Supplements

Precursor direct biosynthesis of odd chain polyunsaturated fatty acids by oomycete *Pythium*

Andrea Palyzová[1], Markéta Kulišová[2], Katarína Majtán[2,3], Karel Fous[2,3], Irena Jarošová Kolouchová[2], Tomáš Řezanka[1,]

[1]Institute of Microbiology, Czech Academy of Sciences, Vídeňská 1083, 142 00 Prague, Czech Republic

[2]Department of Biotechnology, University of Chemistry and Technology, Technická 5, 166 28 Prague, Czech Republic

[3]Research Institute of Brewing and Malting, Lípová 511, 120 44 Prague, Czech Republic

Correspondence: rezanka@biomed.cas.cz

Table 1Sa. Describing data of biological replicates (three cultivations).

	dry weight (mg)				
	first cultivation	second cultivation	third cultivation	mean	S.D.
14/X42PDB	2533	2489	2421	2481	±56
14/X42PDB+Pr+TW	1371	1345	1309	1342	±31

Table 1Sb. Describing data of technical replicates (nine chromatographic analyses).

Fatty acid	14/X42PDB	14/X42PDB+Pr+TW
14:0	4.2 ± 0.08	0.8 ± 0.05
16:0	14.5 ± 0.05	8.4 ± 0.08
16:1ω7	8.1 ± 0.07	4.8 ± 0.06
17:2ω6	0.0 ± 0	3.6 ± 0.03
18:0	3.9 ± 0.06	2.3 ± 0.07
18:1ω9	19.7 ± 0.07	11.6 ± 0.09
17:3ω3	0.0 ± 0	5.5 ± 0.04
17:4ω3	0.0 ± 0	7.6 ± 0.07
18:2ω6	12.5 ± 0.08	7.4 ± 0.05
18:3ω6	1.1 ± 0.06	0.8 ± 0.06
18:3ω3	1.2 ± 0.05	0.8 ± 0.02
19:2ω5	0.0 ± 0	2.9 ± 0.03
20:0	0.4 ± 0.07	0.2 ± 0.04
20:1ω9	1.1 ± 0.02	0.7 ± 0.05
19:3ω5	0.0 ± 0	5.2 ± 0.07
19:4ω5	0.0 ± 0	6.8 ± 0.04
19:5ω2	0.0 ± 0	10.7 ± 0.08
20:3ω6	0.0 ± 0	0.5 ± 0.02
20:4ω6	12.9 ± 0.08	7.6 ± 0.03
20:5ω3	20.4 ± 0.07	11.8 ± 0.08

As can be seen from this table, the chromatographic analysis shows at least one order of magnitude lower ±S.D., compared to the fungal cultures, so it is not essential for the interpretation of the results.

Table 2S. Solvent elution scheme for the recovery of the ethyl ester derivatives (10 mg) of the fatty acids of *Pythium* from a Bond Elute SCX solid-phase extraction column in the silver ion mode.

	Solvents (%)				Fraction
No.	CH_2Cl_2	acetone	acetonitrile	Volume	of FAs
1	100			25 mL	saturated
2	91	9		30 mL	monoenes
3		100		30 mL	dienes
4		96	4	45 mL	trienes
5		92	8	50 mL	tetraenes
6		86	14	35 mL	pentaenes
7		58	42	40 mL	hexaenes

Table 3S. Fatty acid content (%) of *Pythium* strains from published articles.

Fatty acid	(Lio & Wang, 2013)	(Gandhi & Weete, 1991)	(Stinson et al.,, 1991)	(Galanina & Konova, 2000)	(Ghasemi, et al., 2012)	(Russo et al., 2023)	(Cantrell & Walker, 2009)	(Athalye, et al., 2009)	(Roe & Wynn, 2015)	(Bhatia, et al., 1972)	(O'Brien, et al., 1993)	(Singh & Ward, 1998)	mean	STDEV
14:0	1.3	9.3	9.2	9.7	0.0	6.9	11.1	7.8	6.9	6.7	6.8	6.6	6.9	3.25
16:0	12.6	12.0	16.6	18.8	26.5	19.1	23.8	25.3	0.0	30.9	13.4	18.5	18.1	8.20
16:1ω7	1.1	6.4	5.7	1.0	12.0	5.8	6.7	13.6	31.1	11.5	2.7	0.0	8.1	8.51
18:0	4.7	2.0	1.1	2.5	4.3	9.7	5.1	3.2	0.0	8.9	1.8	2.0	3.8	2.98
18:1ω9	20.6	33.0	15.8	20.4	15.3	22.9	22.5	16.7	27.6	20.1	9.3	36.3	21.7	7.61
18:2ω6	42.2	13.2	14.0	19.1	17.1	9.9	10.7	18.4	10.4	7.0	15.6	13.6	15.9	9.04
18:3ω6	0.2	6.5	0.9	2.4	3.6	0.0	1.0	0.0	0.0	1.6	0.0	0.1	1.4	1.98
18:3ω3	6.9	0.8	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.6	1.98
20:0	0.0	0.0	0.4	0.0	0.0	0.0	0.7	0.0	0.0	7.2	0.0	0.0	0.7	2.06
20:1ω9	0.0	0.0	3.1	0.0	2.2	0.0	1.3	0.0	0.0	4.6	5.2	0.0	1.4	1.95
20: ω6	0.0	1.3	1.3	0.0	2.0	3.8	0.0	0.0	0.0	0.0	0.0	0.0	0.7	1.20
20:4ω6	3.5	3.3	7.1	13.5	9.2	2.1	5.3	6.8	3.3	0.0	10.4	7.2	6.0	3.83
20:5ω3	3.2	11.0	12.3	10.7	7.8	6.9	10.9	12.6	6.4	0.0	25.2	2.1	9.1	6.55

Athalye, S. K., Garcia, R. A., & Wen, Z. (2009). Use of biodiesel-derived crude glycerol for producing eicosapentaenoic acid (EPA) by the fungus *Pythium irregulare*. *Journal of Agricultural and Food Chemistry, 57*(7), 2739-2744.

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Cantrell, K. B., & Walker, T. H. (2009). Influence of temperature on growth and peak oil biosynthesis in a carbon-limited medium by *Pythium irregulare*. *Journal of the American Oil Chemists' Society, 86*, 791-797.

Galanina, L., & Konova, I. (2000). Effect of exogenous sterols on the growth and fatty acid composition of the oomycete *Pythium debaryanum*. *Microbiology, 69*, 530-535.

Gandhi, S., & Weete, J. (1991). Production of the polyunsaturated fatty acids arachidonic acid and eicosapentaenoic acid by the fungus *Pythium ultimum*. *Microbiology, 137*(8), 1825-1830.

Ghasemi, M., Atakishiyeva, Y., & Asadi, A. (2012). The changes in lipid composition of *Pythium irregulare* LX oomycetes at a stressful situation created with crude oil. *Molecular Biology Research Communications, 1*(1), 33-38.

Lio, J., & Wang, T. (2013). *Pythium irregulare* fermentation to produce arachidonic acid (ARA) and eicosapentaenoic acid (EPA) using soybean processing co-products as substrates. *Applied Biochemistry and Biotechnology, 169*, 595-611.

O'Brien, D. J., Kurantz, M. J., & Kwoczak, R. (1993). Production of eicosapentaenoic acid by the filamentous fungus *Pythium irregulare*. *Applied Microbiology and Biotechnology, 40*, 211-214.

Roe, C. L., & Wynn, J. P. (2015). Production of omega-3 fatty acids from *Pythium* species. *Canadian Patent* No. 2 904 038.

Russo, G. L., Langellotti, A. L., Martín-García, B., Verardo, V., Romano, R., Sacchi, R., & Masi, P. (2023). New biotechnological production of EPA by *Pythium irregulare* using alternative sustainable media obtained from food industry by-products and waste. *Sustainability, 15*(2), 1147.

Singh, A., & Ward, O. (1998). Docosapentaenoic acid (C22:5, ω-3) production by *Pythium acanthicum*. *Journal of Industrial Microbiology and Biotechnology, 20*(3-4), 187-191.

Stinson, E. E., Kwoczak, R., & Kurantz, M. J. (1991). Effect of cultural conditions on production of eicosapentaenoic acid by *Pythium irregulare*. *Journal of Industrial Microbiology, 8*, 171-178.

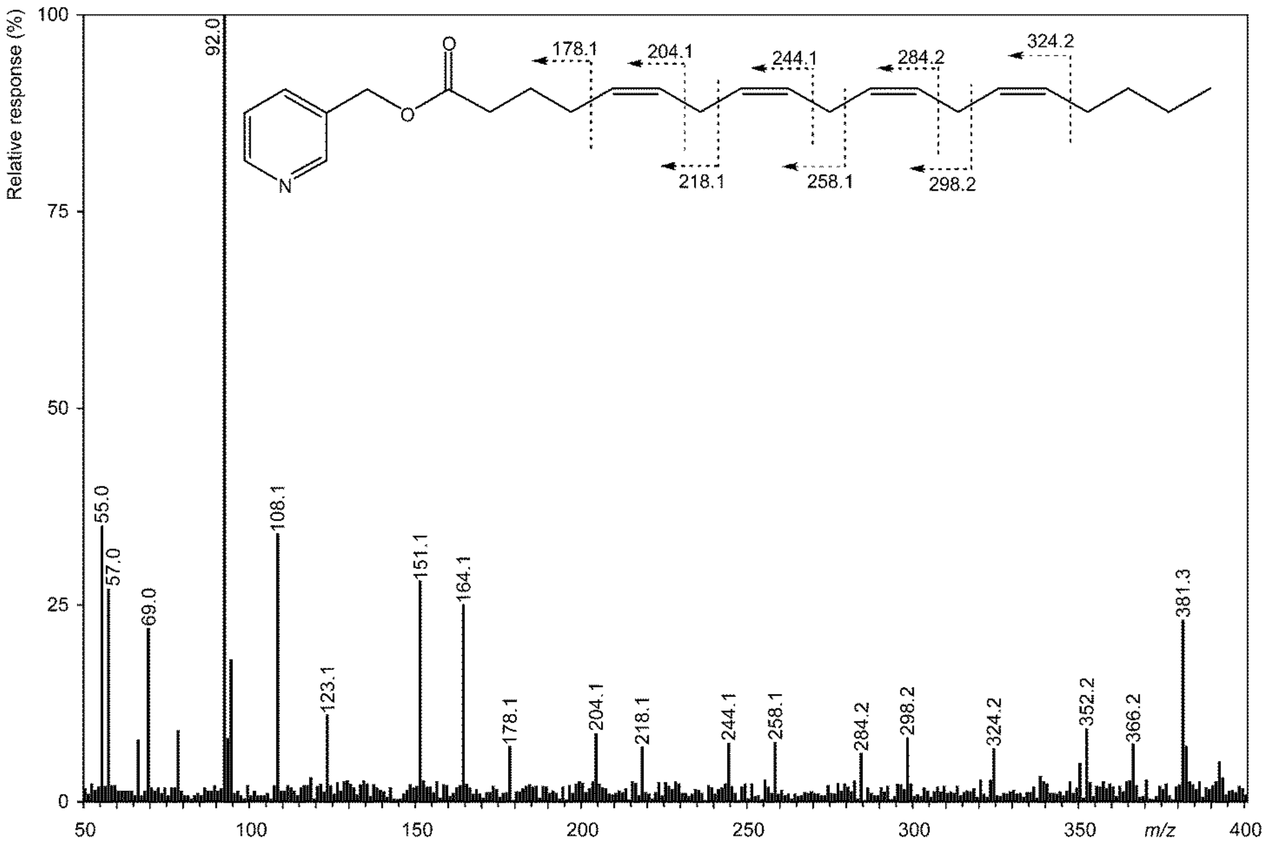


Fig. 1S. The mass spectrum of the 3-pyridyl carbonyl ester of 5Z,8Z,11Z,14Z-nonadecatetraenoic acid (19:4ω5).

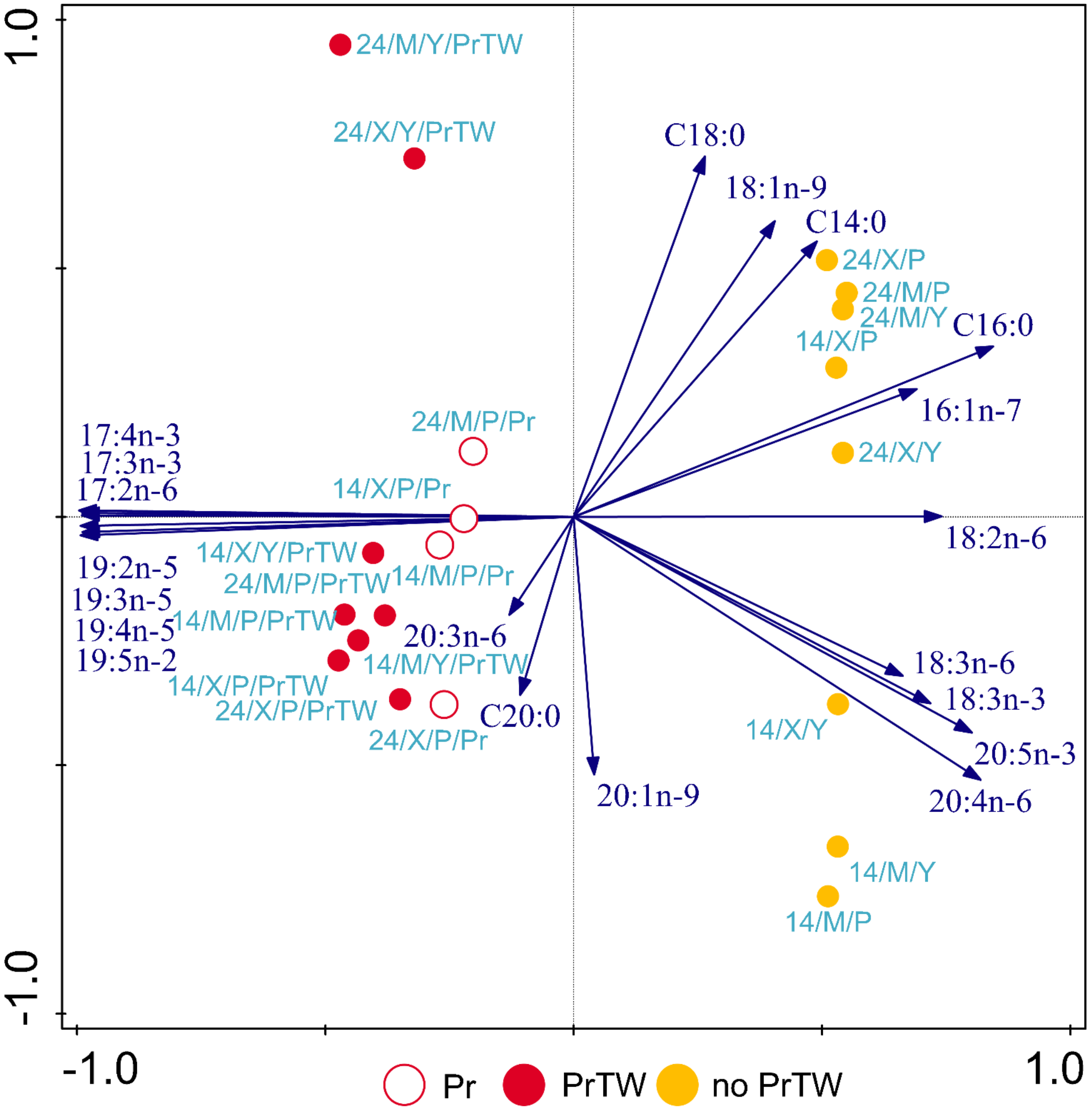


Fig. 2S. Principal component analysis (PCA) of the FA composition in the two strains cultivated in different media and temperature. The first two canonical axes are plotted; the first axis explained 57.0% and the second 12.6% of the variability. Sample codes indicate cultivation temperature (14°C or 24°C), strain identification (X42 or M1) and media composition (P- potato dextrose broth, Y – waste brewery yeast, Pr – propionic acid, TW – TWEEN 80), see experimental section.

Method: PCA, The total variation is 400.00000

Summary Table:

Statistic Axis 1 Axis 2 Axis 3 Axis 4;

Eigenvalues 0.5701 0.1260 0.0902 0.0641;

Explained variation (cumulative) 57.01 69.61 78.63 85.04.

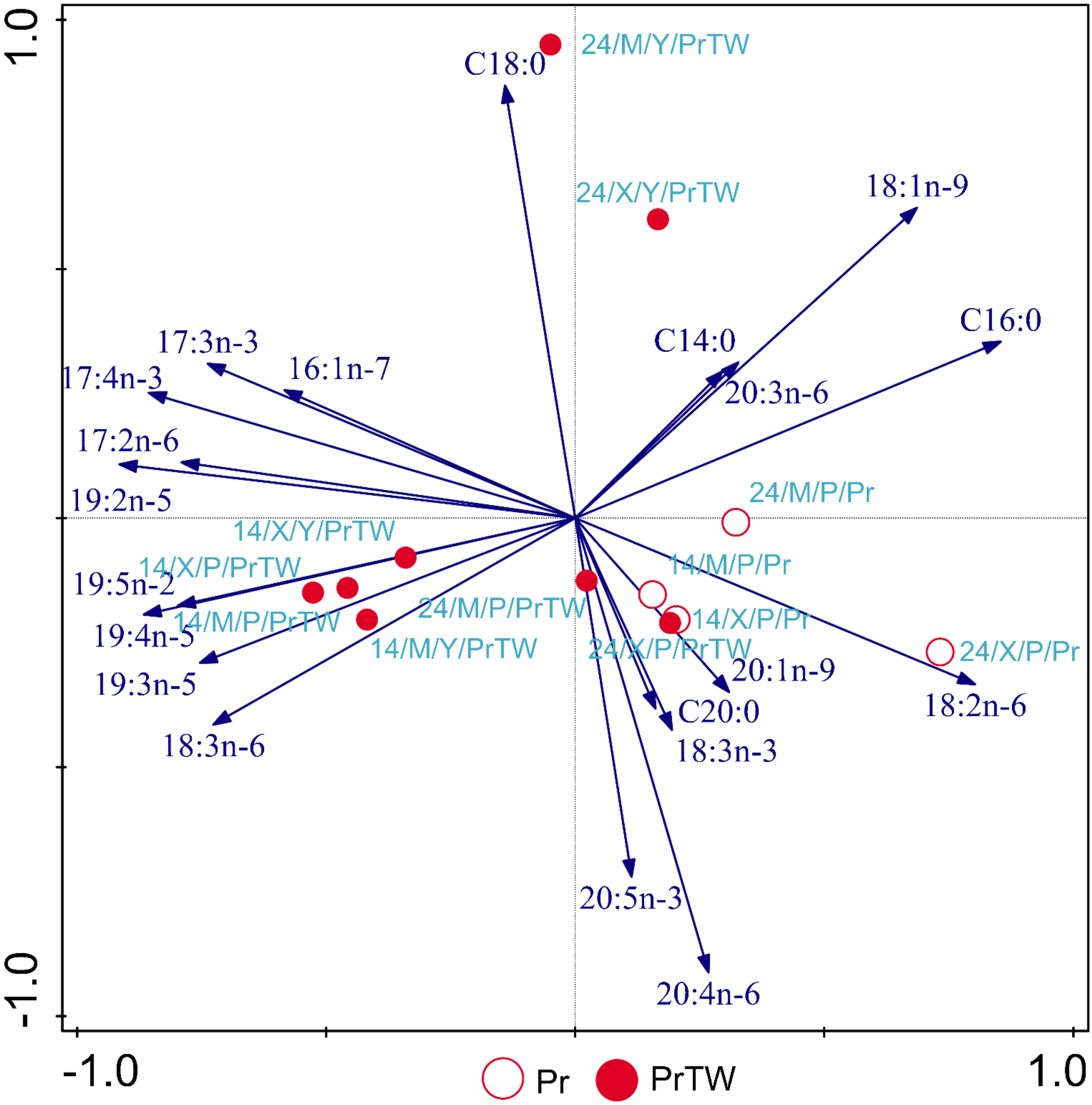
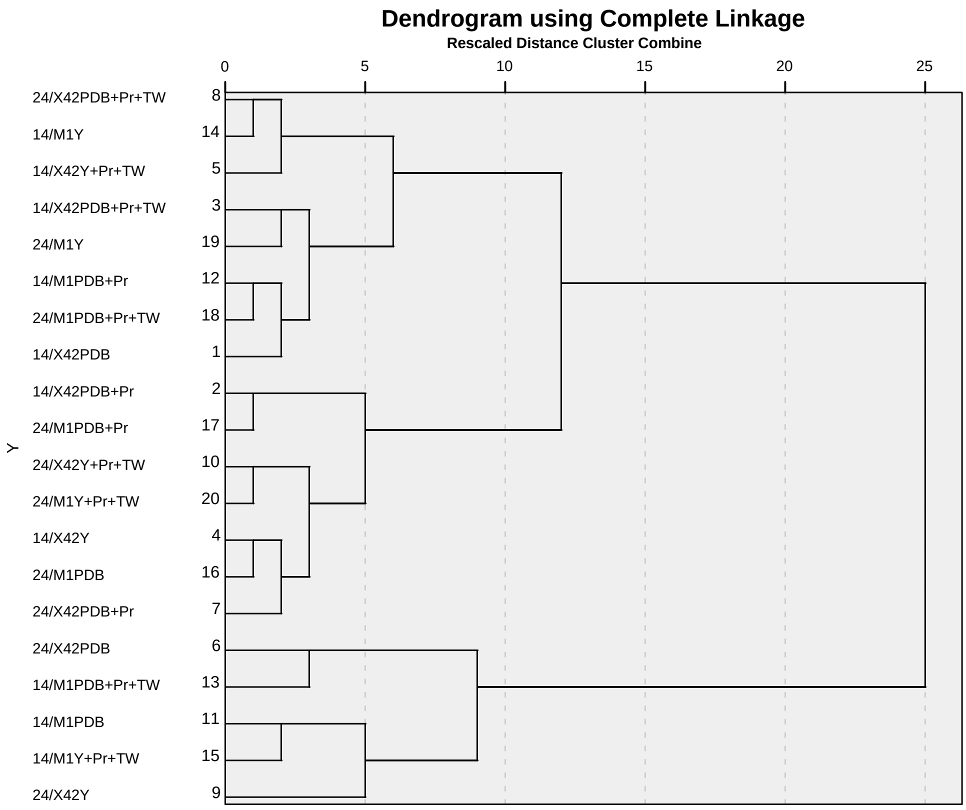


Fig. 3S. Principal component analysis (PCA) of the FA composition in the two strains cultivated in different media and temperature. Only the samples with the addition of propionic acid (Pr) and TWEEN 80 (Pr+TW) are shown. The first two canonical axes are plotted; the first axis explained 39.3% and the second 14.6% of the variability

Analysis 'Analysis 2'
Method: PCA
The total variation is 240.00000
Abbreviations, see Fig. 2S
Summary Table:
Statistic Axis 1 Axis 2 Axis 3 Axis 4
Eigenvalues 0.3934 0.1968 0.1464 0.0931
Explained variation (cumulative) 39.34 59.02 73.66 82.97


Dendrogram using Complete Linkage
Rescaled Distance Cluster Combine
0
5
10
15
20
25
Y
24/X42PDB+Pr+TW 8
14/M1Y 14
14/X42Y+Pr+TW 5
14/X42PDB+Pr+TW 3
24/M1Y 19
14/M1PDB+Pr 12
24/M1PDB+Pr+TW 18
14/X42PDB 1
14/X42PDB+Pr 2
24/M1PDB+Pr 17
24/X42Y+Pr+TW 10
24/M1Y+Pr+TW 20
14/X42Y 4
24/M1PDB 16
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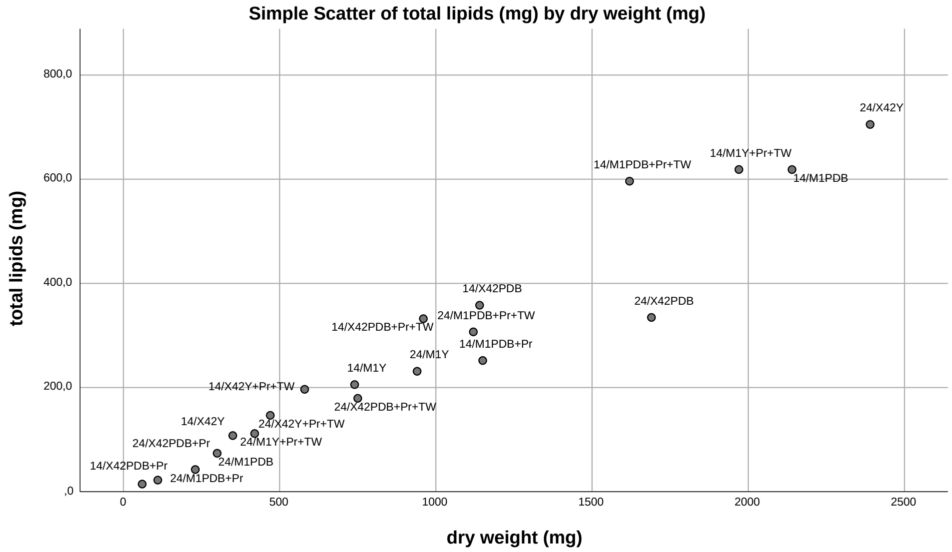
24/X42Y 9


Simple Scatter of total lipids (mg) by dry weight (mg)
total lipids (mg)
dry weight (mg)
800,0
600,0
400,0
200,0
,0
0
500
1000
1500
2000
2500
24/X42Y
14/M1Y+Pr+TW
14/M1PDB+Pr+TW
14/M1PDB
14/X42PDB
24/X42PDB
24/M1PDB+Pr+TW
14/X42PDB+Pr+TW
14/M1PDB+Pr
24/M1Y
14/M1Y
14/X42Y+Pr+TW
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14/X42Y
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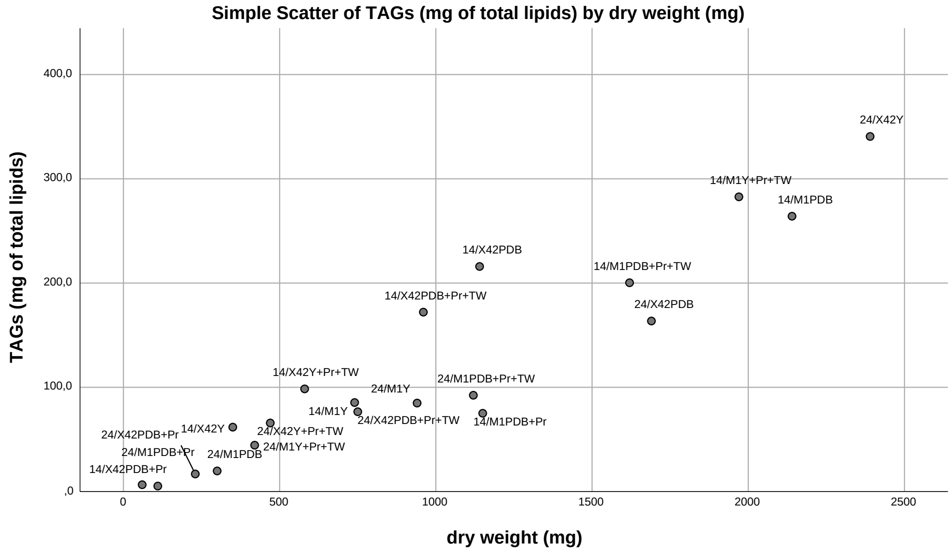
24/M1PDB+Pr



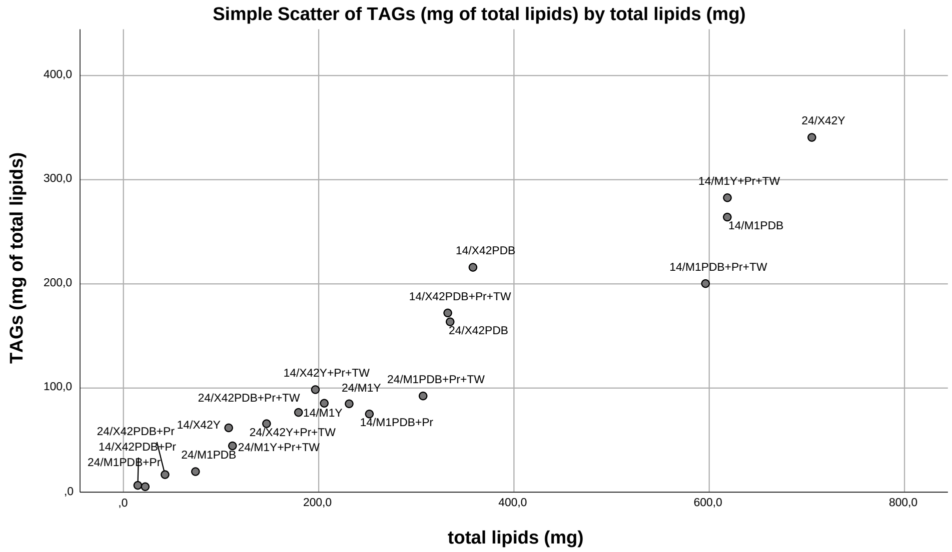


Fig. 4S. Dendrogram using the complete linkage of *Pythium* and different statistical methods.