Additional experimental data

**Figure 1S** - (a) Photograph of the area where the strain of *Penicillium roqueforti* was collected (b) Rocky outcrop on the beach of La Laja, Gran Canaria, Spain.

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**CABI BIOSCIENCE**
IDENTIFICATION SERVICES

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**Identification and comments**

**PM 001**
*Penicillium variabile* Pilát. Description based on Doerk et al. *Compendium of Novel Fungi*, Academic Press, 202-204 p. A common contaminant in air and is often isolated from natural environments such as composting and agricultural soils. This isolate has been described. Report from Dr. F. Lawrence.

**PA 002**
*Penicillium roqueforti* Thost. A weakly acidified agent of *P. roqueforti*. Description based on Dr. J.L. Lawrence, personal communication and *Penicillium and related species*. Academic Press, 345-350 p. This species is capable of growing at low oxygen levels. It is found on sulphur-oxidizing rocks caused by natural disasters, as well as from cheese. Also isolated from soil, pine needles, and tree trunks. Report from Dr. F. Lawrence.

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**Figure 2S** – CABI Bioscience (Surrey, UK) identification report of the fungal strain.
**Figure 3S** - View from above of the mass cultivation of *Penicillium roqueforti*.

**Figure 4S** - Solvent-solvent processing scheme applied to partition the culture broth of *Penicillium roqueforti*, adapted from Kupchan et al. (1973).
Description of the chemical analysis of the culture broth from the derived-marine *Penicillium roqueforti* fungus.

Twelve liters of culture broth were extracted in a separatory funnel with 2.5 L of CH$_2$Cl$_2$ (x3) to obtain 175 mg of crude extract (Pr-L-0), which was subjected to the partitioning scheme described above (Figure 4S).

The $^1$H-NMR spectrum resulted in seven protons of carboxylic acids at $\delta$ 12.5-11.2, aromatics at $\delta$ 8.5-7.0; olefins at $\delta$ 5.9-5.0; geminals to heteroatoms at $\delta$ 4.3-3.5 and aliphatics at $\delta$ 3.0-0.6.

This crude extract was dissolved in 20 ml of water and 180 ml of MeOH and was extracted in a decantation funnel with hexane until it was colorless (10 extractions). The evaporation of the solvent gave 106 mg of the liposoluble fraction (Pr-L-1).

The hydrosoluble fraction was diluted with additional water (160 ml) to obtain an aqueous phase of composition 50% H$_2$O-50% MeOH, which was reextracted with CH$_2$Cl$_2$ (x3) to give the fractions Pr-L-2 (soluble in CH$_2$Cl$_2$, 52 mg) and Pr-L-3 (17 mg).

**Study of the liposoluble fraction-1 (Pr-L-1)**

By TLC (normal-phase, Hexane: EtOAc, 80: 20) six major substances were detected. The same was observed by HPLC (normal-phase, Hexane: EtOAc, 80: 20) (Figure 5S):

![Analytical chromatography by TLC and HPLC of the Pr-L-1 fraction.](image)

In the $^1$H-NMR spectrum of this mixture, we detected: aromatic protons ($\delta$ 7.70 and 7.53),...
olefinic (δ 5.38), germinal to heteroatom (δ 4.31) and aliphatic hydrocarbon chains (δ 2.9-0.7).

In the $^{13}$C-NMR spectrum, the following were to be observed: carbonilic carbons (δ 178.187), aromatics (δ 167.753), olefinic (δ 130.939 and 128.859), geminals to heteroatoms (δ 77.543-65.914) and from aliphatic chains (δ 37.469-14.132).

This mixture was submitted to a normal-phase semipreparative HPLC (Hexane: EtOAc, 80: 20, UV detector adjusted to 254 nm) giving two fractions: Pr-L-1-1 and Pr-L-1-2.

**Pr-L-1-1 fraction**

A colourless oil was obtained (50 mg), and the signals were only observed at δ 0.85-1.86 using $^1$H-NMR spectroscopy, leading to the suspicion that it is a mixture of alkanes. A study by GC-MS allowed for the identification and quantification of the following volatile compounds:

- Pentadecane, $C_{15}$ (I; n= 12; $R_t$= 13.990; 0.037 mg)
- Heptadecane, $C_{17}$ (I; n= 14; $R_t$= 15.642; 0.030 mg)
- Eicosane, $C_{20}$ (I; n= 17; $R_t$= 17.393; 0.063 mg)
- Cyclo-ProLeu (14; Tr= 17.56; 0.211 mg)
- Heneicosane, $C_{21}$ (I; n= 18; $R_t$= 18.327; 0.141 mg)
- Docosane, $C_{22}$ (I; n= 19; $R_t$= 18.978; 0.142 mg)
- Tricosane, $C_{23}$ (I; n= 20; $R_t$= 19.744; 0.250 mg)
- Hexacosane, $C_{26}$ (I; n= 23; $R_t$= 23.242; 1.766 mg)
- Heptacosane, $C_{27}$ (I; n= 24; $R_t$= 25.07; 0.588 mg)

This gave a total of 46.772 mg of non-volatile material, that was assigned to the alkanes of higher molecular weight, that did not volatilize at the injector temperature used or were outside the predetermined scanning time in the method.

**Pr-L-1-2 fraction**

This colourless oil (50 mg), when observed by $^1$H-NMR, revealed the following: aromatic
protons (δ 7.70 and 7.53), olefins (δ 5.48-5.26), geminal to heteroatoms (δ 4.42), in alpha to carbonyls (δ 2.80), of methylenes/aliphatic methines (δ 1.83-1.20), and methyls (δ 0.81). Thus, it appears to be a mixture of alkyl phthalates and unsaturated fatty acids. Indeed, a study by GC-MS showed the presence of the following volatile compounds:

Dibutyl Phthalate (15; n= 3; R<sub>t</sub>= 17.649; 5.126 mg)
7,10-Octadecadienoic acid, methyl ester (7; n= 6, m= 5; R<sub>t</sub>= 18.378; 0.178 mg)
Hexadecanoic acid, octadecyl ester (9; R<sub>t</sub>= 18.646; 0.251 mg)
Hexadecanoic acid, 2-(octadecyloxi)-ethyl ester (12; R<sub>t</sub>= 19.106; 0.119 mg)
9-Octadecenoic acid, (E)- (5; R<sub>t</sub>= 19.807; 0.039 mg)
Bis(4-methylpentyl) phthalate (16; n= 3; R<sub>t</sub>= 20.407; 0.968 mg)
2-(Octadecyloxi) ethanol (11; R<sub>t</sub>= 21.805; 0.555 mg)
Bis(2-ethylhexyl) phthalate (17; R<sub>t</sub>= 22.645; 0.214 mg)
Dihexyl phthalate (15; n= 5; R<sub>t</sub>= 22.735; 2.919 mg)
Dioctyl phthalate (15; n= 7; R<sub>t</sub>= 26.367; 2.349 mg)

There was a total weight of 37.282 mg of non-volatile material that was assigned, using <sup>1</sup>H-RMN spectroscopy, to a mixture of alkyl phthalates of high molecular weight, and to free unsaturated fatty acids that did not volatilize at the injector temperature used or were outside the predetermined scanning time in the method.

Indeed, rechromatography of this fraction through silica eluting with hexane-ethyl acetate yielded a pure sample (homogeneous by TLC) of one of these phthalates. From its <sup>1</sup>H-RMN spectrum, it was assigned the structure of the Dinonyl phthalate (15; n= 8): δ 7.70 (2H, dd); 7.53 (2H, dd); 4.32 (4H, t); 1.75 (4H, m); 1.48-1.20 (24H, m) and 0.89 (6H, t).

**Study of the liposoluble fraction-2 (Pr-L-2)**

52 mg of a mixture was obtained that, by analytical TLC, gave eight substances, with the R<sub>t</sub> coinciding with five components of the previous fraction Pr-L-1.

The <sup>1</sup>H-RMN spectrum showed aromatic protons (δ 7.78-7.47), geminal to heteroatom (δ 4.28 on
one side and 3.90-3.15 on the other) and typical saturated hydrocarbonated 
ch_{HMS} (\delta 2.50-0.75), leading to the suspicion, therefore, of the presence of phthalates and saturated lipid components. A GC-MS analysis of this fraction identified/ quantified as follows:

Hexadecanoic acid (2-pentadecyl-1,3-dioxolan-4-yl) methyl ester (8; R_t= 12.154; 0.0088 mg)
2-Butyl-1-octanol (3; n= 3, m= 3; R_t= 12.519; 0.0025 mg)
Tetradecanoic acid (4, n= 12; R_t= 14.776; 0.0063 mg)
1-Tridecanol (2; n= 11; R_t= 14.867; 0.0023 mg)
Diethyl phthalate (15; n= 1; R_t= 15.062; 0.0083 mg)
1-Hexadecanol (2; n= 14; R_t= 16.348; 0.003 mg)
Bis(2-methylpropyl) phthalate (16; n= 1; R_t= 17.009; 0.0167 mg)
Dibutyl phthalate (15; n= 3; R_t= 17.653; 1.3935 mg)
Stearic acid, 3-(octadecyloxy)-propyl ester (10; R_t= 17.948; 0.0024 mg)
9-Octadecenamide (6; R_t= 18.358; 0.0181 mg)
9-Octadecenoic acid, (E)- (5; R_t= 19.814; 0.0087 mg)

The total weight of 50.529 mg of non-volatile material was assigned by \textsuperscript{1}H-NMR spectroscopy to a mixture of phthalates and saturated lipid components of the above type but of larger molecular size that, as occurred previously, either did not volatilize at the injector temperature used or went outside the default analysis time for the method. Thus, by rechromatography column, some different enriched fractions were separated:

a) alkanes (1; \delta 1.62-0.77), b) Dinonyl phthalate { (15; n= 8); \delta 7.70 (2 H, dd); 7.53 (2 H, dd); 4.32 (4 H, t); 1.75 (4 H, m); 1.48-1.20 (24 H, m) and 0.89 (6 H, t)}, c) Dibutyl phthalate { (15; n= 3); \delta 7.70 (2 H, dd); 7.53 (2 H, dd); 4.31 (4 H, t); 1.72 (4 H, m); 1.44 (4 H, m); 0.96 (6 H, t)}, d) saturated fatty acids (4; \delta 1.85-0.80) e) saturated 1-alkanols (2; \delta 3.80-3.50; 1.85-0.80).

\textbf{Study of the liposoluble fraction-3 (Pr-L-3)}

The crude fraction (17 mg) has an \textsuperscript{1}H-NMR spectrum which shifts germinal protons to heteroatoms (\delta 4.10-3.15), \textsuperscript{-}NH_2 groups (\delta 3.03) and aliphatic chains (\delta 2.50-0.75).
The GC-MS analysis of this fraction allowed the identification/quantification of the following volatile organic compounds:

2,6-Octadienal, 3,7-dimethyl-\((E)\) (13; \(R_t = 12.179\); 0.079 mg)
Pentadecane, \(C_{15}\) (1; \(n= 12\); \(R_t = 13.992\); 0.005 mg)
9-Octadecenamide (6; \(R_t = 18.366\); 0.0489 mg)
9-Octadecenoic acid, (E)- (5; \(R_t = 19.809\); 0.03 mg)
2-Ethylhexyl adipate (18; \(R_t = 20.714\); 0.143 mg)

That gives a total of 16.694 mg that was assigned by \(^1\)H-NMR to a mixture of unsaturated glycerides (δ 5.46-5.14; 4.37-4.06; 2.53-2.23; 1.84-0.80) and polyhydroxy compounds (δ 4.10-3.15) that, as before, either did not volatilize at the injector temperature used or went outside the default analysis time for the method.

Final note: The GC-MS digital fingerprints of the compounds 13, 15 (\(n= 1, 3 \text{ and 7}\)); and the \(^1\)H-NMR spectra of the compound 15 (\(n= 3\)) can be seen below. The GC-MS spectra of the other compounds, and other spectroscopic data, can be obtained by contacting the authors.
Figure 6S – Typical Gas chromatography–mass spectrometry (GC-MS) chromatogram.
Figure 7S – Typical GC-MS enlarged chromatogram.
Figure 8S – The GC-MS fingerprint of the 2,6-Octadienal, 3,7-dimethyl-, (E)- (13).
Figure 9S - GC-MS fingerprint of the Diethyl phthalate (15; n= 1).
Figure 10S – The GC-MS fingerprint of the Dibutyl phthalate (15; n=3)
Figure 11S – The GC-MS fingerprint of the Dioctyl phthalate (15, n= 7).
Figure 12S - The $^1$H-NMR spectrum of the Dibutyl phthalate (15; n = 3).