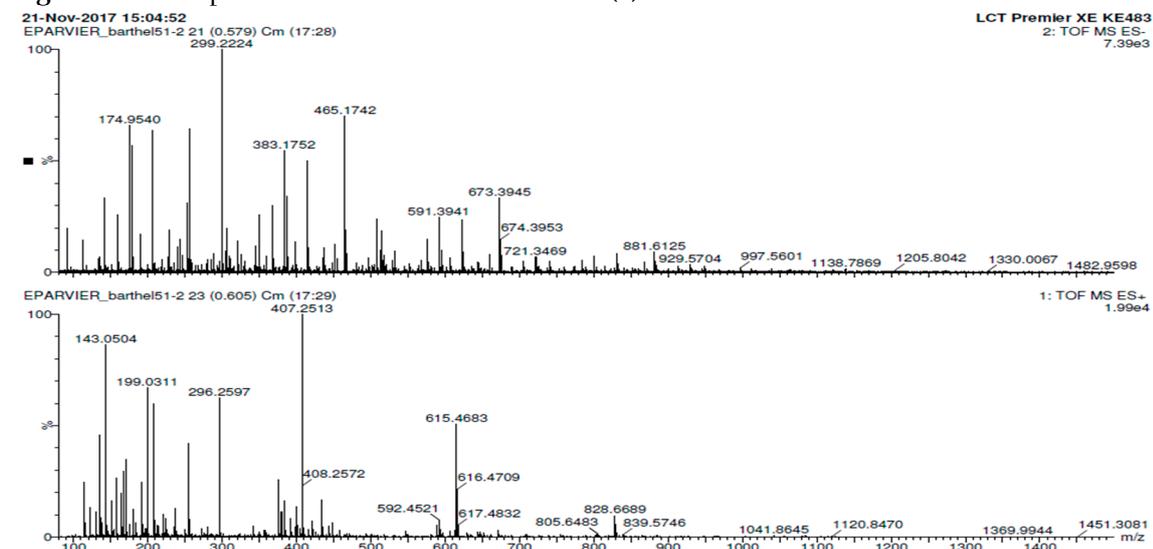
**Figure S24.**  $^1\text{H}$  NMR spectrum of 9Z-hexadecenoic acid (**7**) in  $\text{CD}_3\text{OD}$



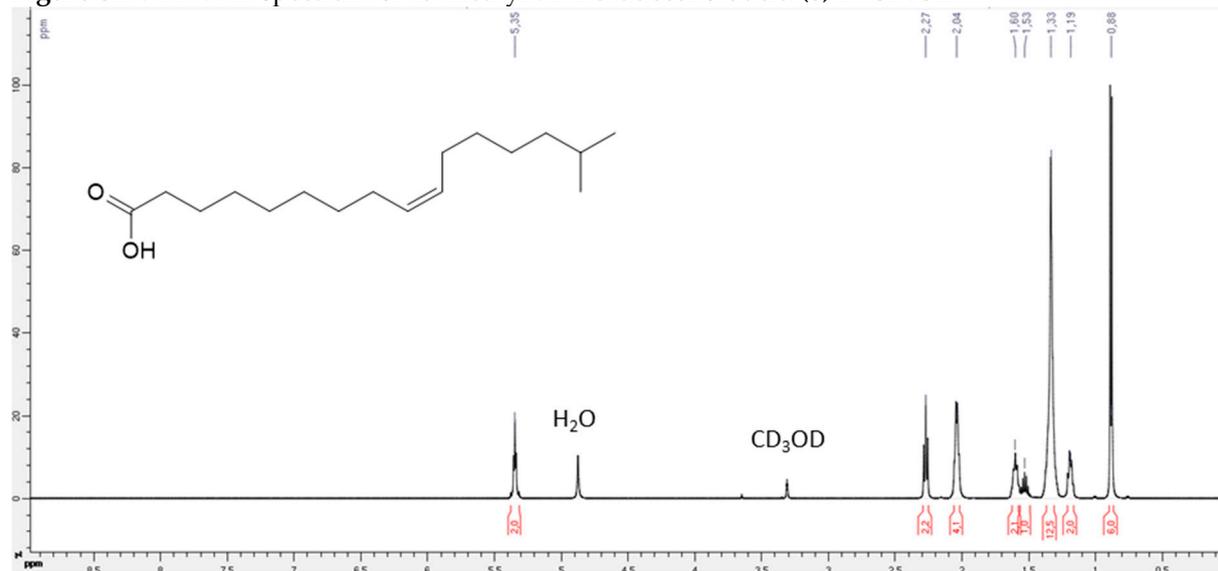
**Figure S25.**  $^{13}\text{C}$  NMR spectrum of 9Z-hexadecenoic acid (**7**) in  $\text{CD}_3\text{OD}$



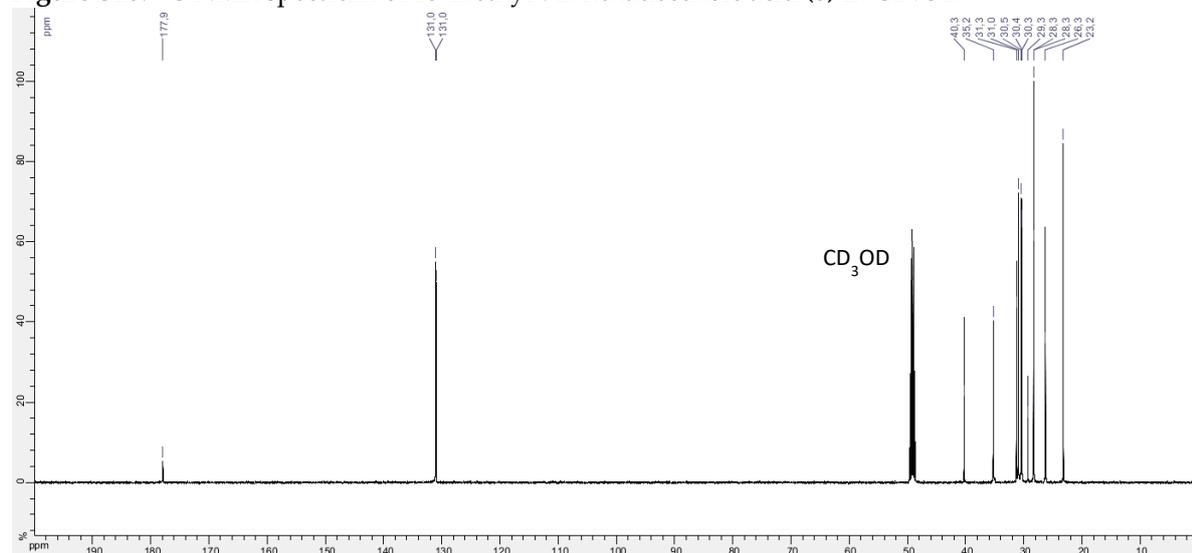
**Figure S26.** MS spectrum of 9Z-hexadecenoic acid (**7**)



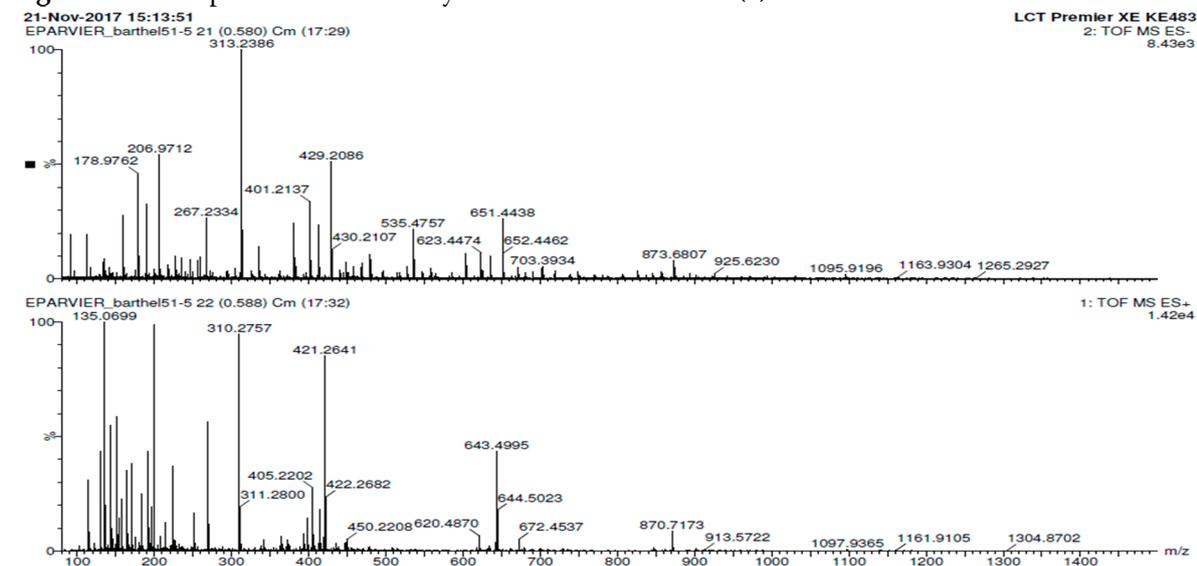
**Figure S27.**  $^1\text{H}$  NMR spectrum of 15-methyl-9Z-hexadecenoic acid (**8**) in  $\text{CD}_3\text{OD}$



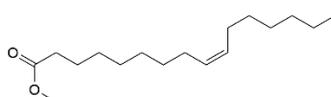
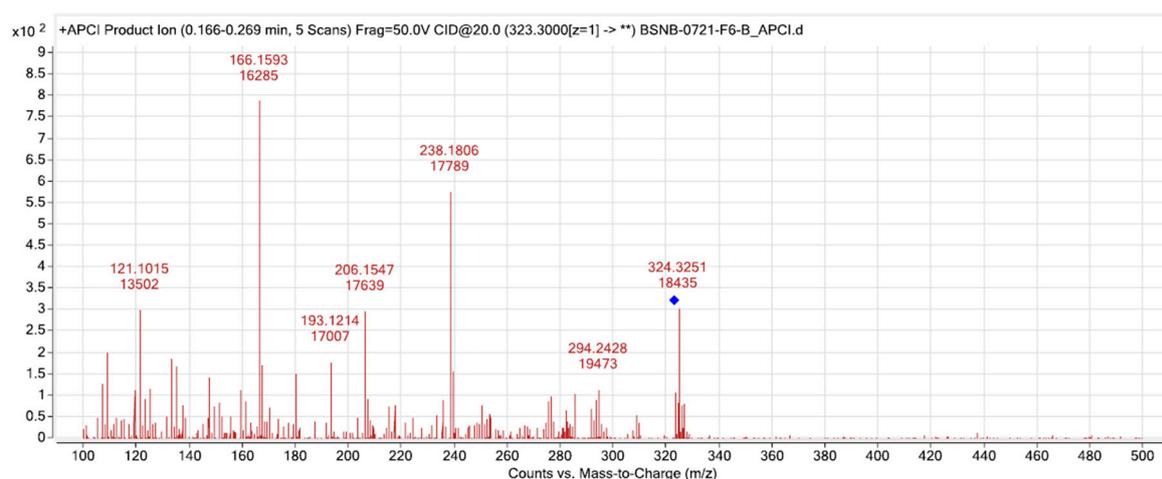
**Figure S28.**  $^{13}\text{C}$  NMR spectrum of 15-methyl-9Z-hexadecenoic acid (**8**) in  $\text{CD}_3\text{OD}$



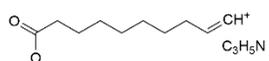
**Figure S29.** MS spectrum of 15-methyl-9Z-hexadecenoic acid (**8**)



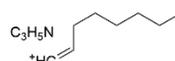
**Figure S30.** Fragmentation of 9Z-hexadecenoic acid (7)



Chemical Formula: C<sub>17</sub>H<sub>32</sub>O<sub>2</sub>  
Exact Mass: 268.2402

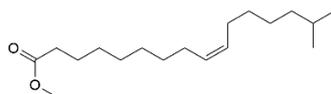
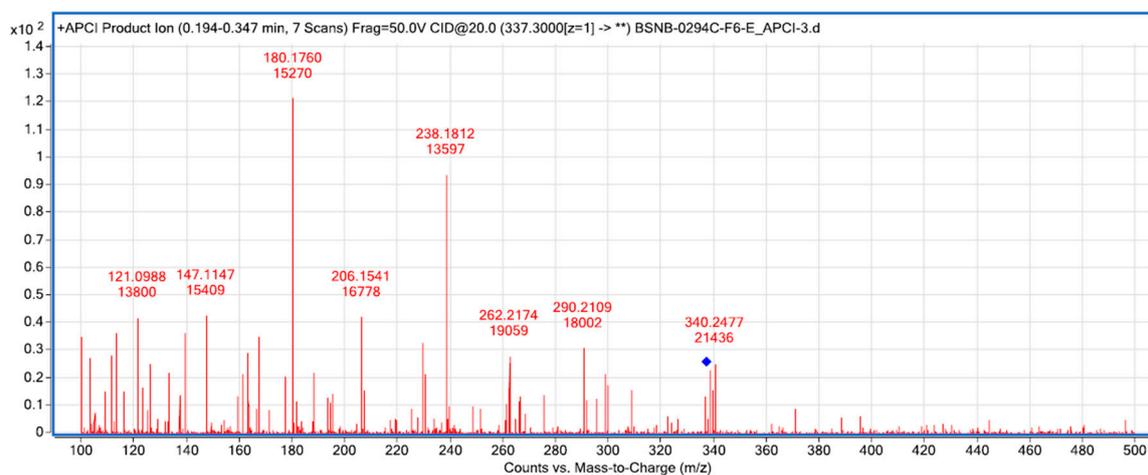


Chemical Formula: C<sub>14</sub>H<sub>24</sub>NO<sub>2</sub><sup>+</sup>  
Exact Mass: 238.1802

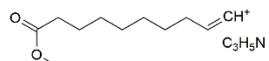


Chemical Formula: C<sub>11</sub>H<sub>20</sub>N<sup>+</sup>  
Exact Mass: 166.1590

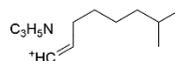
**Figure S31.** Fragmentation of 15-methyl-9Z-hexadecenoic acid (8)



Chemical Formula: C<sub>18</sub>H<sub>34</sub>O<sub>2</sub>  
Exact Mass: 282.2559



Chemical Formula: C<sub>14</sub>H<sub>24</sub>NO<sub>2</sub><sup>+</sup>  
Exact Mass: 238.1802



Chemical Formula: C<sub>12</sub>H<sub>22</sub>N<sup>+</sup>  
Exact Mass: 180.1747

**Table S1.** Identification of endophytic microorganisms associated with *Astrocaryum sciophilum* and biological activities of their respective EtOAc extracts: antibacterial on methicillin-resistant *S. aureus* (MIC in  $\mu\text{g/ml}$ ) and cytotoxicity on MRC-5 cells (viability percentage at the concentration of 10  $\mu\text{g/ml}$ )

ID codes	Closest species in NCBI (accession number)	NCBI accession number	MRSA ( $\mu\text{g/ml}$ )	MRC-5	Selectivity index
BSNB-0575	<i>Fusarium</i> sp. (EF687915)	MK279517	32	20.2 $\pm$ 1.5	0.63
BSNB-0651	<i>Fusarium</i> sp. (EF687946)	MK279518	32	23.9 $\pm$ 3.0	0.75
BSNB-0303	<i>Fusarium decemcellular</i> (MH857667)	MK279519	16	14.8 $\pm$ 3.7	0.92
BSNB-0721	<i>Luteibacter</i> sp. (JQ723723)	MK279522	64	102.4 $\pm$ 4.5	1.60
BSNB-0730	<i>Bacillus subtilis</i> (CP029461)	MK279521	64	106.5 $\pm$ 3.5	1.66
BSNB-0732	<i>Akanthomyces attenuatus</i> (MH872738)	MK279520	< 8	5.0 $\pm$ 0.1	> 0.62

Positive Control: Vancomycin against MRSA (MIC = 1  $\mu\text{g/ml}$ ) and Docetaxel against MRC-5 cells ( $\text{IC}_{50}$  = 5  $\times$  10<sup>-10</sup> M, 0% viability at 10  $\mu\text{g/ml}$ )

**Table S2.** Antibacterial activity of AcOEt extract of *Luteibacter* sp. and its fractions on MRSA (Minimum Inhibitory Concentration in  $\mu\text{g/mL}$ )

ID codes	Fractions	MRSA ( $\mu\text{g/mL}$ )
BSNB-0721	Crude extract	64
	F1	> 256
	F2	> 256
	F3	> 256
	F4	128
	F5	256
	F6	32
	F7	256
	F8	> 256

Positive control: Vancomycin (MIC = 1  $\mu\text{g/mL}$ )