Identification of a novel fatty acid in the cell membrane of *Chryseobacterium frigidisoli* PB4T isolated from an East Antarctic glacier forefield

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**Abstract**

The cell membrane phospholipid fatty acid (PLFA) inventory of *Chryseobacterium frigidisoli* PB4, a psychrotolerant, non-motile, gram-negative, aerobic bacterium isolated from ice free permafrost deposits in continental Antarctica contains a FA with a novel methyl ester mass spectrum. In a temperature cultivation experiment of *C. frigidisoli* at 0 °C this unknown FA was the dominant PLFA. The molecular mass indicates a C₇₂ FA with two double bond equivalents (DBEs). A 3-pyridylcarbinol derivatization experiment as well as hydrogenation of the double bonds allowed structural assignment of the respective FA as 14-methyl-hexadeca-9,13-dienoic acid (anteiso-heptadeca-9,13-dienoic acid or ai-17:2\(^a\)) and one unusual anteiso-o3-FA with bis-methylene interrupted double bonds (ai-17:2o3,7 or ai-17:2n-3,7). The iso-counterpart of this unsaturated FA also occurred in trace amount. The close vicinity of the o3 double bond to the anteiso branch and also to the iso branch seems to be responsible for a shift in the gas chromatography retention times of these compounds, resulting in elution shortly after the saturated counterparts. The dominance of the ai-17:2o3,7 at 0 °C cultivation temperature suggests its importance for cell membrane adaptation of *C. frigidisoli* with respect to low ambient temperature conditions.

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1. Introduction

Continental Antarctica is one of the most inhospitable habitats on the planet. It is dominated by the coldest climate on Earth, with summer temperatures usually < 0 °C as well as limited water and nutrient availability, representing a challenge for all life. Microorganisms in the region are adapted to the extreme conditions and psychrophilic and psychrotolerant microorganisms are found to tolerate even the extreme climate in the Arctic and Antarctic regions (Yi et al., 2005; Loveland-Curtze et al., 2010; Bajerski and Wagner, 2013; Bajerski et al., 2013). To cope with cold temperatures, microorganisms change their cell membrane lipid inventory in order to maintain the membrane fluidity and therefore membrane functionality (Sinensky, 1974; Russell, 1989; Suutari and Laakso, 1994; Mangelsdorf et al., 2009). For instance, bacteria can increase the relative amount of unsaturated or anteiso-branched membrane phospholipid fatty acids (PLFAs; Russell, 1989; Kaneda, 1991) and/or enhance the proportion of short chain membrane FAs (Russell, 1989; Suutari and Laakso, 1994; Mangelsdorf et al., 2009). All these changes lead to a lowering of the cell membrane melting temperature, which is required in a cold environment to maintain membrane fluidity. Furthermore, specific proteins and enzymes are used to maintain metabolism and prevent freezing of the cytoplasm (Jones and Inouye, 1994; Phadtare et al., 1999; Rothschild and Mancinelli, 2001; Deming, 2002; Georlette et al., 2004).

In recent years Chryseobacteria such as *Chrysobacterium frigidisoli*, *C. greenlandense*, *C. Antarctica* and *C. jeonii* have been isolated from polar habitats (Yi et al., 2005; Kämpfer et al., 2009a, 2009b; Loveland-Curtze et al., 2010; Bajerski et al., 2013). The genus Chryseobacterium was first introduced in 1994 as a new classification of the family Flavobacteriaceae by Vandamme et al. (1994) and belongs to the phylum Bacteriodetes. Thus, the type strain of *C. frigidisoli* with respect to low ambient temperature conditions.

### References

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Thus, they occur not only in polar habitats, but also in many other environments. For instance *C. flavum*, *C. soli* and *C. jejeunese* were found in soil (Zhou et al., 2007; Weon et al., 2008), *C. indoltheticum* in marine environments (Campbell and Williams, 1951), *C. aquaticum* in water reservoirs (Kim et al., 2008) and *C. gleum* and *C. indologenes* have a clinical source (Kim et al., 2008; Chen et al., 2013; Brki et al., 2015). Many of these bacteria have been investigated with respect to their PLFA inventory. Bacteria in the genus *Chryseobacterium* are namely yellow pigmented (colonies) rods that react negatively in gram-staining, indicating that their cell wall consists only of one thin peptidoglycan layer embedded between two lipid cell membranes. A detailed characterization of *Chryseobacteria* is given in the genus (Vandamme et al., 1994) and type strain descriptions (Holmes et al., 1984). Their isolation from cold habitats indicates that representatives of the genus can adapt to a life at low temperature. Usually, the FA inventory of the cell membrane of bacterial strains is analyzed and given in the framework of the species description, but detailed information on unknown or rare compounds is often missing, especially with regard to non-optimal growth conditions. To our knowledge this is the first study analyzing the FA inventory of a *Chryseobacterium* at sub-optimal growth temperature, identifying two unknown compounds.

*C. frigidisoli* strain PB4 was isolated from Antarctic ice-free permafrost deposits (Bajerski et al., 2013), is a psychrotolerant, non-motile, gram-negative, shiny yellow, rod shaped, aerobic bacterium. During investigation of the cell membrane lipid inventory of *C. frigidisoli* two unknown phospholipid FAs (PLFAs) with almost the same mass spectra were recognized. Moreover, the more abundant one dominated the PLFA pattern of *C. frigidisoli* cultivated at 0°C. This dominance led to the suggestion that this FA might play an important role for the cell membrane adaptation of the bacterium to cold environmental conditions. In particular, since it did not play such a significant role when cultivated at optimum growth conditions of 20°C. Furthermore, the fact that this component seems to occur only rarely suggests potential that it may act as a characteristic biomarker for *C. frigidisoli* or possibly for the genus *Chryseobacteria*. Thus, it could be a valuable marker compound for taxonomic evaluation of microbial ecosystems. Therefore, in a first step we present here the identification of the two novel FAs. Their structures have been elucidated using hydrogenation and double bond derivatization. The information from the derivatization experiments was used to interpret the unusual Me ester mass spectra of these two unknown FAs.

2. Material and methods

2.1. Sampling and cultivation of *C. frigidisoli*

Within the scope of expedition ANT-XXIII/9 with the research vessel Polarstern to Antarctica in March 2007 *C. frigidisoli* strain PB4 was isolated from permafrost deposits gathered from a transect taken from a glacier forefield on Broknes Peninsula, which is part of the Larsemann Hills (S 69°30’, E 76°20’) in East Antarctica (Bajerski and Wagner, 2013). The hills represent the second largest ice free area (ca. 50 km²) on the East Antarctic coast. Due to past glaciation and deglaciation cycles, the landscape is dominated by erratic boulders, pattered ground and glacial tafoni, and is divided by steep valleys and > 150 partially ice free lakes (Stiwe et al., 1989; Gillessen, 1991). The area is characterized by a marine influenced continental climate (Burgess et al., 1994). Winter air temperature in the coastal region ranges between −18°C and −29°C. In summer, a temperature of around 0°C prevails. The area is dry, with precipitation of ca. 250 mm/yr, mainly as snow (Australian National Antarctic Research Report (ANARE), 2000).

*C. frigidisoli* strain PB4 was also isolated from sandy dry permafrost at 1–6 cm depth. Its cultivation is described in detail by Bajerski et al. (2013); here, it was cultivated close to the summer mean temperature of 0°C and at the optimum growth temperature of 20°C for comparison.

2.2. Lipid extraction

A cell pellet of *C. frigidisoli* cultivated at 0°C (and at 20°C for comparison) was extracted using a flow blending system with a mixture of MeOH/dichloromethane (DCM)/NH₄OAc buffer (pH 7.6), 2:1:0.8 (v/v). Subsequently, the extract was collected in a separation funnel and for phase separation DCM and water were added to achieve a ratio of MeOH/DCM/NH₄OAc buffer (pH 7.6) of 1:1:0.9 (v/v). Afterwards the organic phase was removed and the water phase was re-extracted 2× with DCM. The organic phases were combined and the solvent was evaporated using a TurboVap® 500 system (Biotage) and finally a gentle stream of N₂.

The extract was separated into fractions of different polarity (low polarity lipids, free FAs, glycolipids and phospholipids) using a silica column (1 g silica gel 63–200 μm) and a Florisil® column (1 g magnesium silica gel 150–250 μm) in sequence. The low polarity fraction was eluted with 20 ml CHCl₃, the free FAs with 50 ml of HCO₂Me blended with 12.5 μl glacial MeCO₂H and the glycolipid fraction with 20 ml Me₂CO. After removal of the Florisil® column the phospholipids (PLs) were eluted with 25 ml MeOH from the silica column. To improve the recovery of PLs, the silica column was rinsed with 25 ml of MeOH/water (60:40) and the extract captured in a separation funnel. DCM and water were added for phase separation (MeOH/DCM/water, 1:1:0.9), the organic phase was removed, and the water phase was re-extracted 2× with DCM. Finally, the organic phases were combined and evaporated to dryness and stored at −20°C until analysis. The PL fraction was used for subsequent PLFA analysis. The method applied is described by Zink and Mangelsdorf (2004).

2.3. Ester cleavage of PLs and methylation of PLFAs

The PL fraction was dissolved in 50 μl DCM/MeOH (9:1, v/v) in a 2 ml vial. Trimethylsulphoniumhydroxide (TMSH, 50 μl) was added and the vial sealed before placing it in an oven for 2 h at 70°C (Mueller et al., 1990). After the sample was cooled to room temperature, it was dried under a gentle N₂ stream. For gas chromatography-mass spectrometry (GC–MS) measurements the methylated FAs were dissolved in 50 μl DCM.

2.4. Isolation of target FAs using a GC-preparative fraction collection (PFC) system

The two FAs of interest were isolated from the PLFA inventory of *C. frigidisoli* using an Agilent 6890a gas chromatograph equipped with preparative fraction collection (PFC, Gerstel). A DB1 preparative column (30 m × 0.53 mm i.d., 0.5 μm film thickness) was used with the following program: injector 50–300°C at 12°C/min, column 50–120°C at 10°C/min, then to 220°C at 2.5°C/min and finally to 300°C (held 10 min) at 10°C/min. He was the carrier gas at 4.9 ml/min. Samples were injected by hand. The PFC transfer line and switching device temperature were at 300°C. The compounds were isolated using 6 collection cycles.

2.5. Hydrogenation of target FAs

The isolated FAs (no other FA was present in the GC–MS run) were dissolved in 1 ml MeOH, transferred to a test tube and ca. 1 mg Adams catalyst was added. The test tube was evacuated.
and filled with H2 3× using a three-way valve connected to a vacuum pump and a bladder filled with H2. Finally, a slight overpressure of H2 was applied using the H2 filled bladder. The reaction mixture was vigorously shaken from time to time over 2 h. The Adams catalyst was removed by filtration over a Na2SO4-filled Pasteur pipette (Christie, 1989). The hydrogenated FAs were examined using GC–MS (see below).

2.6. 3-Pyridylcarbinol derivatization of double bonds

Potassium t-butoxide (100 μl) in tetrahydrofuran (1 M) was mixed with 3-hydroxymethylpyridine (200 μl). An aliquot of the PLFA fraction in 1 ml DCM was added, and the test tube closed and held for 30 min at 40 °C. After cooling the mixture to room temperature, 2 ml water and 4 ml n-hexane were added. After phase separation the organic phase was collected, dried over anhydrous Na2SO4 and the solvent removed under a gentle N2 stream. For GC–MS the sample was dissolved in DCM (Destaillets and Angers, 2002).

2.7. GC–MS analysis of PLFA derivatives

The derivatized PLFA aliquots were analyzed using a GC system (Trace GC Ultra, Thermo Fisher Scientific Inc., Waltham, Massachusetts, USA) coupled to a mass spectrometer (DSQ, Thermo Fisher Scientific Inc., Waltham, Massachusetts, USA) operated in the electron impact mode at 70 eV. The gas chromatogram of the cell membrane PLFAs of C. frigidisoli cultivated at 0 °C revealed a series of iso- and anteiso-branched FAs (as Me ester). Most are saturated and some bear additional MeO or hydroxy (OH) groups. Some unsaturated FAs occur, which are either straight chain or iso- and anteiso-branched plus, in small amounts, saturated straight chain FAs. The FAs range from C14 to C18.

After the iso- and anteiso-17:1ω7 a very prominent unknown FA (UK2) eluted from the GC column. Shortly before this peak a less abundant FA (UK1) eluted, with essentially the same mass spectrum. UK2 dominates the PLFA signal of C. frigidisoli cultivated at 0 °C. In contrast, it is significantly smaller in the cultivation at the optimum growth temperature (Bajerski et al., 2013) of 20 °C (Fig. 1b). This suggests that the novel FA is important for the cell membrane adaptation to low ambient temperature, which is essential for a bacterium dwelling in Antarctic soil.

The mass spectrum of the Me ester of UK2 (Fig. 1c), exhibits M+ at m/z 280, suggesting a C17 FA with two double bond equivalents (DBEs, i.e. double bonds and/or rings). This is confirmed by the determination of the molecular formula from FT-ICR-MS, indicating the best fit for C18H33O2 ([M + H]+, m/z 281) with 3 DBEs. This points to a C17 FA methyl ester (FAME) with three DBEs, with one DBE represented by the carbonyl group and two by rings or double bonds in the FA chain.

The FAME mass spectrum is dominated by m/z 83 (Fig. 1c), which is usually a characteristic fragment for a terminal cyclohexyl ring. Thus, at first sight this fragment might suggest that the FA contained one double bond and one terminal ring.

3.2. Hydrogenation of unknown FAs

Prior to hydrogenation, the target FAs (with almost analogous mass spectra) were effectively isolated from the residual PLFA inventory using a GC instrument equipped with PEC in 6 collection cycles (Fig. 2a). Subsequently, n-octadeca-9,12-dienoic Me ester was added to prove that the hydrogenation worked effectively. The hydrogenated FAs (Fig. 2b) were compared with a standard mixture containing iso- and anteiso-C17:0 FAMEs as well as the n-C17:0 and n-C18:0 FAMEs (Fig. 2c). The chromatogram (Fig. 2c) shows a signal at the same retention time as n-C18:0 FAME in the standard run, indicating that the hydrogenation procedure worked well. The n-C18:0 FAME was confirmed from its mass spectrum. Hydrogenation of the dominant target compound led to a signal eluting at the same time as the ai-C17:0 FAME and not with n-C17:0 FAME (Fig. 2b and c) and the identity was confirmed from the mass spectrum. Thus, the unknown FA was a C17 FA with an anteiso-branched and two double bonds. Accordingly, the m/z 83 fragment did not originate from a terminal cyclohexyl ring. The earlier eluting smaller GC peak represents the iso-counterpart (Fig. 2b and c). Hydrogenation of a FAME standard containing a cyclopropyl ring showed that the ring was not cleaved under the mild hydrogenation conditions applied (data not shown). This indicates that the iso- and ai-FAME in the hydrogenation experiment were not the result of cyclopropyl ring cleavage.

3.3. GC–MS analysis of 3-pyridylcarbinol derivative of unknown FA

In mass spectra of 3-pyridylcarbinol esters each carbon-carbon bond is visible by way of its own fragment. Thus, in saturated FAs there are regular differences of 14 mass units between the main fragments. Double bonds can be assigned by a step of 12 mass units counting from the functional group. Furthermore, a branching position is indicated by the lack of the respective fragment in the spectrum (Destaillets and Angers, 2002).

The prominent fragments at m/z 92 and 108 in the spectrum of the target compound derive from the 3-pyridylcarbinol head group part and m/z 151 (m/z 150 + H) as well as 164 are the first visible regular fragments counting from the functional group (Fig. 3a).
Fig. 1. (a) GC–MS chromatogram of the cell membrane PLFAs inventory of *C. frigidisoli* cultivated at 0 °C and (b) at optimal growth temperature of 20 °C. Note that in Bajerski *et al.* (2013) the iso-17:1o7 acid was wrongly assigned as iso-17:1o8. Iso and ai, iso- and anteiso-branched FA; X:Y, numbers of carbon atoms and double bonds; α, double bond position from the tail end; ISTD, internal standard. (c) Mass spectrum of unknown FA (UK2) as Me ester.
Double bonds are indicated by a step of 12 mass units between m/z 234 and 246, as well as between m/z 288 and 300, which correspond to Δ⁹ and Δ¹³, respectively. The allyl fragment between the bis-methylene interrupted double bonds is quite prominent. The fragment is represented by a doublet of m/z 274 (calculated mass) and 275 (H transfer). The counter fragment of this allyl fragment is the above mentioned m/z 83, which is also present in the spectrum of the 3-pyridylcarbinol derivative, indicating that formation of this fragment is favored. The anteiso-branch, verified by hydrogenation, is clearly indicated by the lack of the corresponding fragment at m/z 314.

Thus, together with the hydrogenation experiment the 3-pyridylcarbinol derivatization indicates that the novel FA is 14-methyl-hexadeca-9,13-dienoic acid (anteiso-heptadeca-9,13-dienoic Me ester) or in other words anteiso-o3, 7-heptadecadienoic acid (ai-17:2Δ⁹,13) or 14-methyl-hexadeca-9,13-dienoic acid (anteiso-heptadeca-9,13-dienoic Me ester). Hence, the earlier eluting smaller FA is the corresponding iso-isomer.

### 3.4. Additional derivatization experiments

In addition to the derivatization discussed here, OsO₄, dimethyl disulphide (DMDS) and 4,4-dimethyl oxazoline (DMOX) derivatizations of the unknown FAs were conducted (Dunkelblum et al., 1985; Laurent and Richli, 1991; Pepe et al., 1993; Rontani, 1998). Mass spectra from these approaches and explanation of individual fragments are provided in the Supplementary material. Although the earlier derivatization techniques essentially confirm the structure of the unknown FAs, the results of these additional techniques might also be of interest in using one of these techniques for compound identification. In the OsO₄ experiment the two double bond positions at Δ⁹ (only the fragment containing the functional group) and Δ¹³ (only the tail end fragment) are clearly indicated.

DMDS derivatization shows unexpected side effects. The double bond at Δ⁹ shows a clear fragment. In contrast, the double bond at Δ¹³ is not directly obvious. This is due to the fact that the molecule lost one MeS group at the second double bond, preventing cleavage directly at Δ¹³. However, fragments occur which indicate the formation of a thiapentyl ring between the two double bonds, which is known for bis-methylene interrupted double bonds (Carballeira et al., 1994) and which places the second double bond automatically to Δ¹³. The ring formation is confirmed by the presence of at least 6 isomers with identical mass spectra, derived from the chiral centers caused by the ring formation.

The 4,4-DMOX derivative also confirms the two double bond positions at Δ⁹ and Δ¹³ and the allyl fragment between them is clearly indicated by a very prominent signal (compare with 3-pyryldicarbinol derivative), which is typical for DMOX derivatives of bis-methylene interrupted double bonds (Nikolova et al., 2006). Although these additional experiments confirm the double bond positions at Δ⁹ and Δ¹³, they provide no clear indication for the presence of a tail end branch. Thus, the 3-pyridylcarbinol derivatization in combination with hydrogenation seems to be the best technique for visualizing all the structural features of the novel FAs in C. frigidisoli.

### 3.5. Interpretation of mass spectrum of anteiso-heptadeca-9,13-dienoic Me ester

Based on the structural identification, the FAME mass spectrum can be interpreted (Fig. 1c). Since the spectrum is dominated by m/z 83 it was scaled up to improve visibility of the smaller fragments (cf. Figs. 1c and 3b). The M⁺ is represented by m/z 280. m/z 265 reveals loss of a terminal Me group (M-15)⁺ and m/z 249 loss of a MeO group (M-31)⁺. The ion at m/z 251 is the vinyl tail end fragment of the o3 double bond. Cleavage of the o3 double bond is represented by m/z 223 (m/z 224-H), and the counter fragment including the tail end is at m/z 56 and 55 (-H), respectively. Concomitantly, m/z 251 and 223 are also prominent fragments for an anteiso branch [(M-29)⁺ and (M-57)⁺, respectively]. The m/z 210 (m/z 211-H) and 178 (-MeOH, 32 mass units) form the other vinyl fragments of the o3 double bond with the corresponding tail end fragments at m/z 69 and 67 (-2H). The allyl fragment between both double bonds, including the head group side, is usually a very prominent fragment in FAMEs containing bis-methylene interrupted double bonds. In this case the fragment is not present (m/z 197); however, a fragment at m/z 166 represents the allyl fragment after loss of a MeO (31 mass units). In contrast, the counter fragment is the above mentioned m/z 83, which is very prominent. The reason for this preference might be that the 3-methyl pentenyl tail end fragment undergoes cyclisation to form a stable 1-methyl-cyclopropyl cation (Attina et al., 1981). The preference for m/z 83 could therefore explain why m/z 197 [or m/z 166] is not very abundant. The m/z 109 (m/z 110-H) represents the tail end fragment of the o7 double bond and m/z 96 (m/z 97-H), as well as m/z 123 form the vinyl tail end fragments of the o7 double bond. Finally, m/z 137 is the allyl tail end fragment of the o7 double bond.

Thus, the unusual mass spectrum of the FA, essentially dominated by m/z 83, contains an informative fragmentation pattern in the low intensity range. That the spectrum is so different from other FAs with bis-methylene interrupted double bonds is due to the strong presence of the very stable m/z 83 obscuring the spectrum at first sight. The spectrum of the iso-counterpart

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Fig. 2. (a) GC–MS chromatogram of unknown target FAs (UK1 and UK2) isolated from the chromatogram of the cell membrane PLFA pattern of C. frigidisoli (cultivated at 0 °C) using a preparative fraction collection device plus n-octadeca-9,12-dienoic Me ester added to prove that the hydrogenation experiment was successful. (b) GC–MS chromatogram of the unknown FAs and n-octadeca-9,12-dienoic Me ester after hydrogenation. (c) GC–MS chromatogram of iso- and ai-C₁₇:0 as well as n-C₁₇:0 and n-C₁₈:0 Me ester standards for comparison.
not shown) contains an additional signal at m/z 237. This fragment
represents the loss of the terminal iso-group (M-43)+, confirming
the deduced structure.

Very unusual is the chromatographic position of the iso- and ai-
17:2o3,7 compounds. Comparing Figs. 1a and 2 it can be deduced
that the mono-unsaturated iso- and ai-17:1o7 (Fig. 1a) elute as
expected before the saturated iso- and ai-17:0 (Figs. 1 and 2). Thus,
the target iso- and ai-17:2o3,7 are expected to elute even before
iso- and ai-17:1o7. In fact, they elute shortly after their saturated
counterparts (Fig. 2a and c). Since the o3-double bond is at the
same position as in the mono-unsaturated ai-17:1o7, the reason
for the shift seems to originate from the o3 double bond in con-
junction with the anteiso and iso branch. An indication for a shift
in the retention time of compounds bearing a double bond in a
vinyl position to a branch is provided by Cravo-Laureau et al.
(2005). They found a mid-chain branched FA with a directly adja-
cent double bound shifted after its saturated counterpart. Thus, it
is very likely that the close vicinity of the anteiso and iso branch
to the o3 double bond is responsible for the unexpected elution
behavior of the novel FA.

3.6. Function of anteiso-heptadeca-9,13-dienoic acid

The incorporation of double bonds into the cell membrane
PLFAs is an essential process for microorganisms dwelling in cold
environmental regimes such as Antarctica (Sinensky, 1974;
Russell, 1989). The dominance of anteiso-heptadeca-9,13-dienoic
acid in the 0 °C culture of C. frigidisoli suggests that this FA is
important in the adaptation process to keep the cell membrane
in a liquid stage at cooler ambient conditions (Russell, 1989;
Mangelsdorf et al., 2009). To maintain a liquid stage under cold
environmental conditions, microorganisms bring their membrane
phase transition temperature below the ambient temperature by
increasing the proportion of unsaturated FAs (lower melting tem-
perature) in the PLFAs. Most effective is an increase in cis double
bonds (Russell, 1989), thereby suggesting that the
anteiso-heptadeca-9,13-dienoic acid most likely also has the
cis, cis configuration. Currently, a series of cultivation experiments with differ-
et Chryseobacteria at different temperatures is in progress to
investigate the role of the novel FA in the low temperature adapta-
tion in Chryseobacteria.

Since the occurrence of the anteiso-heptadeca-9,13-dienoic
acid is rather rare, it shows potential to be a characteristic biomarker
for the genus Chryseobacterium. Indeed, publications on C. flavum,
C. balustinum, C. gleum, C. indologenes, C. indotheticum, C. jooste,
C. scophthalmum (Yi et al., 2005; Zhou et al., 2007; Bajerski et al.,
2013) and C. antarcticus as well as C. jeonii, former Sejongia antar-
tico and S. jeonii (Yi et al., 2005; Kämpfer et al., 2009), reported an
unknown FA eluting around the same time (16.580 ECL, equivalent
chain length) as the anteiso-heptadeca-9,13-dienoic acid identified

Fig. 3. Mass spectrum and fragmentation interpretation of 3-pyridylcarbinol-derivative of the unknown FA (UK2) from C. frigidisoli. (b) Mass spectrum and interpretation of fragmentation of anteiso-heptadeca-9,13-dienoic Me ester. Note that the mass spectrum is scaled up; o3 and 7, double bond in positions 3 and 7 from the tail end of the FA. Allyl and vinyl, cleavage in allyl (over next bond) or vinyl (adjacent bond) position to the double bond.

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here, suggesting that this FA might also occur in other Chryseobacteria.

4. Conclusions

*C. frigidisoli* PB47 contains a novel FA, dominating the membrane PLFA side chain inventory in a culture grown at 0 °C. The structure was determined to be 14-methyl-hexadeca-9,13-dienoic acid (*anteiso*-heptadeca-9,13-dienoic acid or *anteiso*-o3,7-heptadecadienoic acid) by evaluating methylated, hydrogenated and 3-pyridylcarbinol-derivatives. The 3-pyridylcarbinol derivatization was the best technique for uncovering all the structural moieties of the unknown FA. The *iso*-heptadeca-9,13-dienoic acid also occurs in trace amount. Due to its rare occurrence the major FA shows potential to be a characteristic biomarker for Chryseobacteria.

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Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.orggeochem.2017.01.003.

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