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Thermochemolysis–GC-MS as a tool for chemotaxonomy and predation monitoring of a predatory actinobacteria against a multidrug resistant bacteria

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Abstract

Thermochemolysis-GC-MS was used to characterize Methicillin resistant *Staphylococcus aureus* (MRSA) and a predatory actinobacteria isolated from Moroccan marine water through their membrane fatty acids released as methyl esters (FAMEs).

FAMEs from MRSA were dominated by branched *iso* and *anteiso* C_{15}, straight C_{18} and C_{20} whereas the predatory actinobacteria was dominated by branched C_{14}, *iso* and *anteiso* C_{15}, and straight C_{16}. The *iso* to *anteiso* C_{15} ratio was 0.44 for MRSA and 0.9 for the actinomycete. A co-culture of actinomycetes and bacteria was conducted during 15 days. An increase in the

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iso to *anteiso* ratio, a decrease in C_{18} and C_{20} FAMEs and an increase in branched C_{14} and C_{16} FAMEs demonstrated the predation of MRSA by the actinomycete.

The total amount of FAMEs which was significantly higher in the studied actinobacteria (83 mg/g) compared to MRSA (17 mg/g) increased during the co-culture demonstrating the predation.

Principal Component (PCA) and Hierarchical Cluster Analysis (HCA) were used to correlate fatty acids (FA) distributions with the strains. HCA allowed to discriminate between MRSA and the actinobacteria. With PCA, long chained (>C_{18}) FAMEs correlated with MRSA whereas short branched-chained FAMEs correlated with the actinobacteria and T15. Consequently, a clear distinction in the chemotaxonomy of MRSA and actinobacteria was established by PCA. Additionally, PCA highlighted the predation of MRSA by the actinobacteria through dissimilarity between MRSA and T15 and similarity between actinobacteria and T15.

Keywords:

Predatory actinobacteria, multidrug resistant bacteria, co-culture, Thermochemolysis-GC-MS, fatty acid methyl esters

1. Introduction

For decades, one of the most worrying health problems is the development and spread of antibiotic resistance [1]. Multidrug resistant bacteria are becoming more and more prevalent in hospitals faster than the discovery of new drugs [2]. Therefore, it is urgent to develop a promising strategy to combat multidrug-resistant bacteria. Most of the natural antibiotics are produced from antagonistic actinobacteria through antibiosis interaction [3,4], but it is not the only pathway. According to new researches, predatory bacteria can also kill multidrug-resistant bacteria [5,6]. For instance, numerous studies have demonstrated that

diverse bacteria are known to be predators of other bacteria, such as *Ensifer adhaerens* [7–9], *Cupriavidus necator* [10,11], *Lysobacter sp* [12,13], *Bdellovibrionales (d-BALOs)*, *Myxobacteria* [14,15]. In contrast, few studies have taken a cursory look at the predation by actinobacteria with the only genus *Streptomyces* who expressed this ability [16–18]. Moreover, no study to date has examined actinobacteria predation against multidrug resistant bacteria.

Assessment of microbial biomass in co-culture can be evaluated by several methods including dilution plating and CFU enumeration, ATP assay, measurement of the enzymatic activity, or physiological methods [5,19]. However, the use of biomarkers to monitor bacterial changes in a community is necessary, because it provides an effective and quantitative way to measure their evolution [20]. In bacterial cells, fatty acids are one of the most essential components of cellular membranes [21]. Fatty acids distribution can be different from a strain to another [22,23]. Indeed, fatty acids which are constituent of the cytoplasmic membrane could be used as biomarkers to identify bacteria [19]. These biomarkers could also been employed to estimate the microbial community structure and metabolic activity in the environment [20,24]. Besides, successive changes in micro-organisms within co-culture can be monitored using methylated fatty acids (FAMEs) to confirm the predation of Methicillin-resistant *Staphylococcus aureus* (MRSA) by the predatory actinobacteria. Indeed fatty acids analysis has been found to be a useful technique for the rapid identification of microbial communities in various ecosystems [19,24,25]. Pyrolysis with in situ methylation also called thermochemolysis [26,27] coupled with gas chromatography and mass spectrometry is an efficient and reliable method for the determination of fatty acid profiles in natural environment [28]. However the data obtained by this method can be difficult to correlate and advanced statistics developed to discriminate large data set and to extract utile information [29]. Hence, broad group of multivariate

analyses were introduced like Principal Component Analysis (PCA) and Hierarchical cluster analysis (HCA) [30]. These two methods consider the correlation between different variables, which are analyzed simultaneously, and data are grouping according to a set of specified characteristics [31,32]. The use of PCA and HCA provide easier interpretation and discussion [32]. Additionally, a valid tool to distinguish between bacteria and to observe differences in the structure of the bacterial communities in biological process [19,20].

In the present paper, we aimed at demonstrating that thermochemolysis with tetramethyl ammonium hydroxide (TMAH) as methylating agent, coupled with gas chromatography and mass spectrometry can be used for chemotaxonomy of bacteria. In a second time actinobacteria isolated from Moroccan marine water were tested in co-culture with multidrug-resistant bacteria. We then aimed at demonstrating that it can be used to evaluate the ability of a predatory actinobacteria to prey on methicillin resistant *Staphylococcus aureus* (MRSA). Although, we evaluated the use of Principal Component and Hierachical Clustering Analysis to confirm the filiation observed above between FAMEs and strains and confirmed the predation after 8 and 15 days.

2. MATERIALS AND METHODS

2.1. Chemotaxonomy

The analysis of experimental design elements was first conducted to generate a database from which the FAMEs biomarkers were selected. Then, FAMEs profiles from pure cultures of MRSA and pure cultures of a predatory actinobacteria, were analyzed to monitor the predicted presence of biomarkers. Finally, the selected biomarkers were used to study the chemotaxonomy and to monitor their fate amongst predation activity against multidrug- resistant bacteria (RMSA) during co-culture.

2.2. Co-culture

2.2.1. Preparation of the prey species and predatory actinobacteria

A single colony of *Staphylococcus aureus* (MRSA) NCTC 12493 and predatory actinobacteria was used as an inoculums of 500 ml erlenmeyer flasks containing 100 mL of Nutrient broth for *Staphylococcus aureus* (MRSA) NCTC 12493, Bennett medium for actinobacteria, incubated respectively for 24 hours at 37°C and for 15 days at 28 °C, on rotary shaker set at 200 rev min^{-1}. Prey and predatory cells were harvested by centrifugation and washed three times in sterile saline water. The final cell suspension represented a concentration of 10^{12} CFU/ml.

2.2.2. Preparation of co-culture

The inoculum of co-culture was prepared by 100ml of washed predatory actinobacteria cells with a concentration of 10^{12} CFU/ml and 100ml of washed prey cells of methicillin-resistant *Staphylococcus Aureus* with a concentration of 10^{12} CFU/ml, the final volume of each Erlenmeyer was 200ml. Co-culture trials were conducted for 2 weeks at 28 °C under constant shaking. Samples were collected at the beginning of the experiment (T0), after 8 days (T8) and after 15 days (T15). The samples were centrifuged at 4000 rpm for 10 min at 10°C, biomasses were collected and lyophilized.

2.3. Thermochemolysis-GC-MS

Multidrug resistant bacteria strains (MRSA), actinobacteria and coculture (T0, T8 and T15) were analyzed by analytical pyrolysis with in situ methylation. The pyrolyzer was a Frontier Lab EGA 2020 pyrolyzer equipped with an AS-1020E auto-shot sampler coupled with GC-MS (Shimadzu QP 2010 Ultra). GC separations were done using a SLB-5MS (Supelco) capillary column (30 m long, 0.25 mm i.d, 0.25 µm phase thickness). The injector temperature was set to 250 °C. The column temperature was programmed from 50 °C to 300 °C with a rate of 5 °C.min-1 and held at 300 °C for 9 min. The ionization mode was electron

impact (70 eV) and the source temperature was 220°C. The acquisition was realised in full scan mode from 50 to 600 uma at 0.2 uma/sec

Thermochemolysis was realised in the isothermal mode during 1 min. at 600 °C on the sample moistened with a TMAH 50/50 v/v solution in methanol used as alkylating agent. 0.5 mg of sample were mixed with 5 µL of TMAH methanolic solution and placed in an inox cup and the sample is placed in the autosampler.

2.4. Quantification

The quantification was conducted as described by Collard et al [33]. Hexadecanoic acid (purchased from Sigma Aldrich) was used as calibration standard. 2 µL of a solution of hexadecanoic acid in dichloromethane (10mg/ml) were mixed with 2 µL of a TMAH methanolic solution (50/50 v/v) and placed in an inox cup. The solvent was evaporated and the inox cup placed into the pyrolyzer.

The amount (in mg) of a given compound was calculated using the equation of the calibration curve : [standard in µg] = ([standard area]-b)/a where a is the slope and b is the intercept of the calibration curve. The linear regression was y=3171329x-2709871. All the samples and standards were pyrolyzed in triplicates. The amount of each FAME (mg/g) expressed relatively to the mass of the pyrolyzed sample and the standard deviations relative to the triplicates are presented in table 2.

2.5. Statistical analysis

In order to confirm the result of our experiment statistically, we studied the variation of quantity (mg/g) of every FAMEs inside the groups (actinobacteria, *Staphylococcus aureus* (MRSA), T0, T8 and T15) and to highlight the specific FAMEs biomarkers of each group, we applied a multivariate analysis (PCA and the hierarchical cluster classification method). PCA was applied to the correlation matrix between FAMEs

variables. The experiments were repeated three times with each independent assay. The statistical treatments were carried out using Xlstat.

3. Results and discussion

3.1. Chemotaxonomy of Methicillin-resistant Staphylococcus aureus (MRSA) and predatory actinobacteria

Methicillin-resistant *Staphylococcus aureus* (MRSA) and a predatory actinobacteria were analysed using pyrolysis with TMAH as methylation reagent. Fatty acids constituting the cell membranes of the bacteria were thus released and detected as FAMEs. FAMEs were the main compounds detected on the pyrograms (Fig. 1 and 2). The relative percentages of each FAMEs were reported on histograms (Fig. 3 and 4) which showed distinct FAMEs profiles for methicillin-resistant *Staphylococcus aureus* (MRSA) and predatory actinobacteria. This difference in distribution of FAMEs traduces different cell wall composition and different biosynthetic capabilities [34,35]. Alan McNabb et al. [36] suggested that bacteria can be separated into two groups. Membrane cell of type I bacteria mainly contain branched-chain whereas type II composition is dominated by saturated or monounsaturated straight-chain fatty acids.

In our study, methicillin-resistant *Staphylococcus aureus* (MRSA) and predatory actinobacteria exhibited respectively 76% and 70 % of branched chain FAMEs (Fig. 3 and 4). Thus, both of the two studied bacteria belong to type I according to McNabb's classification. Branched-chain FAMEs such as *iso*- and *anteiso*- C_{15}, and C_{17} and branched C_{16} are the main compounds of Gram-positive bacteria [24,37,38] whereas straight chained FAMEs are predominant in Gram-negative bacteria.

FAMEs identified for Methicillin-resistant *Staphylococcus aureus* (MRSA) were ranging from C_{12} to C_{21}. Branched *iso* and *anteiso* C_{15} are the major compounds (57.2 % of

the identified FAMEs) which is consistent with literature [39]. The ratio *iso* to *anteiso* is 0.44. However, FAMEs observed for predatory actinobacteria, are dominated by a short mode in the C_{12} to C_{18} range with branched C_{16} as the major compound (26.2%). Moreover, the *iso* to *anteiso* C_{15} ratio is 0.9 which is twice the value observed for MRSA. Branched C_{14} FAME is also one of the main compounds (9.6%) whereas it was minor (4.2%) in MRSA. The distribution observed for the studied actinobacteria is consistent with previous reports [19,36,40–42]. Another feature of MRSA compared to predatory actinobacteria is the presence of long chained FAMEs (C_{19} to C_{21}) which are not detected in the studied predatory actinobacteria. In addition, C_{18} FAME is more abundant (6.6%) in MRSA than in the predatory actinobacteria. (0.8%). At the contrary C_{15}, $C_{16:1}$ and $C_{17:1}$ FAMEs were detected in the predatory actinobacteria but were present as traces in MRSA.

3.2. Predation monitoring

The differences in the FAMEs distributions for the two studied bacteria were used for evaluating the ability of actinobacteria to prey on methicillin resistant *Staphylococcus aureus* (MRSA).

The changes in the distributions of FAMEs during the co-culture after 0, 8 and 15 days were reported in Fig. 5. At the beginning of our experiment (T0), FAMEs strongly resembled the signature of the predatory actinobacteria (Fig.4) with a high amount of short chained branched fatty acids (C_{14}, C_{15}, C_{16}). The *iso* to *anteiso* C_{15} ratio which has been demonstrated (3.1) to be two time more elevated for the actinobacteria than for MRSA increased during the process going from 0.75 for T0 to 0.96 for T15 (Table 1). The latest value was close to those observed for the actinomycete. The branched C_{14} and branched C16 fatty acids which have been shown to be predominant for the actinomycete and minor for MRSA (3.1) increased both from T0 to T15. At the contrary the straight chained FAMEs C_{18} and C_{20} which are abundant for MRSA decreased from T0 to T15.

Finally, at the end of the experiment after two weeks, the distribution of FAMEs ranged from C_{12} to C_{18} and was dominated by the same fatty acids observed at T0 and T8. Therefore, the C_{20} FAME that have disappeared at T15 has been identified above as specific of *Staphylococcus aureus* (MRSA) (see section chemotaxonomy).

Changes in FAMEs distribution during the co-culture were especially marked by an increase in chemotaxonomic FAMEs indicator of predatory actinobacteria, parallel to a decrease in the chemotaxonomic FAMEs indicator of the prey *Staphylococcus aureus* (MRSA). This evolution confirmed the predation activity.

3.3. Quantification and statistical data analysis approach

3.3.1. Quantification

Semi-quantitative measurements based on normalization (concentration = compound area/ total areas) give rise to misinterpretations because the different classes of compounds have very different response factors in mass spectrometry [33]. For this purpose, standardization is required to conduct a statistical analysis of FAMEs. In our study, we used hexadecanoic acid as calibration standard [33] to quantify FAMEs (mg/g) (Table 2). The amount of FAMEs is significantly higher in the predatory actinobacteria (83.1 mg/g) than in MRSA (17.2 mg/g). This is consistent with previous studies what have shown that actinobacteria are an important source of Fatty acids [34]. A promising finding was the evolution of the quantity of FAMEs during the co-culture, which rose from 43.9 mg/g at T0 to 73.8 mg/g after 15 days of co-culture. This result highlights the replacement of the prey (MRSA) population by the predatory actinobacteria.

3.3.2. Statistical analysis

PCA was used in order to reduce our large data set of interrelated variables to a small set that still contains most of the information from the initial data set. The variables were FAMEs (24 in total) present in MRSA, actinobacteria and co-culture (T0, T8 and T15).

In addition, the principal component was used to relate FAMEs with their appropriate bacteria and co-culture, also to provide an overview of similarities and differences among bacteria and co-culture.

In Fig.6 the first eigenvalue equals 24.78 and represents 90.77% of the total variability. This means that if we represent the data on only one axis, we will still be able to see 90.77% of the total data's variability. The second eigenvalue equals 1.84 and represents 7.77% of the total variability and 98.48% for the cumulative variability. These two eigenvalues are significant because they are higher than 1. As a consequence, the first two eigenvalues will correspond to 98.48% of the variance, ensuring that the maps based on the first two factors are a good quality projection of our initial data. As a result, the application of PCA reduced the initial number of eigenvalues who was constituted by N= 24 variables (FAMEs) to a lower number of dimensions (N=2) (Fig.6) allowing to an easier interpretation.

The results of the PCA run between the FAMEs profiles of bacteria and during co-culture are reported in Fig.7.

The first two factors (F1 and F2) accounted for 98.48% of the total inertia of data cloud; this means that 98% of the total variability of FAMEs profiles is represented in this plan, which is an extremely high percentage. The first component, (PC_1) explains 90.78% and the second component (PC_2) explains 7.70% of the variability between domains of FAMEs profiles for bacteria and during co-culture. Domain I gather (FAMEs MRSA indicator) iC_{19}, aC_{19}, C_{19}, C_{20}, C_{21} which are negatively and positively and influenced by PC_1 and PC_2, respectively. It coincides with MRSA. The domain II gathers only a single variable for non-specific bacteria which is C_{18}, is apart and presented negative influence by both PC_1 and PC_2. It coincides with T0 and T8. The last domain III gathers (FAMEs predatory actinobacteria indicator) C_{15}, $C_{16:1}$ and C_{17} which are positively correlated with PC_1 and

yielded small negative correlation with PC_2 except C16:1which is positively correlated with both PC_1 and PC_2. Domain III includes also (non specific bacterial FAMEs brC_{12}, C_{13}, brC_{14}, C_{14}, iC_{15}, brC_{16}, $C_{16:1}$, C_{16}, iC_{17}, aC_{17}, $C_{17:1}$ and brC_{18} which are positively correlated with both PC_1 and PC_2, except brC_{16} and C_{16} who yielded a small negative correlation with PC_2. It coincides with predatory actinobacteria and T15.

A clear distinction in the chemotaxonomy of MRSA and actinobacteria was thus confirmed and obtained by PCA. Consequently, PCA is a valid tool to facilitate the complexity in large data while maintaining the current trends and models. Additionally, PCA provided an overview of dissimilarity between MRSA and the co-culture at T15, similarity between Actinobacteria and the co-culture at T15, which highlighted the predation of MRSA by the actinobacteria. Therefore, PCA is a useful method to observe differences in the structure of the bacterial communities present in a co-culture that will help to monitor the predation process.

These approaches for FAMEs were also confirmed by the method of hierarchical cluster analysis (HCA) of the observations and the variables (Fig.8) who were separated into three clusters. Moreover, HCA of FAMEs from predatory actinobacteria confirmed the strong correlation with the co-culture at the end of the experiment (T15).

4. Conclusion

Thermochemolysis-GC-MS was used to characterize methicillin resistant staphylococcus aureus (MRSA) and a predatory actinobacteria isolated from Moroccan marine water. The total amounts and distributions of fatty acids released by the pyrolytic method and detected as methyl esters (FAMEs) were different for bacteria and actinobacteria thus allowing chemotaxonomy. The predation activity of the actinobacteria towards MRSA was

demonstrated by an increase in the total amount of FAMEs and by a dramatic change in their distribution that after 15 days of co-culture.

The post-treatment of thermochemolysis data using a statistical approach by Principal Component Analysis (PCA) and Hierarchical Cluster Analysis (HCA) allowed a reliable filiation between FAMEs and each strains and confirmed the changes in FAMEs distribution traducing the predation during the co-culture.

This study demonstrated the potentiality of thermochemolysis-GC-MS to be used as a chemotaxonomic tool for bacteria identification and to monitor the fate of bacteria amongst predation activity. We also put in highlighted the interest of statistical tools to improve the interpretation of the thermochemolysis data. The ability of predatory actinobacteria to prey and reduce multidrug resistant bacteria was thus demonstrated which indicates that predatory actinobacteria constitutes a promising new strategy to combat multidrug resistant bacteria. The metabolite produced during the predation as well as molecular characterization of the predatory actinobacteria are under investigation.

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Fig.1. Pyrogram 600°C of Methicillin-resistant *Staphylococcus aureus* (MRSA)

Fig.2. Pyrogram 600°C of the predatory actinobacteria

Fig.3. Relative abundance (%) of FAMEs in Methicillin-resistant *Staphylococcus aureus* (MRSA).

Fig.4. Relative abundance (%) of FAMEs in predatory actinobacteria.

Fig.5. Relative abundance (%) of FAMEs during co-culture (T0, T8, T15).

Fig.6. Scree plot of the eigenvalues from principal components analysis

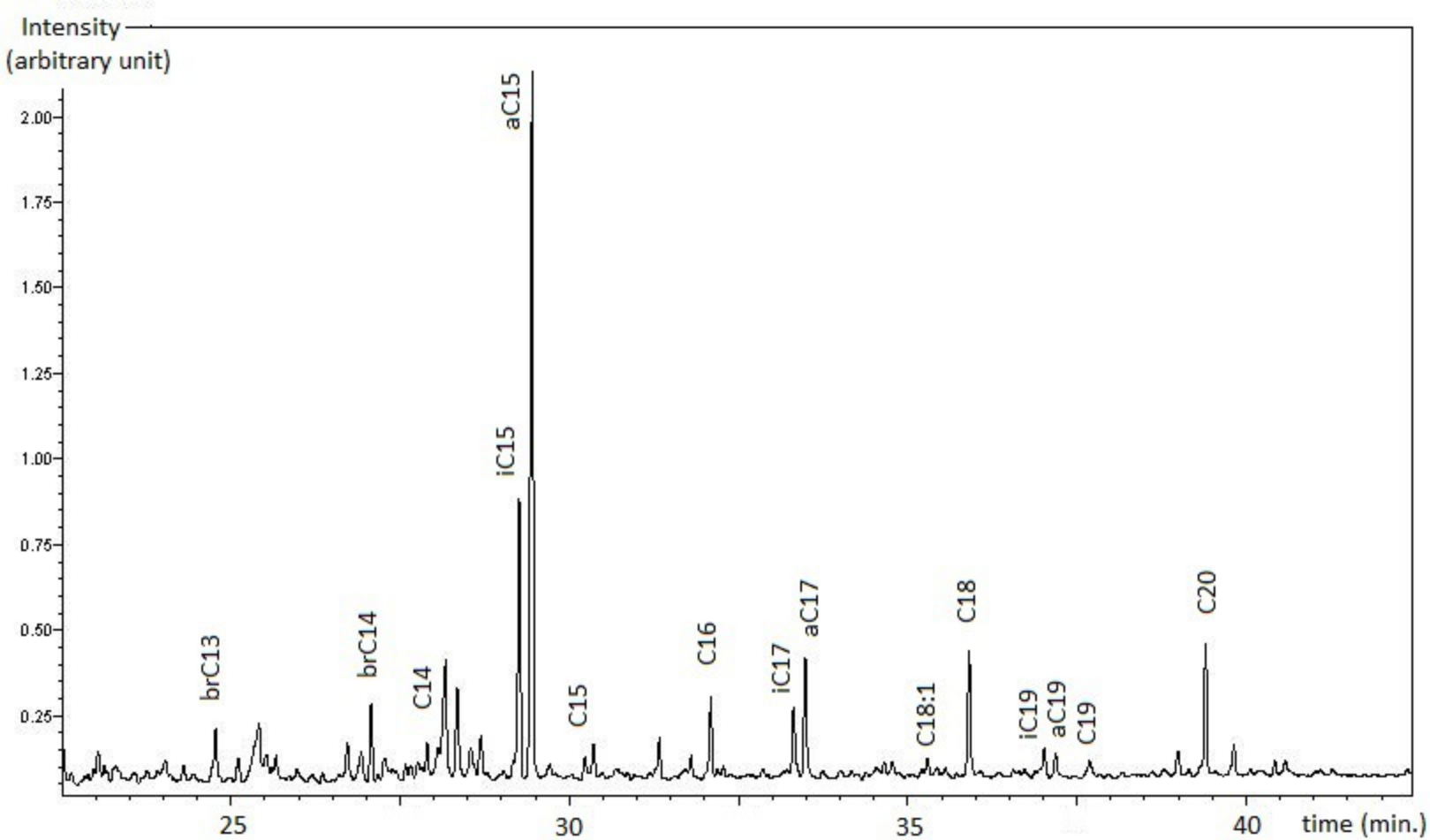
Fig.7. PCA for the normalized dataset. The first two ordination axes (PC1 and PC2) are shown for MRSA, predatory actinobacteria and co-culture (T0-T8-T15)

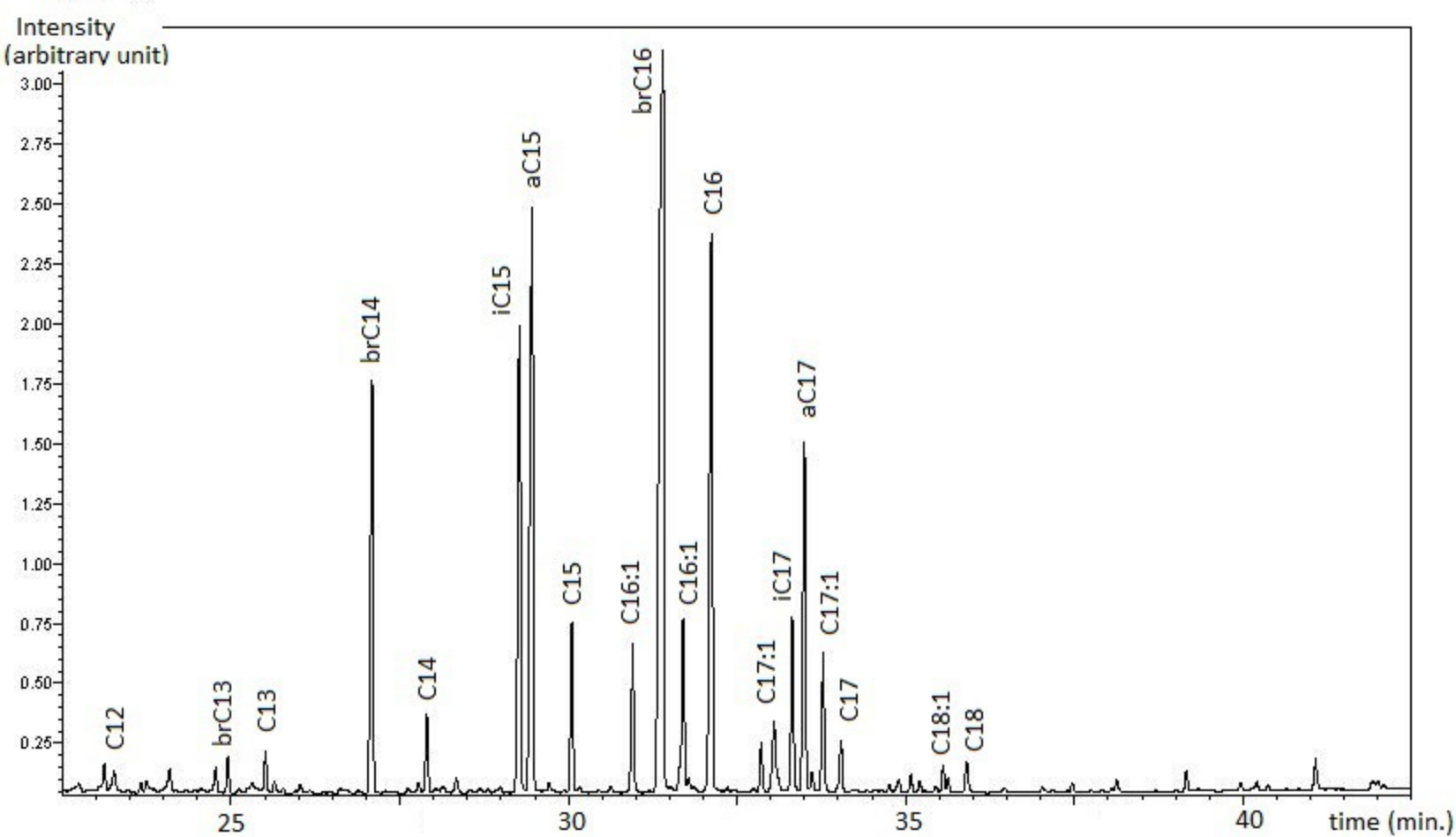
Fig.8. Hierarchical cluster analysis, showing the complete linkage dendrograms of the normalized data based on the fatty acids of MRSA, predatory actinobacteria and co-culture (T0-T8-T15)

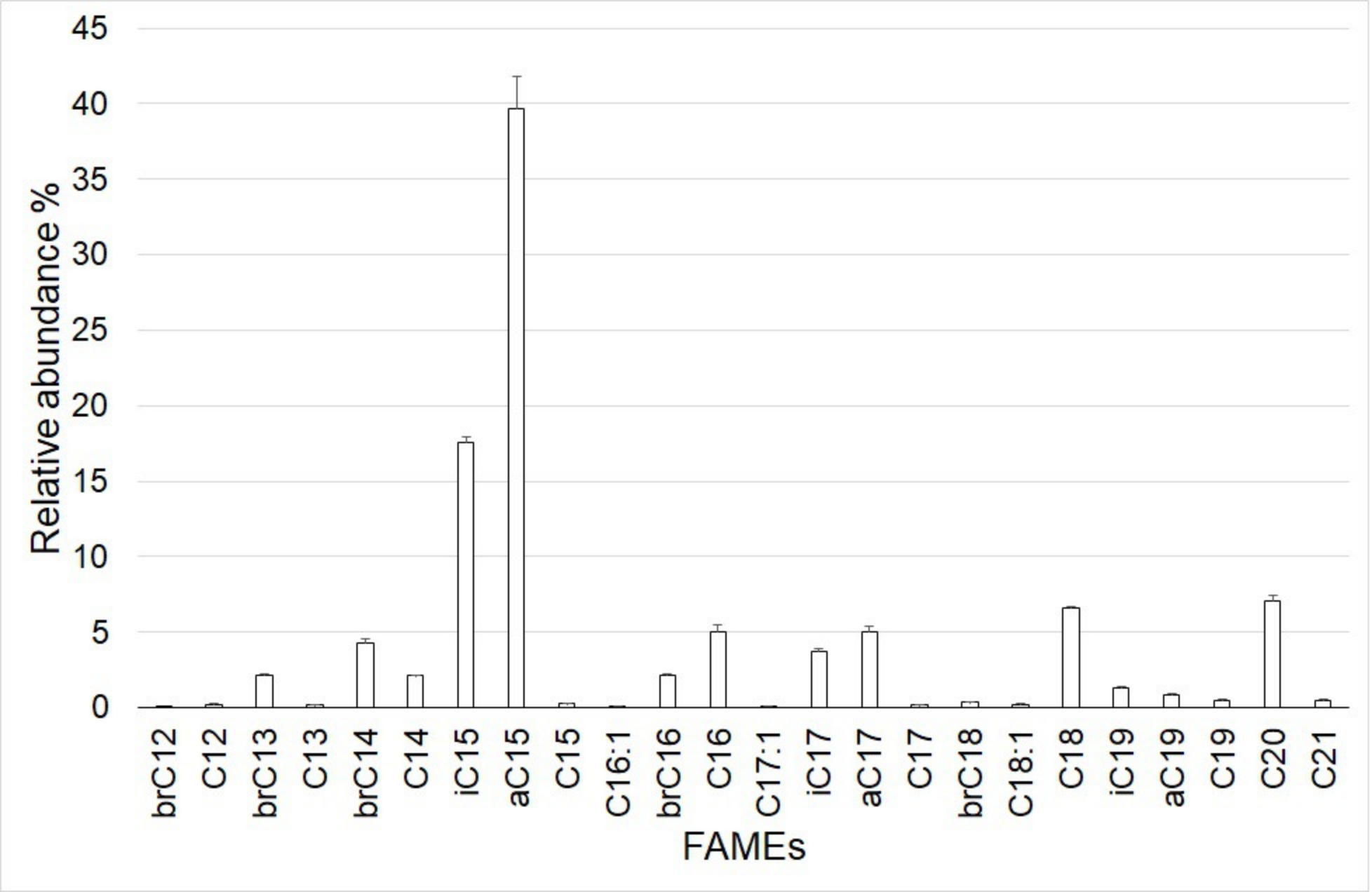
Tables caption

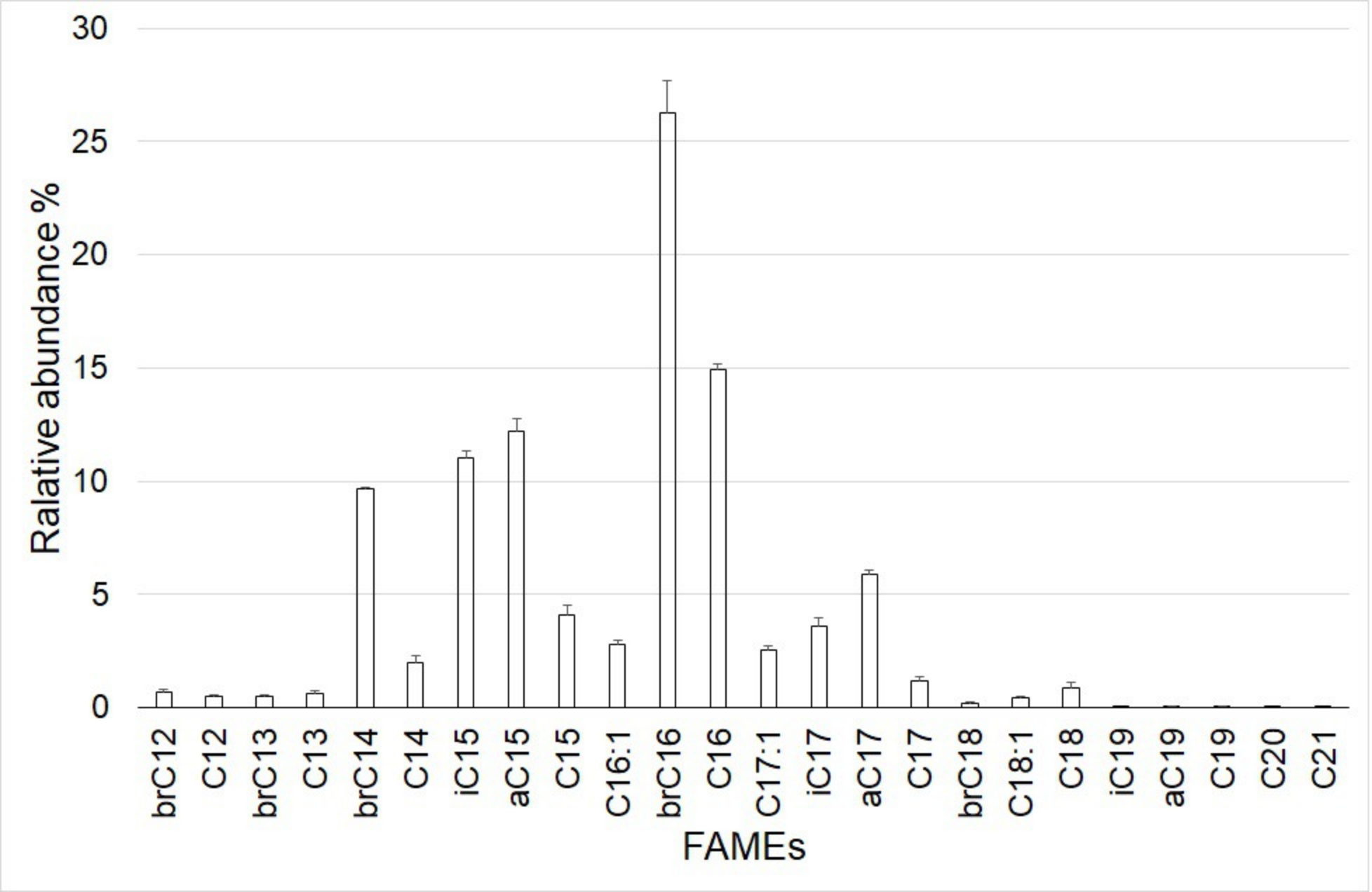
Table 1: Representative ratios **: [**branched C_{14} + branched C_{16}] to total FAMEs ; [C_{18} + C_{20}] to total FAMEs ; iso-C15 to anteiso-C_{15}

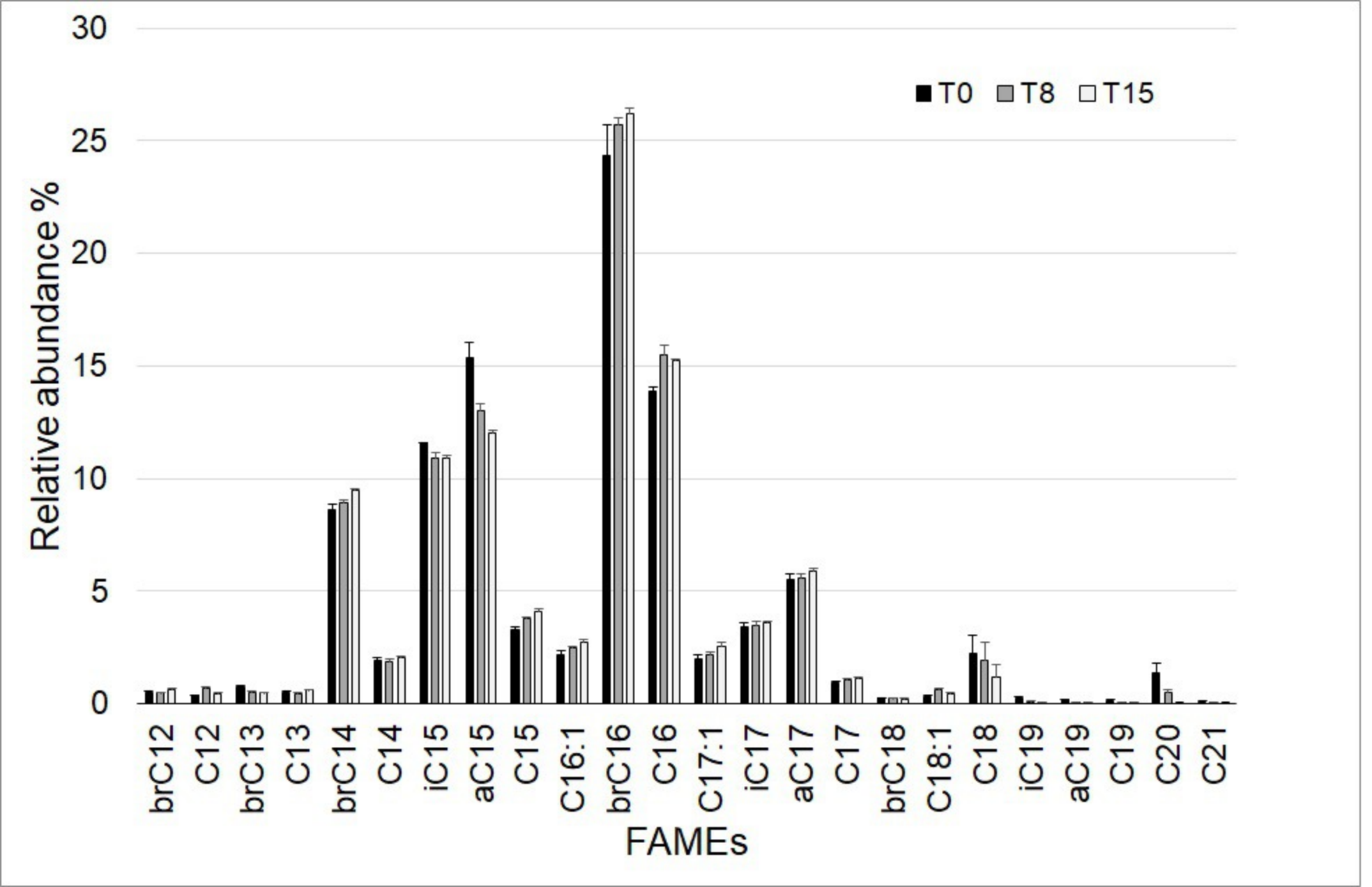
Table 2: Amount of FAMEs obtained by thermochemolysis-GCMS of methicillin-resistant s*taphylococcus aureus* (MRSA) and predatory actinobacteria during co-culture (mg/g)

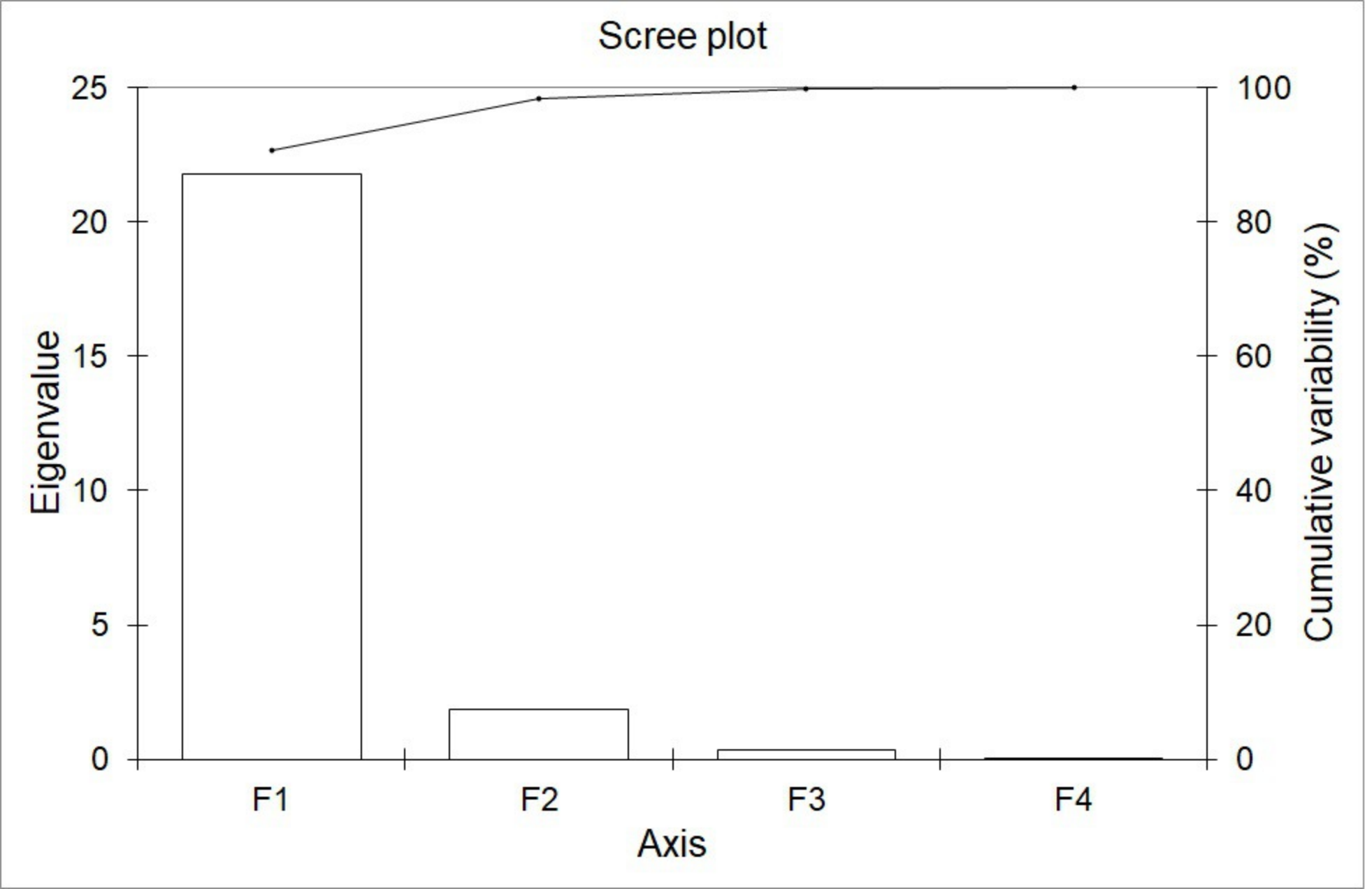

Intensity
(arbitrary unit)
2.00
1.75
1.50
1.25
1.00
0.75
0.50
0.25
brC13
brC14
C14
iC15
aC15
C15
C16
iC17
aC17
C18:1
C18
iC19
aC19
C19
C20
25
30
35
40
time (min.)

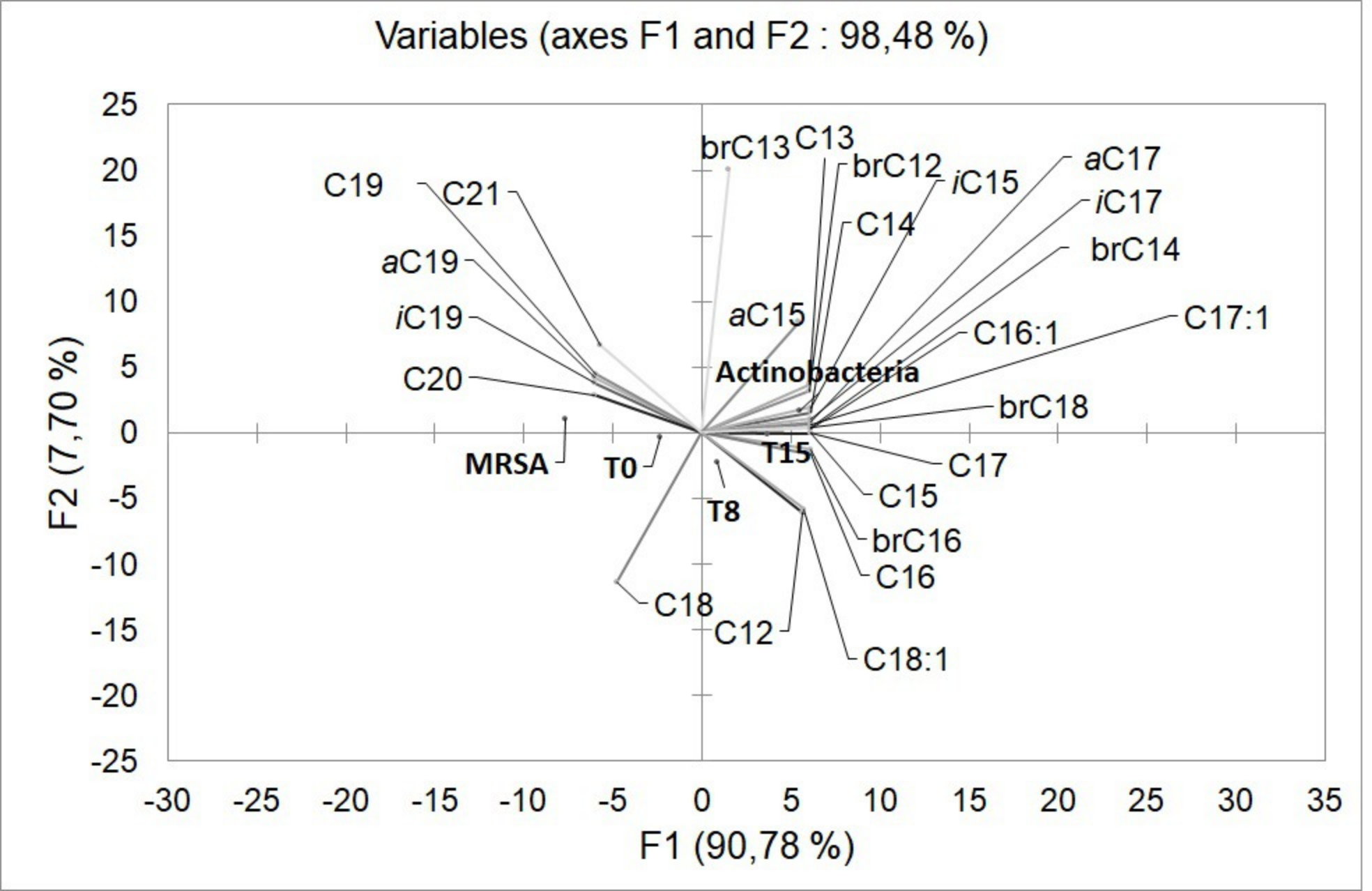

Intensity
(arbitrary unit)
3.00
2.75
2.50
2.25
2.00
1.75
1.50
1.25
1.00
0.75
0.50
0.25
C12
brC13
C13
brC14
C14
iC15
aC15
C15
C16:1
brC16
C16:1
C16
C17:1
iC17
aC17
C17:1
C17
C18:1
C18
25
30
35
40
time (min.)

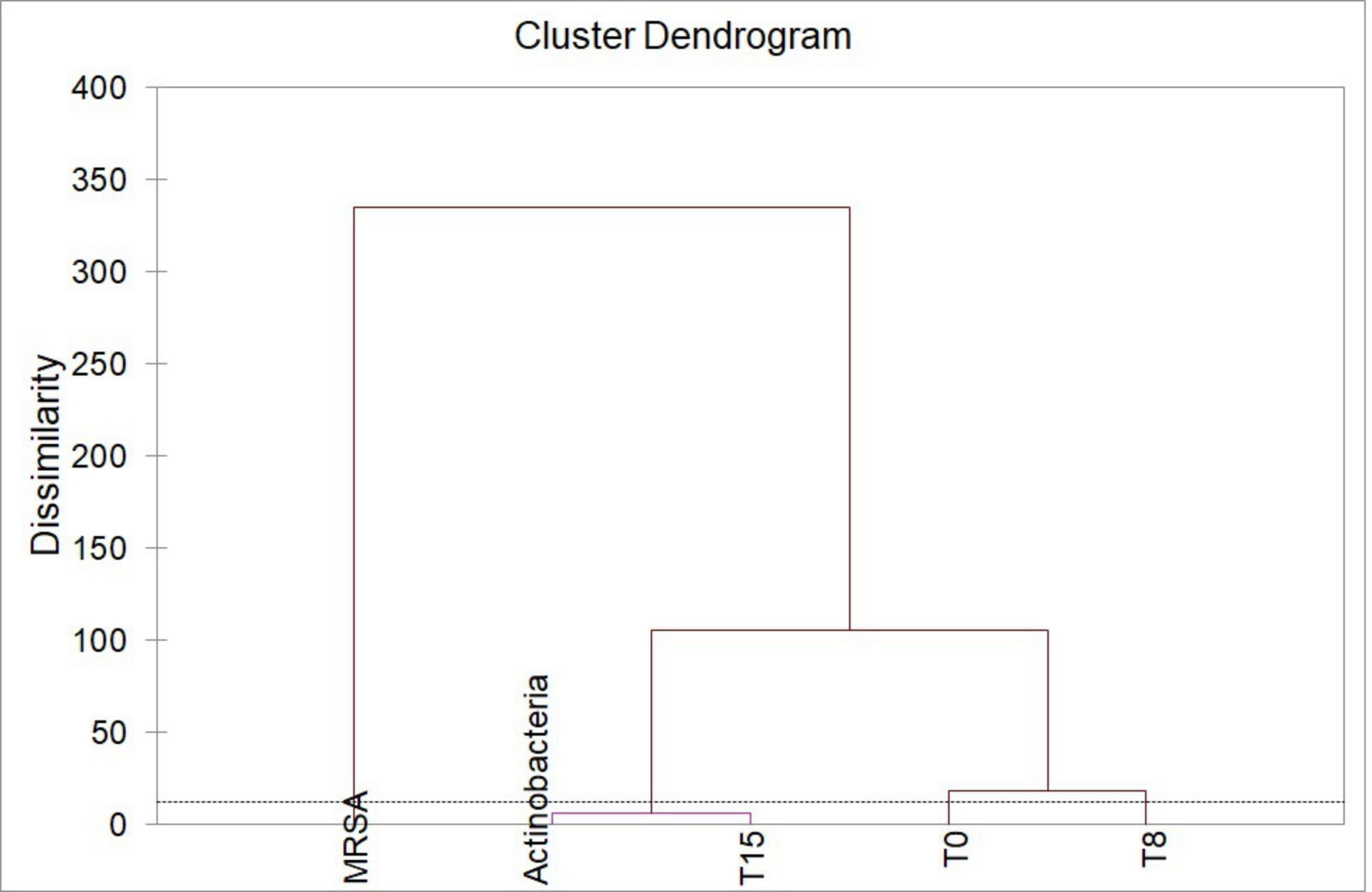

Relative abundance %
45
40
35
30
25
20
15
10
5
0
brC12
C12
brC13
C13
brC14
C14
iC15
aC15
C15
C16:1
brC16
C16
C17:1
iC17
aC17
C17
brC18
C18:1
C18
iC19
aC19
C19
C20
C21
FAMEs


Ralative abundance %
30
25
20
15
10
5
0
brC12
C12
brC13
C13
brC14
C14
iC15
aC15
C15
C16:1
brC16
C16
C17:1
iC17
aC17
C17
brC18
C18:1
C18
iC19
aC19
C19
C20
C21
FAMEs


T0
T8
T15
Relative abundance %
30
25
20
15
10
5
0
brC12
C12
brC13
C13
brC14
C14
iC15
aC15
C15
C16:1
brC16
C16
C17:1
iC17
aC17
C17
brC18
C18:1
C18
iC19
aC19
C19
C20
C21
FAMEs


Scree plot
Eigenvalue
Cumulative variability (%)
Axis
F1
F2
F3
F4
0
5
10
15
20
25
0
20
40
60
80
100


Variables (axes F1 and F2 : 98,48 %)
F2 (7,70 %)
F1 (90,78 %)
25
20
15
10
5
0
-5
-10
-15
-20
-25
-30
-25
-20
-15
-10
-5
0
5
10
15
20
25
30
35
brC13
C13
brC12
iC15
aC17
iC17
brC14
C14
C19
C21
aC19
iC19
C20
aC15
Actinobacteria
C16:1
C17:1
brC18
MRSA
T0
T15
T8
C17
C15
brC16
C16
C18
C12
C18:1


Cluster Dendrogram
Dissimilarity
400
350
300
250
200
150
100
50
0
MRSA
Actinobacteria
T15
T0
T8

				Co-culture	
	MRSA	Actinobacteria	T0	T8	T15
[brC_{14}+ brC_{16}]/ Total	6.4	35.9	33	34.6	35.6
[C_{18}+C_{20}]/Total	13.6	0.8	3.6	2.4	1.1
iC_{15}/aC_{15}	0.4	0.9	0.7	0.8	0.9

Compound	Actinobacteria	MRSA	T0	T8	T15
brC_{12}	0.5±0.1	-	0.2±0	0.2±0	0.4±0
C_{12}	0.4±0.1	-	0.1±0	0.4±0	0.4±0
brC_{13}	0.4±0.1	0.3±0	0.3±0	0.3±0	0.3±0
C_{13}	0.5±0.1	-	0.2±0	0.2±0	0.4±0
brC_{14}	8±0.9	0.7±0.1	3.7±0.6	5.2±0.7	6.9±0.6
C_{14}	1.6±0.4	0.3±0	0.8±0.1	1.1±0.1	1.5±0.1
iC_{15}	9.1±0.8	3±0.3	5±0.8	6.4±0.9	8±0.7
aC_{15}	10.1±0.7	6.8±0.9	6.7±1.2	7.6±1	8.8±0.9
C_{15}	3.4±0.8	-	1.4±0.2	2.2±0.3	3±0.3
$C_{16:1}$	2.2±0.2	-	1.3±0	1.4±0.1	2±0.1
brC_{16}	21.6±1.2	0.3±0	10.7±2.1	15.1±2	19.3±1.9
C_{16}	12.4±1.3	0.8±0	6.1±1	9.1±1.2	11.2±1.1
$C_{17:1}$	2±0.1	-	0.5±0	0.8±0	0.12±0
iC_{17}	3±0.6	0.6±0	1.5±0.1	2±0.3	2.6±0.3
aC_{17}	4.9±0.7	0.8±0	2.4±0.3	3.3±0.5	4.3±0.5
C_{17}	0.9±0.3	-	0.4±0	0.6±0	0.8±0.1
brC_{18}	0.1±0	-	0.1±0	0.1±0	0.1±0
$C_{18:1}$	0.3±0	-	-	-	0.2±0
C_{18}	0.7±0.2	1.1±0.1	0.9±0.3	1.1±0.4	0.8±0.4
iC_{19}	-	0.2±0	0.1±0	-	-
aC_{19}	-	0.1±0	-	-	-
C_{19}	-	-	-	-	-
C_{20}	-	1.2±0.1	0.6±0.2	0.2±0	-
C_{21}	-	<0.1	-	-	-

C_n: Fatty acids containing n carbon atoms; i*: iso;* a*: anteiso* ; br : branched ; - : not detected