Supplementary Information

Genomics- and Metabolomics-Based Investigation of the Deep-Sea Sediment-Derived Yeast, *Rhodotorula mucilaginosa* 50-3-19/20B

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Table S1. Bioactivity screening of extracts derived from six deep-sea *Rhodotorula* spp.

| Sample          | Cell culture | ESKAPE panel | ESKAPE panel | ESKAPE panel | ESKAPE panel | ESKAPE panel | ESKAPE panel | ESKAPE panel | ESKAPE panel | ESKAPE panel | ESKAPE panel | ESKAPE panel | ESKAPE panel | ESKAPE panel | ESKAPE panel | ESKAPE panel | ESKAPE panel | ESKAPE panel |
|-----------------|--------------|--------------|--------------|--------------|--------------|--------------|--------------|--------------|--------------|--------------|--------------|--------------|--------------|--------------|--------------|--------------|--------------|--------------|--------------|
|                 | MB231 | A375 | HaCaT | Efm | exp. | MRSA | exp. | Kp | exp. | Ab | Psa | Ec | Pss | Ea | Rs | Ca | Cn | Pi | Po | Bc | Tr | Tm |
| 50-3-19/20B_W   | -     | -     | -     | 32   | 1    | 59   | 99   | 96 | -    | -  | -   | -  | -   | -  | -   | -  | -   | -  | -   | -  | -   | -  |
| 50-3-19/20B_P   | 74    | 73    | 20    | -    | 2    | 37   | -    | -  | -    | -  | -   | -  | -   | -  | -   | -  | -   | -  | -   | -  |
| 52-1-0/1B_W     | -     | -     | -     | 24   | 1    | 100  | 99   | 99 | -    | -  | -   | -  | -   | -  | -   | -  | -   | -  | -   | -  |
| 52-1-0/1B_P     | -     | -     | -     | 78   | 1    | 100  | 99   | 99 | 100  | 100| 22  | 22| 59  | 99 | 22 | 22| 59  | 99 | 22 | 22 |
| 54-4-0/1B_W     | -     | -     | -     | 91   | 1    | 100  | 99   | 99 | -    | -  | -   | -  | -   | -  | -   | -  | -   | -  | -   | -  |
| 54-4-0/1B_P     | -     | -     | -     | 100  | 1    | 100  | 99   | 99 | -    | -  | -   | -  | -   | -  | -   | -  | -   | -  | -   | -  |
| LR 28-14-1-1-1_W | -     | -     | -     | 62   | 1    | 86   | 99   | 96 | -    | -  | -   | -  | -   | -  | -   | -  | -   | -  | -   | -  |
| LR 28-14-1-1-1_P | -     | -     | -     | 100  | 1    | 71   | 94   | 93 | -    | -  | -   | -  | -   | -  | -   | -  | -   | -  | -   | -  |
| LR 28-17-4-1_W  | -     | -     | -     | 61   | 1    | 90   | 99   | 99 | -    | -  | -   | -  | -   | -  | -   | -  | -   | -  | -   | -  |
| LR 28-17-4-1_P  | -     | -     | -     | 85   | 1    | 100  | 94   | 97 | -    | -  | -   | -  | -   | -  | -   | -  | -   | -  | -   | -  |
| LR 5-2-4-1_W    | -     | -     | -     | 79   | 1    | 79   | 98   | 96 | -    | -  | -   | -  | -   | -  | -   | -  | -   | -  | -   | -  |
| LR 5-2-4-1_P    | -     | -     | -     | 83   | 1    | 53   | 88   | 92 | -    | -  | -   | -  | -   | -  | -   | -  | -   | -  | -   | -  |
| WSP-30blank     | -     | -     | -     | -    | -    | -    | -    | -  | -    | -  | -   | -  | -   | -  | -   | -  | -   | -  | -   | -  |
| PDA blank       | 40    | 28    | 44    | 24   | 1    | 47   | 40   | -  | -    | -  | -   | -  | -   | -  | -   | -  | -   | -  | -   | -  |
| positive control| 83    | 97    | 66    | 97   | 1    | 91   | 97   | 96 | 97   | 96 | 99  | 99 | 42  | 99 | 96 | 64 | 92 | 94 | 98 | 99 |
| solvent control | -     | -     | -     | -    | -    | -    | -    | -  | -    | -  | -   | -  | -   | -  | -   | -  | -   | -  | -   | -  |

MB231: breast cancer; A375: lung carcinoma; HaCaT: non-cancerous keratinocyte; Efm: *Enterococcus faecium*; MRSA: methillicin-resistant *Staphylococcus aureus*; Kp: *Klebsiella pneumoniae*; Ab: *Acinetobacter baumannii*; Psa: *Pseudomonas aeruginosa*; Ec: *Escherichia coli*; Pss: *Pseudomonas syringae*; Ea: *Erwinia amylovora*; Rs: *Ralstonia solanacearum*; Ca: *Candida albicans*; Cn: *Cryptococcus neoformans*; P: *Phytophthora infestans*; Po: *Pyricularia oryzae*; Bc: *Botrytis cinerea*; Tr: *Trichophyton rubrum*; Tm: *T. mentagrophytes*
### Table S2. Overview of the genomic locus for exo-inulinase enzyme in marine \textit{R. mucilaginosa} 50-3-19/20B, as deduced via Omicsbox and Pfam annotations.

| Name | Description | Protein Length | Pfam annotation | Pfam Domain | Pfam ID | E-value | Start | End |
|------|-------------|----------------|-----------------|-------------|---------|---------|-------|-----|
| g1614.t1 | Endonuclease/exonuclease/phosphatase domain-containing protein | 1604 | Endonuclease/Exonuclease/phosphatase family | Exo_endo_phos | PF03372.22 | 9.0E-6 | 99 | 472 |
| g1615.t1 | hypothetical protein RHOSPDRAFT_19222 | 463 | Translation initiation factor SUI1 | SUI1 | PF01253.21 | 3.6E-27 | 353 | 429 |
| g1616.t1 | ---NA--- | 663 | Permease for cytosine/purines, uracil, thiamine, allantoin | Transp_cyt_pur | PF02133.14 | 3.7E-42 | 326 | 579 |
| g1617.t1 | hypothetical protein BMF94_3082 | 66 | | | | | |
| g1618.t1 | FAD/NAD(P)-binding domain-containing protein | 592 | GDP dissociation inhibitor | GDI | PF00996.17 | 2.2E-32 | 5 | 284 |
| g1619.t1 | ---NA--- | 101 | Vacuolar protein sorting 55 | Vps55 | PF04133.13 | 1.2E-24 | 6 | 97 |
| g1620.t1 | hypothetical protein RHOSPDRAFT_34835 | 812 | | | | | |
| g1621.t1 | actin-like ATPase domain-containing protein | 1956 | | | | | |
| g1622.t1 | NAD-P-binding protein | 1910 | short chain dehydrogenase | adh_short | PF00106.24 | 5.1E-24 | 1641 | 1861 |
| g1623.t1 | ---NA--- | 1041 | | | | | |
| g1624.t1 | ARM repeat-containing protein | 1943 | | | | | |
| g1625.t1 | hypothetical protein RHOSPDRAFT_35524 | 1198 | | | | | |
| g1626.t1 | L-iditol 2-dehydrogenase | 617 | Alcohol dehydrogenase GroES-like domain | ADH_N | PF08240.11 | 3.5E-24 | 59 | 171 |
| g1627.t1 | ferric reductase transmembrane component | 778 | Ferric reductase like transmembrane component | Ferric_reduct | PF01794.18 | 6.5E-18 | 186 | 302 |
| g1628.t1 | protein of ctr copper transporter family | 216 | Ctr copper transporter family | Ctr | PF04145.14 | 6.2E-33 | 22 | 193 |
| g1629.t1 | beta-fructofuranosidase | 679 | Glycosyl hydrolases family 32 N-terminal domain | Glyco_hydro_32N | PF00251.19 | 6.5E-76 | 178 | 485 |
| g1630.t1 | Dynactin, subunit p25 | 696 | RING-variant domain | RINGv | PF12906.6 | 9.5E-13 | 341 | 391 |
| Gene   | Description                                      | Start | End | Description                     | Accessory | Score 1 | Score 2 | Score 3 | Score 4 |
|--------|--------------------------------------------------|-------|-----|---------------------------------|-----------|---------|---------|---------|---------|
| g1631.t1 | gpi anchored protein                             | 275   |     |                                 |           |         |         |         |         |
| g1632.t1 | lipoyl(octanoyl) transferase                    | 356   |     |                                 |           |         |         |         |         |
| g1633.t1 | hypothetical protein RHOSPDRAFT_32393            | 744   |     | Glycine-rich domain-containing protein-like | GRDP-like |         |         |         |         |
| g1634.t1 | voltage-gated chloride channel                  | 1809  |     | Voltage gated chloride channel   | Voltage_CLC|         |         |         |         |
|         |                                                  |       |     | Rab-GTPase-TBC domain            | RabGAP-TBC|         |         |         |         |
|         |                                                  |       |     | CBS domain                       | CBS       |         |         |         |         |
| g1635.t1 | hypothetical protein RHOSPDRAFT_24861            | 600   |     |                                 |           |         |         |         |         |
| g1636.t1 | E3 ubiquitin-protein ligase UBRI                | 2049  |     | Putative zinc finger in N-recogin (UBR box) | zf-UBR   |         |         |         |         |
|         |                                                  |       |     | ATP-dependent Clp protease adaptor protein ClpS | ClpS     |         |         |         |         |
| g1637.t1 | carbon-nitrogen hydrolase                        | 332   |     | Carbon-nitrogen hydrolase        | CN_hydrolase|         |         |         |         |
| g1638.t1 | hypothetical protein RHOSPDRAFT_24864            | 805   |     |                                 |           |         |         |         |         |
| g1639.t1 | hypothetical protein RHOSPDRAFT_24865            | 920   |     |                                 |           |         |         |         |         |
| g1640.t1 | hypothetical protein RHOSPDRAFT_24865            | 564   |     |                                 |           |         |         |         |         |
| g1641.t1 | NAD(P)-binding protein                           | 303   |     | Enoyl-(Acyl carrier protein) reductase | adh_short_C2|         |         |         |         |
| g1642.t1 | Other/IRE protein kinase                         | 1382  |     | Ribonuclease 2-5A               | Ribonuc_2-5A|         |         |         |         |
|         |                                                  |       |     | Protein kinase domain            | Pkinase   |         |         |         |         |
|         |                                                  |       |     | Protein kinase domain            | Pkinase   |         |         |         |         |
| g1643.t1 | C6 transcription factor                          | 1042  |     |                                 |           |         |         |         |         |
| g1644.t1 | taurine catabolism dioxygenase                   | 377   |     | Taurine catabolism dioxygenase TauD, TfdA family | TauD     |         |         |         |         |

**Score Notes:**
- **Score 1:** E-value
- **Score 2:** Protein length
- **Score 3:** ClustalW score
- **Score 4:** SWISS-PROT similarity score
Table S3. Annotation summary of putative exo-inulinase enzyme from *Rhodotorula mucilaginosa* 50-3-19/20B and its homologs in different fungi

| Sequence ID | Species Description | Protein Length | e-Value sim mean | GO IDs | GO Names | Enzyme Codes | Enzyme Names | InterPro IDs | InterPro GO IDs | InterPro GO Names |
|-------------|---------------------|---------------|-----------------|--------|----------|--------------|--------------|--------------|----------------|-----------------|
| g1629.t1 R. mucilaginosa 50-3-19/20B | glycoside hydrolase family 32 protein | 679 | 0.07414 | 3 | P:GO:0005987; F:GO:0004575; C:GO:000324 | P:sucrose catabolic process; F:sucrose alpha-glucosidase activity; C:fungal-type vacuole | EC:3.2.1.20; EC:3.2.1.26; EC:3.2.1.48 | Alpha-glucosidase; Beta-fructofuranosidase; Sucrose alpha-glucosidase | IPR013189 (PFAM); G3DSA:2.60.120.560 (GENE3D); IPR023296 (G3DSA:2.115.10.GENE3D); IPR013148 (PFAM); mobidb-lite (MOBIDB_LITE); PTHR42800:SF2 (PANTHER); PTHR42800 (PANTHER); cd18622 (CDD); IPR013320 (SUPERFAMILY); IPR023296 (SUPERFAMILY) | no GO terms | no GO terms |
| AZR37516.1 Rhodotorula paludigena | beta-fructofuranosidase | 617 | 0.0687 | 3 | P:GO:0005987; F:GO:0004575; C:GO:000324 | P:sucrose catabolic process; F:sucrose alpha-glucosidase activity; C:fungal-type vacuole | EC:3.2.1.20; EC:3.2.1.26; EC:3.2.1.48 | Alpha-glucosidase; Beta-fructofuranosidase; Sucrose alpha-glucosidase | IPR023296 (G3DSA:2.115.10.GENE3D); IPR013148 (PFAM); G3DSA:2.60.120.560 (GENE3D); mobidb-lite (MOBIDB_LITE); mobidb-lite (MOBIDB_LITE); PTHR42800:SF2 (PANTHER); PTHR42800 (PANTHER); IPR018053 (PROSITE_PATTERNS); | P:carbohydrate metabolic process; F:hydrolase activity, hydrolyzing O-glycosyl compounds | 6 |
| Accession    | Organism              | Protein Name                        | M / E | Percent Identity | Percent Similarity | GO Terms                                      | Domain/Taxa                                                                 |
|--------------|-----------------------|-------------------------------------|-------|------------------|-------------------|-----------------------------------------------|----------------------------------------------------------------------------|
| TKA53735.1   | Rhodotorula sp. CCFEE 5036 | glycoside hydrolase family 32 protein | 676   | 0                | 74.85             | P:carbohydrate metabolic process; F:hydrolase activity, hydrolyzing O-glycosyl compounds | IPR023296 (SUPERFAMILY); IPR013320 (SUPERFAMILY)                           |
|              |                       |                                     |       |                  |                   | P:GO:0005975; F:GO:0004553                   |                                                                            |
|              |                       |                                     |       |                  |                   |                                               |                                                                            |
| KWU45911.1   | Rhodotorula sp. JG-1b | glycoside hydrolase family 32 protein | 559   | 0                | 74.92             | P:carbohydrate metabolic process; F:hydrolase activity, hydrolyzing O-glycosyl compounds | IPR013189 (PFAM); IPR013148 (PFAM); IPR023296 (G3DSA:2.115.10.GENE3D); IPR013148 (PFAM); mobidb-lite (MOBIDB_LITE); mobidb-lite (MOBIDB_LITE); PTHR42800:SF2 (PANTHER); PTHR42800 (PANTHER); IPR018053 (PROSITE_PATTERNS); cd18622 (CDD); IPR013320 (SUPERFAMILY); IPR023296 (SUPERFAMILY) |
|              |                       |                                     |       |                  |                   |                                               |                                                                            |
| Accession | Organism                  | Enzyme Name                      | Activity Scores | GO Terms | EC Numbers                      |
|-----------|---------------------------|----------------------------------|-----------------|----------|---------------------------------|
| POY72393.1 | Rhodotorula taiwanensis  | beta-fructofuranosidase          | 614 0.07396 2   | P:carbohydrate metabolic process; F:hydrolase activity, hydrolyzing O-glycosyl compounds | G3DSA:2.60.120.560 (GENE3D); IPR023296 (G3DSA:2.115.10.GENE3D); IPR013189 (PFAM); IPR013148 (PFAM); mobidb-lite (MOBIDB_LITE); PTHR42800 (PANTHER); PTHR42800.SF2 (PANTHER); IPR018053 (PROSITE_PATTERNS); cd18622 (CDD); IPR013320 (SUPERFAMILY); IPR023296 (SUPERFAMILY) |
| GFZ51952.1 | Saitozyma sp. JCM 24511  | beta-fructofuranosidase          | 523 0.07708 4   | P:sucrose catabolic process; F:sucrose alpha-glucosidase activity; C:fungal-type vacuole; C:integral component of membrane | IPR013189 (PFAM); IPR023296 (G3DSA:2.115.10.GENE3D); IPR013148 (PFAM); G3DSA:2.60.120.560 (GENE3D); mobidb-lite (MOBIDB_LITE); PTHR42800.SF2 (PANTHER); PTHR42800 (PANTHER); IPR018053 (PROSITE_PATTERNS); cd18622 (CDD); IPR023296 (SUPERFAMILY) |

P: carbohydrate metabolic process; F: hydrolase activity, hydrolyzing O-glycosyl compounds
| Accession  | Species         | Description                              | P:GO:0005975; F:GO:0004553 | (SUPERFAMILY); IPR013320 (SUPERFAMILY) |
|------------|-----------------|------------------------------------------|-----------------------------|----------------------------------------|
| KKY24889.1 | *Diplodia*      | glycoside hydrolase family 32 protein    | P:carbohydrate metabolic process; F:hydrolase activity, hydrolyzing O-glycosyl compounds |                                         |
|            | *seriata*      |                                          |                            | IPR023296 (G3DSA:2.115.10.GENE3D); IPR013148 (PFAM); IPR013189 (PFAM); G3DSA:2.60.120.560 (GENE3D); PTHR42800:SF2 (PANTHER); PTHR42800 (PANTHER); IPR018053 (PROSITE_PATTERNS); cd18622 (CDD); IPR013320 (SUPERFAMILY); IPR023296 (SUPERFAMILY) |
| OMP85659.1 | *Diplodia*      | glycoside hydrolase family 32 protein    | P:carbohydrate metabolic process; F:hydrolase activity, hydrolyzing O-glycosyl compounds |                                         |
|            | *seriata*      |                                          |                            | IPR013148 (PFAM); IPR013189 (PFAM); IPR023296 (G3DSA:2.115.10.GENE3D); G3DSA:2.60.120.560 (GENE3D); PTHR42800:SF2 (PANTHER); PTHR42800 (PANTHER); IPR018053 (PROSITE_PATTERNS); cd18622 (CDD); IPR013320 (SUPERFAMILY); IPR023296 (SUPERFAMILY) |

The table lists the accessions, species, descriptions, and additional information for two glycoside hydrolase proteins from *Diplodia seriata*. Each entry includes the accession number, species name, protein type, and a set of functional annotations provided by GO and other databases such as SUPERFAMILY, IPRs, and PROSITE patterns.
| Accession   | Organism                | Gene Name       | E value | Score | GO Terms                          | Domain Information                                                                 |
|-------------|-------------------------|-----------------|---------|-------|-----------------------------------|----------------------------------------------------------------------------------|
| XP_035362863.1 | Lasiodiplodia theobromae | glycoside hydrolase family 32 protein | 562     | 0     | 86.11 2                          | P:carbohydrate metabolic process; F:hydrolase activity, hydrolyzing O-glycosyl compounds |
| XP_02129257.1   | Diplodia corticola       | glycoside hydrolase family 32 protein | 563     | 0     | 85.81 2                          | P:carbohydrate metabolic process; F:hydrolase activity, hydrolyzing O-glycosyl compounds |
| KAF4312946.1    | Botryosphaeria dothidea | glycoside hydrolase family 32 protein | 562     | 0     | 85.72 3                          | P:carbohydrate metabolic process; F:hydrolase activity,                          |

**Domain Information:**
- IPR013148 (PFAM); IPR013189 (PFAM); G3DSA:2.115.10.GENE3D; G3DSA:2.60.120.560 (GENE3D);
- PTHR42800:SF2 (PANTHER); PTHR42800 (PANTHER); IPR018053 (PROSITE_PATTERNS);
- cd18622 (CDD); IPR023296 (SUPERFAMILY); IPR013320 (SUPERFAMILY)
| KJ52043.1 | Sphaerobulus stellatus SS14 | glycoside hydrolase family 32 protein | 562 | 0 | 84.23 | 2 | hydrolyzing O-glycosyl compounds; Cintegral component of membrane | PTHR42800 (PANTHER); PTHR42800:SF2 (PANTHER); IPR018053 (PROSITE_PATTERNS); cd18622 (CDD); IPR013320 (SUPERFAMILY); IPR023296 (SUPERFAMILY) |  |
| THU96730.1 | Dendrothele bispora CBS 962.96 | glycoside hydrolase family 32 protein | 523 | 0 | 84.95 | 3 | P:carbohydrate metabolic process; F:hydrolase activity, hydrolyzing O-glycosyl compounds | IPR013148 (PFAM); IPR013189 (PFAM); IPR023296 (G3DSA:2.115.10.GENE3D); G3DSA:2.60.120.560 (GENE3D); PTHR42800 (PANTHER); PTHR42800:SF2 (PANTHER); IPR018053 (PROSITE_PATTERNS); cd18622 (CDD); IPR013320 (SUPERFAMILY); IPR023296 (SUPERFAMILY) | P:carbohydrate metabolic process; F:hydrolase activity, hydrolyzing O-glycosyl compounds |
| Accession    | Species            | Type                     | MSA | E-value | Location                                                                 | GO Terms                                      |
|--------------|--------------------|--------------------------|-----|---------|----------------------------------------------------------------------------|-----------------------------------------------|
| XP_008034329.1 | Trametes versicolor | glycoside hydrolase family 32 protein | 540 | 0       | 83.59 3                                                                  | P:carbohydrate metabolic process; F:hydrolase activity, hydrolyzing O-glycosyl compounds; C:integral component of membrane | cd18622 (CDD); IPR013320 (SUPERFAMILY); IPR023296 (SUPERFAMILY) |
| KAF5355425.1  | Tetrapyrgos nigripes | glycoside hydrolase family 32 protein | 521 | 0       | 83.08 3                                                                  | P:carbohydrate metabolic process; F:hydrolase activity, hydrolyzing O-glycosyl compounds; C:integral component of membrane | IPR013148 (PFAM); G3DSA:2.60.120.560 (GENE3D); IPR023296 (G3DSA:2.115.10.GENE3D); IPR013189 (PFAM); PTHR42800 (PANTHER); PTHR42800:SF2 (PANTHER); IPR018053 (PROSITE_PATTERNS); cd18622 (CDD); IPR023296 (SUPERFAMILY); IPR013320 (SUPERFAMILY) |
| Accession   | Species               | Type                  | Entrez Gene | Score  | Identity | E-value | GO:0005987 | GO:0004575 | GO:0000324 | EC:3.2.1.20 | EC:3.2.1.126 | EC:3.2.1.48 | Description                                                                 |
|-------------|-----------------------|-----------------------|-------------|--------|----------|---------|-------------|-------------|-------------|--------------|--------------|--------------|-----------------------------------------------------------------------------|
| KAE8543285.1| Cryptococcus cf. gattii| beta-fructofuranosidase| 519         | 0      | 97.55    | 3       | P             | F           | C           | Alpha-glucosidase; Beta-fructofuranosidase; Sucrose alpha-glucosidase      |
|             |                       |                       |             |        |          |         | P:0005987    | F:0004575   | C:0000324   | G3DSA:2.60.120.560 (GENE3D); IPR013189 (PFAM); IPR023296 (GENE3D); IPR013148 (PFAM); mobidb-lite (MOBIDB_LITE); PTHR42800 (PANTHER); PTHR42800:SF2 (PANTHER); IPR018053 (PROSITE_PATTERNS); cd18622 (CDD); IPR023296 (SUPERFAMILY); IPR013320 (SUPERFAMILY) | P:carbohydrate metabolic process; F:hydrolase activity, hydrolyzing O-glycosyl compounds |
| OSD00128.1  | Trametes coccinea BRFM310| glycoside hydrolase family 32 protein| 524         | 0      | 83.56    | 3       | P:0005975    | F:0004553   | C:016021    | IPR013148 (PFAM); G3DSA:2.60.120.560 (GENE3D); IPR013189 (PFAM); IPR023296 (GENE3D); PTHR42800 (PANTHER); PTHR42800:SF2 (PANTHER); IPR018053 (PROSITE_PATTERNS); cd18622 (CDD); IPR023296 (SUPERFAMILY); IPR013320 (SUPERFAMILY) | P:carbohydrate metabolic process; F:hydrolase activity, hydrolyzing O-glycosyl compounds |
| EOD51241.1 | Neofusicoccum parvum UCRNP2 | glycoside hydrolase family 32 protein | 563 | 0 | 84.03 | 2 | P:GO:0005975; F:GO:0004553 | P:carbohydrate metabolic process; F:hydrolase activity, hydrolyzing O-glycosyl compounds | IPR013148 (PFAM); IPR023296 (G3DSA:2.115.10.GENE3D); G3DSA:2.60.120.560 (GENE3D); mobildb-lite (MOBIDB_LITE); PTHR42800:SF2 (PANTHER); PTHR42800 (PANTHER); IPR018053 (PROSITE_PATTERNS); cd18622 (CDD); IPR023296 (SUPERFAMILY); IPR013320 (SUPERFAMILY) | P:carbohydrate metabolic process; F:hydrolase activity, hydrolyzing O-glycosyl compounds |
|----------|---------------------------------|------------------------------------|-----|----|--------|---|---------------------------|---------------------------------------------|-------------------------------------------------------------------------------------------------|--------------------------------------------------------------------------------------------------|
| KAF2091127.1 | Saccharatus proteae CBS 121410 | SCF E3 ubiquitin ligase complex F-box protein | 537 | 0 | 79.88 | 4 | P:GO:0005975; P:GO:0006629; F:GO:0004553; F:GO:0008081 | P:carbohydrate metabolic process; P:lipid metabolic process; F:hydrolase activity, hydrolyzing O-glycosyl compounds; F:phosphoric diester hydrolase activity | G3DSA:2.60.120.560 (GENE3D); IPR023296 (G3DSA:2.115.10.GENE3D); IPR013148 (PFAM); PTHR42800:SF2 (PANTHER); PTHR42800 (PANTHER); IPR018053 (PROSITE_PATTERNS); cd18622 (CDD); IPR023296 (SUPERFAMILY); IPR013320 (SUPERFAMILY) | P:carbohydrate metabolic process; F:hydrolase activity, hydrolyzing O-glycosyl compounds |
| KIR51126.1 | Cryptococcus gattii Ru294 | beta-fructofuranosidase | 519 | 0 | 97.81 | 3 | P:GO:0005987; F:GO:0004557; C:GO:000324 | P:sucrose catabolic process; F:sucrose alpha-glucosidase EC:3.2.1.20; EC:3.2.1.26; EC:3.2.1.48 | Alpha-glucosidase; Beta-fructofuranosidase; IPR023296 (G3DSA:2.115.10.GENE3D); G3DSA:2.60.120.560 (GENE3D) | P:carbohydrate metabolic process; F:hydrolase activity, hydrolyzing O-glycosyl compounds |
| Accession  | Organism          | Enzyme Family | Gene ID   | Description                                      | GO Terms                          |
|------------|-------------------|---------------|-----------|--------------------------------------------------|-----------------------------------|
| XP_567775.1 | Cryptococcus neofor... | beta-fructofuranosidase | 519 0 96.37 3 | P: sucrase catabolic process; F: sucrase alpha-glucosidase activity; C: fungal-type vacuole | P: GO:0005987; F: GO:0004575; C: GO:0000324 |
| KIR83202.1 | Cryptococcus gattii VGIV IND107 | beta-fructofuranosidase | 519 0 97.96 3 | P: sucrase catabolic process; F: sucrase alpha-glucosidase activity; C: fungal-type vacuole | P: GO:0005987; F: GO:0004575; C: GO:0000324 |

**activity; C:fungal-type vacuole**

Sucrose alpha-glucosidase

IPR013189 (PFAM); IPR013148 (PFAM); PTHR42800 (PANTHER); PTHR42800:SF2 (PANTHER); IPR018053 (PROSITE_PATTERNS); cd18622 (CDD); IPR023296 (SUPERFAMILY); IPR013320 (SUPERFAMILY)

**F: hydrolase activity, hydrolyzing O-glycosyl compounds**

G3DSA:2.60.120.560 (GENE3D); IPR023296 (G3DSA:2.115.10.GENE3D); IPR013189 (PFAM); IPR013148 (PFAM); PTHR42800:SF2 (PANTHER); PTHR42800 (PANTHER); IPR018053 (PROSITE_PATTERNS); cd18622 (CDD); IPR023296 (SUPERFAMILY); IPR013320 (SUPERFAMILY)

**P: carbohydrate metabolic process; F: hydrolase activity, hydrolyzing O-glycosyl compounds**

G3DSA:2.115.10.GENE3D; IPR023296 (G3DSA:2.115.10.GENE3D); IPR013189 (PFAM); IPR013148 (PFAM); PTHR42800:SF2 (PANTHER); PTHR42800 (PANTHER); IPR018053 (PROSITE_PATTERNS); cd18622 (CDD); IPR023296 (SUPERFAMILY); IPR013320 (SUPERFAMILY)

**P: carbohydrate metabolic process; F: hydrolase activity, hydrolyzing O-glycosyl compounds**
glycosyl compounds
Table S4. Cytotoxic activity (%-inhibition at 100 µg/mL) of the fractionated PDA-K-DCM subextract of *R. mucilaginosa* 50-3-19/20B against the breast cancer cell line MDA-MB-231

| Fraction | MDA-MB-231 |
|----------|------------|
| F1       | -          |
| F2       | -          |
| F3       | -          |
| F4       | 27         |
| F5-8     | 22         |
| F9-12    | -          |
| F13-16   | -          |
| F17      | -          |
| F18      | -          |
| F19      | 44         |
| F20      | 51         |
| **F21**  | **86**     |
| F22      | 94         |
| F23      | 88         |
| F24      | 97         |
| F25      | 52         |
| F26      | -          |
| F27      | -          |
| F28      | -          |
| F29      | -          |
| F30      | -          |
| F31      | -          |
Table S5. Putative annotations of compounds detected in deep-sea *R. mucilaginosa* 50-3-19/20B extracts. Annotations were based on GNPS and manual dereplication of m/z ([M+H]+ or [M+Na]+), retention time (ts), fragmentation pattern and predicted molecular formula against Dictionary of Natural Products, DEREP_NP and other literature data. Confidence levels of putative identification according to Sumner et al. [1] and Blaženović et al. [2].

| LD  | m/z   | ts (min) | MS/MS (m/z)* | Ion type | Molecular formula (M) | Identified compound | Compound class | Confidence | Reference |
|-----|-------|----------|--------------|----------|----------------------|--------------------|---------------|------------|-----------|
| 261.1304 | 2.15  | 233.17; 216.14; 188.14; 120.08 | [M+H]+ | C₃₀H₃₁NO₂ | Cyclo-(Leu-Phe) | Diketopiperazine | 2           | [3]       |
| 188.0713 | 3.83  | 170.06; 146.06; 118.07 | [M-H₂O+H]+ | C₁₅H₂₀NO₂ | DL-Indole-3-lactic acid | Indole alkaloid | 2           | [4]       |
| 160.0764 | 3.84  | 132.08; 118.07 | [M+H]+ | C₁₅H₁₅NO₂S | 1-Hydroxy-2-(1H-indol-3-yl)ethanesulfonic acid | Indole alkaloid | 3           |           |
| 206.08  | 3.94  | 188.07; 170.06; 160.06; 146.06; 130.07 | [M+H]+ | C₁₅H₁₅NO₂ | DL-Indole-3-lactic acid | Indole alkaloid | 2           | [4]       |
| 603.5312 | 9.54  | 273.10; 235.21; 231.09; 217.20; 189.08; 171.07; 161.13; 153.06; 135.12; 121.10; 111.04; 95.09; 81.07 | [M+H]+ | C₄₀H₅₈O₄C | Tetrahydroxydihydrolycopene | Carotenoid | 3           | [5]       |
| 571.6362 | 10.37 | 263.24; 245.23; 231.09; 189.08; 175.15; 161.13; 153.06; 147.12; 133.10; 121.10; 111.05; 93.03; 81.07 | [M+H]+ | C₁₅H₁₅NO₂ | Dihydroxylycopene | Carotenoid | 3           | [5]       |
| 553.5593 | 15.17 | 451.34; 391.32; 293.28; 275.27; 233.23; 219.21; 201.08; 177.16; 163.15; 149.13; 141.06; 135.12; 121.10; 109.10; 97.10; 81.03 | [M+H]+ | C₁₅H₁₅NO₂ | Cryptoxanthin | Carotenoid | 3           | [5]       |
| 5      | 220.0983 | 202.09; 170.06; 160.08; 132.08; 118.07 | [M+H]+ | C₁₅H₁₅NO₂ | Methyl 2-hydroxy-3-(1H-indol-2-yl)propanoate | Indole | 1           | [6]       |
| 411.3263 | 14.01  | 21635.14; 191.11; 173.10; 145.10 | [M-H₂O+H]+ | C₁₅H₁₅NO₂ | 5α,8α-Epidioxyergosta-6,22-dien-3β-ol | Sterol | 2           | [7]       |
| 288.2906 | 7.76   | 270.28; 106.09; 88.08 | [M+H]+ | C₁₅H₁₅NO₂ | C₁₇-Sphinganine | Aminolipid | 2           | [8]       |

PEFA (Polyol ester of fatty acid) glycolipid annotations (in molecular clusters as per Figure 4 in the main text)

| PEFA (Polyol ester of fatty acid) glycolipid annotations | (in molecular clusters as per Figure 4 in the main text) | 767.4197 | 15.59 | 707.40; 679.37; 647.38; 619.34; 587.36; 517.31; 425.14; 365.12; 337.09; 265.25; 181.09; 153.05; 111.04 | [M+Na]+ | C₁₅H₁₅NO₂ | Mannitol-pentaacetate-3-acetlyoxy-C₂₀ | PEFA | 2 |
| Mass (Da) | Charge | Formula | Name | PEFA | Number | References |
|----------|--------|---------|------|------|--------|------------|
| 739.4256 | 15.48  | [M+Na]⁺ | C₁₈H₃₀O₁₄ | Mannitol-pentaacetate-3-acetyloxy-C₁₈ | PEFA | 3 | [9,10] |
| 695.3984 | 15.40  | [M+Na]⁺ | C₁₈H₃₀O₁₄ | Mannitol-pentaacetate-3-acetyloxy-C₁₈ | PEFA | 3 |       |
| 613.2845 | 11.44  | [M+Na]⁺ | C₁₂H₂₄O₁₃ | Mannitol-tetraacetate-3-acetyloxy-C₁₂ | PEFA | 2 |       |
| 641.3152 | 12.62  | [M+Na]⁺ | C₁₄H₂₈O₁₃ | Mannitol-tetraacetate-3-acetyloxy-C₁₄ | PEFA | 2 | [10,11] |
| 669.4626 | 13.61  | [M+Na]⁺ | C₁₆H₃₂O₁₃ | Mannitol-tetraacetate-3-acetyloxy-C₁₆ | PEFA | 3 | [9-11] |
| 697.3560 | 14.51  | [M+Na]⁺ | C₁₈H₃₂O₁₃ | Mannitol-tetraacetate-3-acetyloxy-C₁₈ | PEFA | 3 | [9-11] |
| 725.4203 | 15.22  | [M+Na]⁺ | C₂₀H₄₀O₁₃ | Mannitol-tetraacetate-3-acetyloxy-C₂₀ | PEFA | 3 | [10]  |
| 555.3145 | 12.70  | [M+Na]⁺ | C₁₆H₃₂O₁₃ | Mannitol-tetraacetate-3-acetyloxy-C₁₆ | PEFA | 2 | [10]  |
| Mass (m/z) | Retention Time (min.) | M+Na⁺ | Molecular Formula | Compound Description | TIC (a.u.) |
|-----------|-----------------------|-------|-------------------|----------------------|------------|
| 583.5362  | 13.83                 | [M+Na⁺] | C₆₃H₁₁₂O₂₀ | Mannitol-diacetate-3-acetyloxy-C₁₈ | PEFA | 3 |
| 529.3364  | 9.33                  | [M+Na⁺] | C₆₃H₁₁₄O₁₁ | Mannitol-diacetate-3-acetyloxy-C₁₂ | PEFA | 3 |
| 557.2955  | 10.97                 | [M+Na⁺] | C₆₃H₁₁₄O₁₁ | Mannitol-diacetate-3-acetyloxy-C₁₄ | PEFA | 2 |
| 585.4353  | 12.32                 | [M+Na⁺] | C₆₃H₁₁₄O₁₁ | Mannitol-diacetate-3-acetyloxy-C₁₆ | PEFA | 3 |
| 613.3577  | 13.51                 | [M+Na⁺] | C₆₃H₁₁₄O₁₁ | Mannitol-diacetate-3-acetyloxy-C₁₈ | PEFA | 2 |

| Mass (m/z) | Retention Time (min.) | M+Na⁺ | Molecular Formula | Compound Description | TIC (a.u.) |
|-----------|-----------------------|-------|-------------------|----------------------|------------|
| 417.2099  | 6.63                  | [M+Na⁺] | C₆₃H₁₁₂O₁₀ | Mannitol-3-acetyloxy-C₁₀ | PEFA | 2 |
| 445.2415  | 8.02                  | [M+Na⁺] | C₆₃H₁₁₂O₁₀ | Mannitol-3-acetyloxy-C₁₂ | PEFA | 2 |
| 473.4443  | 9.44                  | [M+Na⁺] | C₆₃H₁₁₂O₁₀ | Mannitol-3-acetyloxy-C₁₄ | PEFA | 3 |
| 501.2922  | 10.73                 | [M+Na⁺] | C₆₃H₁₁₂O₁₀ | Mannitol-3-acetyloxy-C₁₆ | PEFA | 2 |
| 515.2836  | 10.11                 | [M+Na⁺] | C₆₃H₁₁₂O₁₀ | Mannitol-monocetate-3-acetyloxy-C₁₈ | PEFA | 2 |
| 543.3145  | 11.54                 | [M+Na⁺] | C₆₃H₁₁₂O₁₀ | Mannitol-monocetate-3-acetyloxy-C₁₆ | PEFA | 2 |
| 571.3468  | 12.57                 | [M+Na⁺] | C₆₃H₁₁₂O₁₀ | Mannitol-monocetate-3-acetyloxy-C₁₈ | PEFA | 2 |

| Mass (m/z) | Retention Time (min.) | M+Na⁺ | Molecular Formula | Compound Description | TIC (a.u.) |
|-----------|-----------------------|-------|-------------------|----------------------|------------|
| 655.2946  | 12.32                 | [M+Na⁺] | C₆₃H₁₁₂O₁₀ | Mannitol-pentacetate-3-acetyloxy-C₁₂ | PEFA | 2 |
| 739.3934  | 14.86                 | [M+Na⁺] | C₆₃H₁₁₂O₁₀ | Mannitol-pentacetate-3-acetyloxy-C₁₈ | PEFA | 2 |
| M/z       | Intensity | Exact Mass | Spectrum | Molecular Formula | Compound Name                                                                 | PEFA  |
|-----------|-----------|------------|----------|------------------|-------------------------------------------------------------------------------|-------|
| 683.3955  | 14.87     | 623.38; 563.36; 503.33; 443.30; 435.31; 393.30; 313.09; 293.28; 253.07; 231.09; 211.06; 193.05; 189.08; 171.07; 153.06; 111.05 | [M+Na]^+ | C_{34}H_{60}O_{12} Mannitol-triacetate-3-acetyloxy-C20 | PEFA 2 | [10] |
| 655.3449  | 13.92     | 595.35; 535.33; 475.30; 453.32; 415.28; 365.27; 313.09; 265.25; 253.07; 231.09; 189.08; 153.06; 111.05 | [M+Na]^+ | C_{32}H_{56}O_{12} Mannitol-triacetate-3-acetyloxy-C18 | PEFA 3 | [9-11] |
| 653.3890  | 15.13     | 593.37; 533.35; 473.33; 451.34; 413.30; 393.30; 293.28; 283.08; 223.06; 201.08 | [M+Na]^+ | C_{33}H_{54}O_{12} Mannitol-triacetate-3-acetyloxy-C16 | PEFA 3 | [10] |
| 627.6426  | 12.82     | 567.32; 507.29; 447.27; 387.25; 379.25; 337.24; 313.09; 253.07; 237.22; 23.09; 189.08; 111.05 | [M+Na]^+ | C_{31}H_{54}O_{12} Mannitol-triacetate-3-acetyloxy-C18 | PEFA 3 | [9,10] |
| 625.3367  | 14.40     | 565.34; 505.31; 445.29; 423.31; 385.25; 365.27; 283.08; 265.25; 223.06; 202.08; | [M+Na]^+ | C_{31}H_{54}O_{12} Mannitol-triacetate-3-acetyloxy-C18 | PEFA 3 | [9,10] |
| 599.4146  | 11.65     | 539.28; 479.26; 419.24; 397.26; 359.22; 351.21; 313.09; 253.07; 231.09; 209.19; 189.08; 153.06; 111.05 | [M+Na]^+ | C_{30}H_{52}O_{12} Mannitol-triacetate-3-acetyloxy-C14 | PEFA 3 | [10] |
| 597.3263  | 13.46     | 537.30; 477.28; 417.26; 395.28; 375.24; 337.24; 283.08; 255.11; 237.22; 223.06; 01.08 | [M+Na]^+ | C_{29}H_{50}O_{12} Mannitol-triacetate-3-acetyloxy-C16 | PEFA 3 | [9,10] |
| 571.4611  | 10.55     | 511.25; 451.23; 391.21; 396.23; 331.19; 313.08; 281.17; 253.07; 231.09; 189.08; 181.16; 153.06; 111.05 | [M+Na]^+ | C_{28}H_{48}O_{12} Mannitol-triacetate-3-acetyloxy-C12 | PEFA 3 | |
| 569.3000  | 12.30     | 509.27; 449.25; 389.23; 367.25; 329.21; 309.20; 283.08; 255.11; 223.06; 209.19 | [M+Na]^+ | C_{27}H_{46}O_{12} Mannitol-triacetate-3-acetyloxy-C14 | PEFA 3 | |
| 541.2764  | 10.93     | 481.24; 421.22; 361.20; 339.22; 301.18; 283.08; 223.06; 201.08; 181.16; 141.06 | [M+Na]^+ | C_{26}H_{44}O_{12} Mannitol-triacetate-3-acetyloxy-C12 | PEFA 3 | |
| 655.3052 | 14.16 | 595.35; 535.33; 475.30; 453.32; 415.28; 365.27; 313.09; 265.25; 253.07; 231.09; 189.08; 153.06; 111.05 | [M+Na]^+ | C₃₀H₅₂O₁₂ Mannitol-triacetate-3-acetyloxy-C₁₈ | PEFA | 2 |
| 627.3359 | 12.64 | 567.32; 507.29; 447.27; 425.29; 387.25; 337.24; 313.09; 253.07; 231.09; 189.08; 111.05 | [M+Na]^+ | C₃₀H₅₂O₁₂ Mannitol-triacetate-3-acetyloxy-C₁₆ | PEFA | 2 |
| 599.3064 | 11.65 | 539.28; 479.26; 419.24; 397.26; 359.22; 335.21; 313.09; 309.20; 253.07; 231.09; 209.19; 189.08; 153.06; 111.05 | [M+Na]^+ | C₃₀H₅₂O₁₂ Mannitol-triacetate-3-acetyloxy-C₁₄ | PEFA | 2 |
| 571.2739 | 10.59 | 511.25; 451.23; 391.21; 369.23; 311.13; 331.09; 281.17; 231.09; 197.04; 189.08; 171.01; 153.06; 111.05 | [M+Na]^+ | C₃₀H₅₂O₁₂ Mannitol-triacetate-3-acetyloxy-C₁₂ | PEFA | 2 |
| 639.3936 | 14.00 | 579.32; 519.29; 497.31; 477.28; 459.27; 437.29; 399.31; 395.28; 349.29; 337.24; 325.09; 283.26; 265.07; 237.22; 210.08; 141.06 | [M+Na]^+ | C₃₀H₅₂O₁₂ Arabinol-tetraacetate-3-acetyloxy-C₁₆ | PEFA | 3 | [9,10] |
| 667.4842 | 14.95 | 607.35; 547.33; 525.34; 505.31; 487.30; 465.31; 445.29; 427.28; 423.31; 365.27; 325.09; 303.11; 265.25; 247.24; 210.08; 141.06; 181.16 | [M+Na]^+ | C₃₀H₅₂O₁₂ Arabinol-tetraacetate-3-acetyloxy-C₁₈ | PEFA | 3 | [9,10] |
| 459.2210 | 7.46 | 399.20; 339.18; 253.14; 229.07; 189.08; 169.05; 154.13; 129.06 | [M+Na]^+ | C₃₀H₅₂O₁₀ Mannitol-monoacetate-3-acetyloxy-C₁₀ | PEFA | 2 |
| 487.2520 | 8.85 | 427.23; 367.21; 281.17; 229.07; 189.08; 181.16; 169.05; 129.06; 111.05 | [M+Na]^+ | C₃₀H₅₂O₁₀ Mannitol-monoacetate-3-acetyloxy-C₁₂ | PEFA | 2 |
| 501.2312 | 8.39 | 441.21; 381.19; 321.17; 271.08; 253.14; 211.06; 189.08; 153.13; 129.06; 111.05 | [M+Na]^+ | C₃₀H₅₂O₁₁ Mannitol-diacetate-3-acetyloxy-C₁₀ | PEFA | 2 |
| 529.2628 | 9.56 | 469.24; 409.22; 353.19; 349.20; 323.16; 281.17; 211.06; 189.08; 181.16; 111.05 | [M+Na]^+ | C₃₀H₅₂O₁₁ Mannitol-diacetate-3-acetyloxy-C₁₂ | PEFA | 2 |
| m/z     | Retention Time | m/z Reference | Molecular Formula | Compound Name                          | PEFA | Charge | [10] |
|---------|----------------|---------------|------------------|----------------------------------------|------|--------|------|
| 585.3253| 12.33          | 525.30; 465.28; 409.26; 405.26; 379.25; 337.24; 271.08; 236.22; 211.06; 189.08; 111.05 | [M+Na]^+ | CaH5O11 | Mannitol-diacetate-3-acetyloxy-C16 | PEFA | 2      | [11] |
| 583.2775| 12.28          | 523.25; 463.23; 441.25; 421; 403.21; 381.23; 343.19; 339.22; 325.09; 281.17; 256.07; 201.08; 181.16; 141.06 | [M+Na]^+ | CaH5O11 | Arabitol-tetraacetate-3-methoxy-C14 | PEFA | 3      |      |
| 655.2927| 11.14          | 595.31; 535.29; 513.30; 475.27; 453.29; 433.26; 415.26; 393.27; 353.23; 325.09; 293.21; 265.07; 235.21; 201.08; 141.06 | [M+Na]^+ | CaH5O14 | Mannitol-pentaacetate-3-acetyloxy-C12 | PEFA | 3      |      |
| 683.3435| 12.51          | 623.34; 563.32; 541.34; 521.31; 503.30; 443.27; 481.31; 461.29; 421.28; 381.26; 325.09; 321.24; 303.11; 265.07; 201.08; 141.06 | [M+Na]^+ | CaH5O14 | Mannitol-pentaacetate-3-acetyloxy-C14 | PEFA | 3      | [10] |
| 611.3049| 10.68          | 551.28; 491.26; 431.24; 369.12; 351.21; 309.09; 291.19; 283.08; 223.06; 210.08; 141.05 | [M+Na]^+ | CaH5O10 | Arabitol-triacetate-3-hydroxy-C20 | PEFA | 3      |      |
| 653.3145| 12.27          | 593.29; 533.27; 511.29; 491.26; 473.25; 432.24; 351.21; 325.09; 291.19; 265.07; 223.21; 201.08; 155.14; 141.06 | [M+Na]^+ | CaH5O11 | Arabitol-pentaacetate-3-hydroxy-C20 | PEFA | 3      |      |

* For PEFA annotations, fragments most indicative of loss of acetyl groups and the residual polyol moiety are highlighted in bold
Table S6. Theoretical masses of acetylated PEFAs

|          | Mannitol 0 acetyl hydroxyl | Mannitol 1 acetyl hydroxyl | Mannitol 2 acetyl hydroxyl | Mannitol 3 acetyl hydroxyl | Mannitol 4 acetyl hydroxyl | Mannitol 5 acetyl hydroxyl |
|----------|-----------------------------|-----------------------------|-----------------------------|-----------------------------|-----------------------------|-----------------------------|
| C14      | 431                         | 473                         | 515                         | 557                         | 599                         | 641                         |
| C16      | 459                         | 501                         | 543                         | 585                         | 627                         | 669                         |
| C18      | 487                         | 529                         | 571                         | 613                         | 655                         | 697                         |
| C20      | 515                         | 557                         | 599                         | 641                         | 683                         | 725                         |

|          | Mannitol 0 acetyl methoxy   | Mannitol 1 acetyl methoxy   | Mannitol 2 acetyl methoxy   | Mannitol 3 acetyl methoxy   | Mannitol 4 acetyl methoxy   | Mannitol 5 acetyl methoxy   |
|----------|-----------------------------|-----------------------------|-----------------------------|-----------------------------|-----------------------------|-----------------------------|
| C14      | 445                         | 487                         | 529                         | 571                         | 613                         | 655                         |
| C16      | 473                         | 515                         | 557                         | 599                         | 641                         | 683                         |
| C18      | 501                         | 543                         | 585                         | 627                         | 669                         | 711                         |
| C20      | 529                         | 571                         | 613                         | 655                         | 697                         | 739                         |

|          | Mannitol 0 acetyl acetoxy   | Mannitol 1 acetyl acetoxy   | Mannitol 2 acetyl acetoxy   | Mannitol 3 acetyl acetoxy   | Mannitol 4 acetyl acetoxy   | Mannitol 5 acetyl acetoxy   |
|----------|-----------------------------|-----------------------------|-----------------------------|-----------------------------|-----------------------------|-----------------------------|
| C14      | 473                         | 515                         | 557                         | 599                         | 641                         | 683                         |
| C16      | 501                         | 543                         | 585                         | 627                         | 669                         | 711                         |
| C18      | 529                         | 571                         | 613                         | 655                         | 697                         | 739                         |
| C20      | 557                         | 599                         | 641                         | 683                         | 725                         | 767                         |

|          | Arabitol 0 acetyl hydroxyl  | Arabitol 1 acetyl hydroxyl  | Arabitol 2 acetyl hydroxyl  | Arabitol 3 acetyl hydroxyl  | Arabitol 4 acetyl hydroxyl  |
|----------|-----------------------------|-----------------------------|-----------------------------|-----------------------------|-----------------------------|
| C14      | 401                         | 443                         | 485                         | 527                         | 569                         |
| C16      | 429                         | 471                         | 513                         | 555                         | 597                         |
| C18      | 457                         | 499                         | 541                         | 583                         | 625                         |
| C20      | 485                         | 527                         | 569                         | 611                         | 653                         |

|          | Arabitol 0 acetyl methoxy   | Arabitol 1 acetyl methoxy   | Arabitol 2 acetyl methoxy   | Arabitol 3 acetyl methoxy   | Arabitol 4 acetyl methoxy   |
|----------|-----------------------------|-----------------------------|-----------------------------|-----------------------------|-----------------------------|
| C14      | 415                         | 457                         | 499                         | 541                         | 583                         |
| C16      | 443                         | 485                         | 527                         | 569                         | 611                         |
| C18      | 471                         | 513                         | 555                         | 597                         | 639                         |
| C20      | 499                         | 541                         | 583                         | 625                         | 667                         |

|          | Arabitol 0 acetyl acetoxy   | Arabitol 1 acetyl acetoxy   | Arabitol 2 acetyl acetoxy   | Arabitol 3 acetyl acetoxy   | Arabitol 4 acetyl acetoxy   |
|----------|-----------------------------|-----------------------------|-----------------------------|-----------------------------|-----------------------------|
| C14      | 443                         | 485                         | 527                         | 569                         | 611                         |
| C16      | 471                         | 513                         | 555                         | 597                         | 639                         |
| C18      | 499                         | 541                         | 583                         | 625                         | 667                         |
| C20      | 527                         | 569                         | 611                         | 653                         | 695                         |
Table S7. MS/MS fragment ions indicative of the type of polyol group in PEFAs

|                  | m/z [M+H]^+ | m/z [M+NH4]^+ | m/z [M+Na]^+ |
|------------------|-------------|---------------|--------------|
| Mannitol, 0 Ac   | 165.1       | 182.1         | 187.1        |
| Mannitol, 1 Ac   | 207.1       | 224.1         | 229.1        |
| Mannitol, 2 Ac   | 249.1       | 266.1         | 271.1        |
| Mannitol, 3 Ac   | 291.1       | 308.1         | 313.1        |
| Mannitol, 4 Ac   | 333.1       | 350.1         | 355.1        |
| Mannitol, 5 Ac   | 375.1       | 392.1         | 397.1        |
| Arabitol, 0 Ac   | 135.1       | 152.1         | 157.1        |
| Arabitol, 1 Ac   | 177.1       | 194.1         | 199.1        |
| Arabitol, 2 Ac   | 219.1       | 236.1         | 241.1        |
| Arabitol, 3 Ac   | 261.1       | 278.1         | 283.1        |
| Arabitol, 4 Ac   | 303.1       | 320.1         | 325.1        |
| Position | $\delta_H \text{ Multiplicity (f in Hz)}$ | $\delta_C$ | COSY | HMBC $\text{H} \rightarrow \text{C}$ | TOCSY | NOE |
|----------|--------------------------------|----------|------|--------------------------------|------|-----|
| 1        | 3.63 (m), 3.80 (m)             | 64.8     | H-2  | C-3                              | H-6  | H-1, H-3, H-4, H-5, H-6, H-2, H-5 |
| 2        | 3.79 (m)                       | 70.3     | H-1, H-3 | C-1, C-3, C-4, C-11              |     |     |
| 3        | 3.69 (m)                       | 72.7     | H-2, H-4 | C-9                              | H-5  | H-5 |
| 4        | 3.48 (m)                       | 70.4     | H-3, H-5 | C-5                              | H-2, H-4, H-6, C-4 | H-5 |
| 5        | 3.87 (m)                       | 70.0     | H-4, H-6 | C-4                              | H-2, H-4, H-6, H-5, C-2 | H-5 |
| 6        | 4.16 (m), 4.37 (m)             | 67.7     | H-5  | C-4, C-7                         |     | H-5 |
| 7        | -                              | 172.1    |      |                                  |     |     |
| 8        | 2.05 (s)                       | 20.3 - 20.8 |      | C-7                              |     |     |
| 9        | -                              | 173.1    |      |                                  |     |     |
| 10       | 2.03 (s)                       | 20.3 - 20.8 |      | C-9                              |     |     |
| 11       | -                              | 172.9    |      |                                  |     |     |
| 12       | 2.08 (s)                       | 20.6     |      | C-11                             |     |     |
| 1'       | -                              | 172.3    |      |                                  |     |     |
| 2'       | 2.61 (m), 2.65 (m)             | 39.8     | H-3' | C-1', C-3', C-4'                | H-3', H-4', H-5, H-4' | H-4' |
| 3'       | 5.22 (m)                       | 71.8     | H-2', H-4' | C-1', C-2', C-4', C-5', C-17' | H-2', H-4', H-5 | H-5' |
| 4'       | 1.61 (m), 1.61 (m)             | 34.7     | H-3', H-5' |                                  | H-2', H-3', H-5' | H-5' |
| 5'       | 1.33 (m)                       | 25.9     | H-4' |                                  |     |     |
| 6' - 13' | 1.29 – 1.33 (m)                | 30.2 – 31.0 |      |                                  |     |     |
| 14'      | 1.29 (m)                       | 32.9     | H-13', H-15' |                                  |     |     |
| 15'      | 1.31 (m)                       | 23.5     | H-14', H-16' | C-14', C-16' |     |     |
| 16'      | 0.90 (t, 6.9)                  | 14.1     | H-15' | C-14', C-15'                   |     |     |
| 17'      | -                              | 172.3    |      |                                  |     |     |
| 18'      | 2.02 (s)                       | 20.8     |      | C-17                             |     |     |
Table S9. NMR data for compound 2 (MeOD, 600/150 MHz)

| Position | δ\textsubscript{n} Multiplicity (J in Hz) | δ\textsubscript{C} | COSY | HMBC H → C | NOE |
|----------|----------------------------------------|----------------|-------|------------|-----|
| 1        | 3.63 (m), 3.80 (m)                     | 64.7           | H-2   | C-2        | H-2 |
| 2        | 3.79 (m)                               | 70.3           | H-1, H-3 | C-3     | H-1, H-3, H-5 |
| 3        | 3.69 (m)                               | 72.5           | H-2, H-4 |         | H-2, H-4 |
| 4        | 3.47 (m)                               | 70.7           | H-3, H-5 | C-7     | H-3, H-5 |
| 5        | 3.87 (m)                               | 70.0           | H-4, H-6 | C-4     | H-2, H-4, H-6 |
| 6        | 4.18 (m), 4.39 (m)                     | 67.8           | H-5   |           | H-5 |
| 7        |                                       |                |       |           |     |
| 8        | 2.08 (s)                               | 20.5           |       | C-7      |     |
| 1'       |                                       | 172.3          |       |          |     |
| 2'       | 2.65 (m), 2.65 (m)                     | 39.8           | H-3'  | C-1', C-3', C-4' | H-4' |
| 3'       | 5.22 (m)                               | 71.7           | H-2', H-4' | C-1', C-17' | H-2', H-4', H-5' |
| 4'       | 1.62 (m), 1.62 (m)                     | 34.8           | H-3', H-5' | C-5'   | H-2', H-3', H-5' |
| 5'       | 1.32 (m)                               | 25.9           |       | H-4'     |     |
| 6' - 13' | 1.29 – 1.33 (m)                        | 30.2 – 31.0    |       |          |     |
| 14'      | 1.29 (m)                               | 32.8           | H-13', H-15' |        |     |
| 15'      | 1.31 (m)                               | 23.5           | H-14', H-16' | C-14', C-16' |     |
| 16'      | 0.90 (t, 6.9)                          | 14.2           | H-15' | C-15'    |     |
| 17'      |                                       | 172.3          |       |          |     |
| 18'      | 2.02 (s)                               | 20.8           |       | C-17'    |     |
Table S10. NMR data of compound 5 (MeOD, 600/150 MHz)

| Position | $\delta$ m (J in ppm) | $\delta$ c | HMBC C$\rightarrow$ H | NOE |
|----------|----------------------|-----------|----------------------|------|
| 1        | 175.7 H-1, H-2, H-3, OMe |
| 2        | 4.43 (dd, 6.9, 5.4) 72.6 H-3 H-3 |
| 3        | 3.11 (dd, 14.5, 6.5, 0.7) 31.3 H-2 H-2 |
|          | 3.21 (dd, 14.5, 5.4, 0.7) 137.7 H-4', H-6' |
| 1'       | 124.5 H-3 H-3 |
| 2'       | 7.09 (s) 110.8 H-3, H-2, H-4', H-2' |
| 3'       | 110.8 H-3, H-2, H-4', H-2' |
| 3a'      | 127.3 H-2, H-3, H-6' |
| 4'       | 7.53 (d, 7.8) 119.1 H-6' H-2, H-3, H-5', OMe |
| 5'       | 6.99 (t, 7.8) 119.4 H-7' H-4', H-6' |
| 6'       | 7.07 (t, 7.8) 122.0 H-4' H-5', H-7' |
| 7        | 7.31 (d) 111.9 H-5' H-6' |
| 7a'      | 110.1 H-4, H-2, H-6' |
| OMe      | 3.64 (s) 52.0 H-2', H-4' |
Figure S1. Differential metabolomes of *R. mucilaginosa* 50-3-19/20B (a) on PDA medium; (b) on WSP30 medium

Figure S2. Overview of genomic statistics based on *de novo* genome assembly. (A) Genome Assembly statistics (B) Total repetitive elements

| A | denovo Assembly | Definition |
|---|-----------------|------------|
| Assembled Size (Mb) | 20.02 | Total denovo assembled size |
| Total scaffolds | 265 | Total number of scaffolds in assembled genome |
| N25 (Kb) | 498.8 | Length of the scaffold until which sum of lengths of scaffolds are reached to 25% of 20.02 Mb |
| L25 | 8 | Number of scaffolds in the assembled genome that constitute N25 |
| N50 (Kb) | 295.8 | Length of the scaffold until which sum of lengths of scaffolds are reached to 50% of 20.02 Mb |
| L50 | 21 | Number of scaffolds in the assembled genome that constitute N50 |
| N75 (Kb) | 163.5 | Length of the scaffold until which sum of lengths of scaffolds are reached to 75% of 20.02 Mb |
| L75 | 44 | Number of scaffolds in the assembled genome that constitute N75 |
| %GC | 60.47 | Percentage of total G+C content of assembled genome (20.02 Mb) |

| B | Types of repeats | No. of Repeats* | Total length (bp) | Percentage of genomic sequence |
|---|------------------|-----------------|-------------------|------------------------------|
| Retroelements | 81 | 55667 | 0.28 |
| LINEs | 18 | 3607 | 0.02 |
| LTR elements | 63 | 52060 | 0.26 |
| Ty1/Copia | 28 | 30103 | 0.15 |
| Gypsy/DIRS1 | 33 | 21758 | 0.11 |
| DNA transposons | 9 | 581 | 0.00 |
| Tc1-IF630-Pogo | 1 | 15 | 0.00 |
| Unclassified | 2 | 887 | 0.00 |
| Small RNA | 10 | 7904 | 0.04 |
| Simple repeats | 8318 | 326295 | 1.63 |
| Low complexity | 759 | 37594 | 0.19 |
| Total interspersed repeats | 57135 | | 0.29 |
| Total repeat contents | 428912 | | 2.14 |
Figure S3. GNPS MS/MS mirror plot of experimental and library data of 9-oxo-10E,12Z-octadecadienoic acid

Figure S4. GNPS MS/MS mirror plot of experimental and library data of cyclo-(Leu-Phe)
Figure S5. GNPS MS/MS mirror plot of experimental and library data of DL-Indole-3-lactic acid

Figure S6. GNPS MS/MS mirror plot of experimental and library data of 5α,8α-epidioxyergosta-6,22-dien-3β-ol
Figure S7. GNPS MS/MS mirror plot of experimental and library data of C17-sphinganine

Figure S8. GNPS MS/MS mirror plot of experimental and library data of 9,10-Epoxy-12-octadecenoic acid
Figure S9. (a) HR-MS and (b) MS/MS spectra of 1
Figure S10. $^1$H NMR spectrum of compound 1 (MeOD, 600 MHz)

Figure S11. HSQC spectrum of compound 1 (MeOD, 600/150 MHz)
Figure S12. COSY spectrum of compound 1 (MeOD, 600 MHz)

Figure S13. HMBC spectrum of compound 1 (MeOD, 600/150 MHz)
Figure S14. NOESY spectrum of compound 1 (MeOD, 600 MHz)

Figure S15. TOCSY spectrum of compound 1 (MeOD, 600 MHz)
Figure S16. (a) HR-ESIMS and (b) MS/MS spectra of 2
Figure S17. $^1$H NMR spectrum of compound 2 (MeOD, 600 MHz)

Figure S18. HSQC spectrum of compound 2 (MeOD, 600/150 MHz)
Figure S19. COSY spectrum of compound 2 (MeOD, 600 MHz)

Figure S20. NOESY spectrum of compound 2 (MeOD, 600 MHz)
Figure S21. HMBC spectrum of compound 2 (MeOD, 600/150 MHz)
Figure S22. (a) HR-ESIMS and (b) MS/MS spectra of 3
Figure S23. (a) HR-ESIMS and (b) MS/MS spectra of 4
Figure S24. (a) HR-ESIMS and (b) MS/MS spectra of 5
Figure S25. $^1$H NMR spectrum of compound 5 (MeOD, 600 MHz)

Figure S26. $^{13}$C NMR spectrum of compound 5 (MeOD, 150 MHz)
Figure S27. HSQC spectrum of compound 5 (MeOD, 600/150 MHz)

Figure S28. COSY spectrum of compound 5 (MeOD, 600 MHz)
Figure S29. HMBC spectrum of compound 5 (MeOD, 600/150 MHz)

Figure S30. NOESY spectrum of compound 5 (MeOD, 600 MHz)
Figure S31. Metabolites reported in the Dictionary of Natural Products for the genus *Rhodotorula*

- **10-Undecenoic acid**
- **10-Undecynoic acid**
- **(R)-form, 2E-Hydroxy-3-pentanone**
- **1,5-Dihydroxy-2,2-dimethyl-3-pentanone**
- **3,4-Didehydro-γ-carotene**
- **7,8-Dihydrolycopene**
- **Torularhodin**
- **16'-Aldehyde Torularhodin**
- **16'-Alcohol Torularhodin**
- **3-Hydroxyhexadecanoic acid; (R)-form**
- **3-Hydroxyoctadecanoic acid; (R)-form**

**Saccharide biosynthesis**

- **NRPS/RiPP**
- **3-O-β-D-Mannopyranosyl-D-mannose**
- **4-O-β-D-Mannopyranosyl-D-mannose**

**Terpene/Fatty acid biosynthesis**

- **(S)-form, N-Hydroxy-3-Amino-2-piperidinone**

**PKS Pathway**

- **Oxaspirol A**
- **4-Ketone-Oxaspirol A**
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