Electronic Supplementary Material (ESI) for Chemical Communications.
This journal is © The Royal Society of Chemistry 2020

# Supporting Information

## Manipulating polyketide stereochemistry via exchange of polyketide synthase modules

Jean-Malo Massicard,[a] Claire Soligot,[b,c] Kira J. Weissman*[a] and Christophe Jacob*[a]

[a] *Université de Lorraine, CNRS, IMoPA, F-54000 Nancy, France.*

[b] *Université de Lorraine, UR AFPA, USC 340 INRAE, F-54000 Nancy, France.*

[c] *Université de Lorraine, PASM, F-54000 Nancy, France.*

## Table of Contents

**1. Experimental methods**

*Strains, Plasmids, Media and Growth condition.*

The strains and plasmids used in this study are listed in **Table S1**. *E. coli* strains were grown in LB medium (agar and liquid). Appropriate antibiotics were added to the media when needed at the following concentrations: apramycin, 50 μg/mL; nalidixic acid, 50 μg/mL; kanamycin, 50 μg/mL; chloramphenicol, 25 μg/mL. *Saccharomyces cerevisiae* 23.344c was grown in rich medium (agar and liquid). Transformants were selected on uracil-deficient YNB (Difco) containing 20 mM ammonium and 3% (*w/v*) glucose. *Sac. erythraea* mutant strain JC2 NCIMB 40802 has been described previously.[1] Medium ABB13 was used for conjugation, and screening medium 3 (SM3) was used for triketide lactone production.[2]

*Materials, DNA isolation and DNA manipulation.*

Primers used in this study are listed in **Table S2**. Standard procedures were used for DNA purification, PCR and molecular analysis. PCR was performed using Phusion Hot Start II DNA polymerase (Thermo Scientific, US) in a Mastercycler Pro (Eppendorf, Weseling-Berzdof, Germany). Isolation of DNA fragments from agarose gel and purification of PCR products were carried out with a Nucleo-Spin Extract II kit (Marcherey-Nagel, Hoerdt, France). Other enzymes used in this study were from Thermo Scientific. PCR primers were purchased from Sigma-Aldrich, and DNA sequencing was carried out by GATC Biotech (Mulhouse, France). All kits and enzymes were used according to the manufacturers' recommendations.

*Plasmid construction.*

PCR was used to amplify the fragment containing *URA3* and *CEN6*/*ARS4* sites from plasmid pFL38.[3] The fragment contains a 15 bp homology region to the 5′ end of the *Pci*I site from plasmid pMU3-DEBS 1-TE. After gel purification of the URA3-CEN6/ARS4 fragment and the *Pci*I digested pMU3-DEBS 1-TE plasmid, they were assembled using the In-Fusion HD Cloning kit (Clontech, US) to yield plasmid pMU3-DEBS 1-TE-URA3. The identity of pMU3-DEBS 1-TE-URA3 was confirmed by diagnostic digestion and sequencing. PCR was used to amplify each module from cosmid or genomic DNA template (**Table S3**), using appropriate pairs of primers (**Table S2**) containing 50 bp homologous to the appropriate region in the pMU3-DEBS 1-TE-URA3 plasmid. Yeast homologous recombination was employed to recombine the module fragments with the pMU3-DEBS 1-TE-URA3 plasmid. Each individual module fragment and *Avr*II-digested pMU3-DEBS 1-TE-URA3 were co-transformed into *Saccharomyces cerevisiae* 23.344c as described elsewhere.[4] Correct plasmid assembly was confirmed by diagnostic digestion and sequencing.

*Transformation of* Sac. erythraea JC2 *by transconjugation, and growth for triketide lactone production.*

The pMU3-DEBS 1-TE-URA3 derivatives were transferred into *E. coli* 12567/pUZ8002 cells by heat shock transformation. Transconjugation between *E. coli* ET12567/pUZ8002 and *Sac. erythraea* JC2 was carried out as described previously.[1] The plates were incubated for 14 days at 30 °C, or until exconjugates became visible.

At least 4 clones per plasmid were then grown in liquid TSB for two days at 30 °C. Equal portions of the cultures were then used to inoculate SM3 production plates, which were incubated for 14 days at 30 °C. Equally-sized portions of each SM3 plate (2 cm²) were removed and frozen so that all samples could be analysed together.

*Analysis of triketide fermentation products.*

Extraction of triketides was carried out using the "lactonex" procedure, as previously described:[5] the excised 2 cm² pieces of mycelium and agar were chopped into pieces, and transferred into 2 mL Eppendorf tubes. Ethyl acetate (1.2 mL) and formic acid (20 μL) were added to each tube with mixing. The tubes were incubated at 50 °C for 15 min, and then vortexed for 30 min. The mixtures were centrifuged (20 000*g*) for 1 min, and the supernatants carefully removed and placed in fresh 1.5 mL Eppendorf tubes. After evaporation to dryness under reduces pressure, the residue in each tube was dissolved in ethyl acetate (200 μL), centrifuged for 1 min and the supernatant was transferred to a GC-MS vial. GC-MS was performed on a model 7890A-5975C system (Agilent Technologies) equipped with a DB 5 MS column (30 m × 0.25 mm × 0.25 μm; Agilent technologies): 40 °C for 2 min; 10 °C/min to 250 °C; 25 °C/min to 300 °C, hold for 7 min. The products were analysed by EI-MS in positive-ion MS mode, in both the full-scan and extracted ion modes (*m/z* = 58 and 56).

The identities of all lactones present in each sample were determined by comparison of the observed retention times and mass spectra with those of synthetic (a 2.5:1 mixture of **1a** and **3a**, as well as **2a**) and/or biosynthetic standards (**1a/1b**–**3a/3b** and **5a/5b** from previously-described strains JC2/pJLK25, JC2/pJLK30 and JC2/pJLK35).[5] In order to quantify lactone yields, we constructed a calibration curve with known quantities of the 2.5:1 mixture of lactones **1a** and **3a**. This yielded a linear correlation between lactone quantity and peak area in the total ion chromatogram (TIC). We could further confirm that this correlation held for key extracted ions (58 for reduced lactones **1**–**4**, and 56 for ketolactone **5**), allowing us to also quantify lactone yields based on the EICs (this was critical for lactones **1b** and **5a** whose peaks overlap in the TIC).

## 2. Additional tables and figures

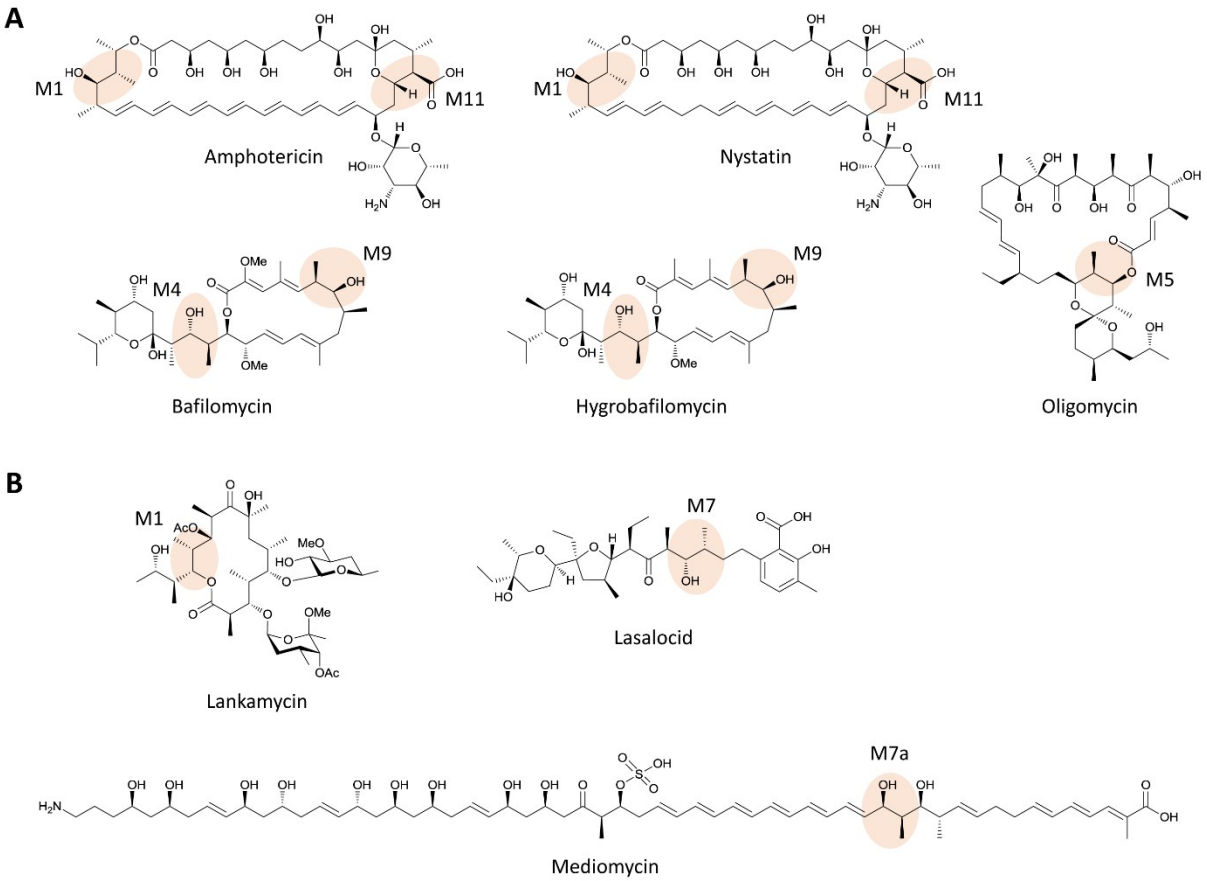



**Fig. S1** Structures of polyketides whose modular polyketide synthases (PKSs) were used as sources of heterologous modules. (A) Sources of A2 modules. (B) Sources of B2 modules. In (A) and (B), the relevant stereocentres and the modules which establish them, are indicated (shading).

**Table S1.** List of strains and plasmids used in this study.

| Name | Description | Source and reference |
|---|---|---|
| DH5α | *Escherichia coli* for routine plasmid maintenance, *supE*44, Δ*lacU*169 (Θ80 *lacZ*Δ*M*15), *hsdR*17, *recA*1, *endA*1, *gyrA*96, *thi*-1, *relA*1 | Life Technologies |
| 23.344c | *Saccharomyces cerevisiae* for yeast homologous recombination, *Matα*, *ura3* | [6] |
| ET12567/pUZ8002 | *Escherichia coli* for conjugation, $F^-$, *dam*$^-$*13::TN9*, *dcm–6*, *hsdM*, $Cml^R$, carrying helper plasmid pUZ8002 | [7] |
| JC2 | *Saccharopolyspora erythraea* for lactone production, Δ*eryA* | [1] |
| pMU3-DEBS 1-TE-URA3 | pMU3-DEBS 1-TE modified to incorporate the URA3-CEN6/ARS4 DNA sequence | This study |
| pMU3-DEBS 1-TE-URA3-Amph$_{M11}$-A | pMU3-DEBS 1-TE-URA3 modified to incorporate Amph M11 (KS → ACP) | This study |
| pMU3-DEBS 1-TE-URA3-Amph$_{M11}$-B | pMU3-DEBS 1-TE-URA3 modified to incorporate Amph M11 (linker KS-AT → ACP) | This study |
| pMU3-DEBS 1-TE-URA3-Amph$_{M11}$-C | pMU3-DEBS 1-TE-URA3 modified to incorporate Amph M11 (KS → ACP$_{SacI}$) | This study |
| pMU3-DEBS 1-TE-URA3-Amph$_{M11}$-D | pMU3-DEBS 1-TE-URA3 modified to incorporate Amph M11 (linker KS-AT → ACP$_{SacI}$) | This study |
| pMU3-DEBS 1-TE-URA3-Bfm$_{M4}$-A | pMU3-DEBS 1-TE-URA3 modified to incorporate Bfm M4 (KS → ACP) | This study |
| pMU3-DEBS 1-TE-URA3- Bfm$_{M4}$-B | pMU3-DEBS 1-TE-URA3 modified to incorporate Bfm M4 (linker KS-AT → ACP) | This study |
| pMU3-DEBS 1-TE-URA3-Bfm$_{M9}$-A | pMU3-DEBS 1-TE-URA3 modified to incorporate Bfm M9 (KS → ACP) | This study |
| pMU3-DEBS 1-TE-URA3- Bfm$_{M9}$-B | pMU3-DEBS 1-TE-URA3 modified to incorporate Bfm M9 (linker KS-AT → ACP) | This study |
| pMU3-DEBS 1-TE-URA3-Hba$_{M4}$-A | pMU3-DEBS 1-TE-URA3 modified to incorporate Hba M4 (KS → ACP) | This study |
| pMU3-DEBS 1-TE-URA3- Hba$_{M4}$-B | pMU3-DEBS 1-TE-URA3 modified to incorporate Hba M4 (linker KS-AT → ACP) | This study |
| pMU3-DEBS 1-TE-URA3- Hba$_{M9}$-A | pMU3-DEBS 1-TE-URA3 modified to incorporate Hba M9 (KS → ACP) | This study |
| pMU3-DEBS 1-TE-URA3- HbaM9-B | pMU3-DEBS 1-TE-URA3 modified to incorporate Hba M9 (linker KS-AT → ACP) | This study |
| pMU3-DEBS 1-TE-URA3-Las$_{M7}$-A | pMU3-DEBS 1-TE-URA3 modified to incorporate Las M7 (KS → ACP) | This study |
| pMU3-DEBS 1-TE-URA3- Las$_{M7}$-B | pMU3-DEBS 1-TE-URA3 modified to incorporate Las M7 (linker KS-AT → ACP) | This study |
| pMU3-DEBS 1-TE-URA3- Las$_{M7}$-C | pMU3-DEBS 1-TE-URA3 modified to incorporate Las M7 (KS → ACP$_{SacI}$) | This study |
| pMU3-DEBS 1-TE-URA3- Las$_{M7}$-D | pMU3-DEBS 1-TE-URA3 modified to incorporate Las M7 (linker KS-AT → ACP$_{SacI}$) | This study |
| pMU3-DEBS 1-TE-URA3-Lkm$_{M1}$-A | pMU3-DEBS 1-TE-URA3 modified to incorporate Lkm M1 (KS → ACP) | This study |
| pMU3-DEBS 1-TE-URA3- Lkm$_{M1}$-B | pMU3-DEBS 1-TE-URA3 modified to incorporate Lkm M1 (linker KS-AT → ACP) | This study |

| | | |
|---|---|---|
| pMU3-DEBS 1-TE-URA3- $Lkm_{M1}$-C | pMU3-DEBS 1-TE-URA3 modified to incorporate Lkm M1 (KS → $ACP_{SacI}$) | This study |
| pMU3-DEBS 1-TE-URA3- $Lkm_{M1}$-D | pMU3-DEBS 1-TE-URA3 modified to incorporate Lkm M1 (linker KS-AT → $ACP_{SacI}$) | This study |
| pMU3-DEBS 1-TE-URA3- $Nys_{M1}$-A | pMU3-DEBS 1-TE-URA3 modified to incorporate Nys M1 (KS → ACP) | This study |
| pMU3-DEBS 1-TE-URA3- $Nys_{M1}$-B | pMU3-DEBS 1-TE-URA3 modified to incorporate NysM1 (linker KS-AT → ACP) | This study |
| pMU3-DEBS 1-TE-URA3-$Nys_{M11}$-A | pMU3-DEBS 1-TE-URA3 modified to incorporate Nys M11 (KS → ACP) | This study |
| pMU3-DEBS 1-TE-URA3- $Nys_{M11}$-B | pMU3-DEBS 1-TE-URA3 modified to incorporate NysM11 (linker KS-AT → ACP) | This study |
| pMU3-DEBS 1-TE-URA3-$Med_{M7a}$-A | pMU3-DEBS 1-TE-URA3 modified to incorporate Med M7a (KS → ACP) | This study |
| pMU3-DEBS 1-TE-URA3- $Med_{M7a}$-B | pMU3-DEBS 1-TE-URA3 modified to incorporate Med M7a (linker KS-AT → ACP) | This study |
| pMU3-DEBS 1-TE-URA3-$Olm_{M5}$-A | pMU3-DEBS 1-TE-URA3 modified to incorporate Olm M5 (KS → ACP) | This study |
| pMU3-DEBS 1-TE-URA3- $Olm_{M5}$-B | pMU3-DEBS 1-TE-URA3 modified to incorporate Olm M5 (linker KS-AT → ACP) | This study |
| pMU3-DEBS 1-TE-URA3- $Olm_{M5}$-C | pMU3-DEBS 1-TE-URA3 modified to incorporate Olm M5 (KS → $ACP_{SacI}$) | This study |
| pMU3-DEBS 1-TE-URA3- $Olm_{M5}$-D | pMU3-DEBS 1-TE-URA3 modified to incorporate Olm M5 (linker KS-AT → $ACP_{SacI}$) | This study |

**Table S2.** List of forward and reverse primers.

| Plasmid | Primers | Sequence (5′ → 3′)[a] |
|---|---|---|
| pMU3-DEBS 1-TE-URA3-Amph$_{M11}$ | Forward KS | AGCAGGCGGCACCGGCGACCACGGCCCCCGTCGACGAGCCGATC GCGATCatcggcatgagctgccgctaccccggcgga |
| | Forward KS-AT linker | GGGAGTGGTCGCCCGCCGCGGACGGGGTGCGCCGGGCAGGTGTG TCGtccttcggcatcagcggtaccaacgcccac |
| | Reverse ACP$_{complete}$ | TAGCCGTCGCGAAGAGCGCTGCTCGCTTCCCGGGCGGGAGTCCC GCTGTCcagttcggagtggaggaagccggccagggc |
| | Reverse ACP$_{SacI}$ | GCCTGCTGGAGCTGGTTGCGCAGCCCGACCGCGGTCAGCGAGTC GAAGCCGAcgtcgcggaaggcgcgctggtcggtgag |
| pMU3-DEBS 1-TE-URA3-Bfm$_{M4}$ | Forward KS | AGCAGGCGGCACCGGCGACCACGGCCCCCGTCGACGAGCCGATC GCGATCgtctcgatggcctgccgctaccccggcggc |
| | Forward KS-AT linker | GGGAGTGGTCGCCCGCCGCGGACGGGGTGCGCCGGGCAGGTGTG TCGgccttcggcgtcagcggcaccaatgcccacctg |
| | Reverse ACP$_{complete}$ | TAGCCGTCGCGAAGAGCGCTGCTCGCTTCCCGGGCGGGAGTCCC GCTGTCcaggccggtctccaggcgcccggcgagcgc |
| pMU3-DEBS 1-TE-URA3-Bfm$_{M9}$ | Forward KS | AGCAGGCGGCACCGGCGACCACGGCCCCCGTCGACGAGCCGATC GCGATCgtggcgatgagctgccgcttccccggcggg |
| | Forward KS-AT linker | GGGAGTGGTCGCCCGCCGCGGACGGGGTGCGCCGGGCAGGTGTG TCGtcgttcggcatgagcggcaccaacgcgcacgtc |
| | Reverse ACP$_{complete}$ | TAGCCGTCGCGAAGAGCGCTGCTCGCTTCCCGGGCGGGAGTCCC GCTGTCcagttcggcgcggatctcccgggcgagcgc |
| pMU3-DEBS 1-TE-URA3-Hba$_{M4}$ | Forward KS | AGCAGGCGGCACCGGCGACCACGGCCCCCGTCGACGAGCCGATC GCGATCgtctccatgggctgccgctaccccggtggc |
| | Forward AT | GGGAGTGGTCGCCCGCCGCGGACGGGGTGCGCCGGGCAGGTGTG TCGgcgttcggcgtcagcggcaccaacgcccac |
| | Reverse ACP$_{complete}$ | TAGCCGTCGCGAAGAGCGCTGCTCGCTTCCCGGGCGGGAGTCCC GCTGTCgaggagggcgcccaggtggcgggcgagggc |
| pMU3-DEBS 1-TE-URA3-Hba$_{M9}$ | Forward KS | AGCAGGCGGCACCGGCGACCACGGCCCCCGTCGACGAGCCGATC GCGATCgcggtcgtctcgatggcctgccgcttcccc |
| | Forward KS-AT linker | GGGAGTGGTCGCCCGCCGCGGACGGGGTGCGCCGGGCAGGTGTG TCGtccttcggcatgagcggcaccaacgcccac |
| | Reverse ACP$_{complete}$ | TAGCCGTCGCGAAGAGCGCTGCTCGCTTCCCGGGCGGGAGTCCC GCTGTCgagttccgtgccgatgtgccgggacagggc |
| pMU3-DEBS 1-TE-URA3-Med$_{M7a}$ | Forward KS | AGCAGGCGGCACCGGCGACCACGGCCCCCGTCGACGAGCCGATC GCGATCgtcgcgatgagctgccgctaccccgggggc |
| | Forward KS-AT linker | GGGAGTGGTCGCCCGCCGCGGACGGGGTGCGCCGGGCAGGTGTG TCGtccttcggcttcagcggcaccaacgcccac |
| | Reverse ACP$_{full}$ | TAGCCGTCGCGAAGAGCGCTGCTCGCTTCCCGGGCGGGAGTCCC GCTGTCgagctcggcgcgcaggtgcctggcgagcac |
| pMU3-DEBS 1-TE-URA3-Nys$_{M1}$ | Forward KS | AGCAGGCGGCACCGGCGACCACGGCCCCCGTCGACGAGCCGATC GCGATCgtcggaatgggctgccgacttcccggcggc |
| | Forward KS-AT linker | GGGAGTGGTCGCCCGCCGCGGACGGGGTGCGCCGGGCAGGTGTG TCGtcgttcggcatcagcggcaccaacgcgcac |
| | Reverse ACP$_{complete}$ | TAGCCGTCGCGAAGAGCGCTGCTCGCTTCCCGGGCGGGAGTCCC GCTGTCcgccgtcgtcttcaggaacgcggcgagcgc |
| pMU3-DEBS 1-TE-URA3-Nys$_{M11}$ | Forward KS | AGCAGGCGGCACCGGCGACCACGGCCCCCGTCGACGAGCCGATC GCGATCatcggcatggcctgccgcttccccggcggc |
| | Forward KS-AT linker | GGGAGTGGTCGCCCGCCGCGGACGGGGTGCGCCGGGCAGGTGTG TCGtcgttcggcatcagcggtaccaacgcccat |
| | Reverse ACP$_{complete}$ | TAGCCGTCGCGAAGAGCGCTGCTCGCTTCCCGGGCGGGAGTCCC GCTGTCcagctcgccgtgcagataggcggcgagcgc |
| pMU3-DEBS 1-TE-URA3-Olm$_{M5}$ | Forward KS | AGCAGGCGGCACCGGCGACCACGGCCCCCGTCGACGAGCCGATC GCGATCatcgtcggcatggcctgccgctaccccggcggc |

| | | |
|---|---|---|
| | Forward KS-AT linker | GGGAGTGGTCGCCCGCCGCGGACGGGGTGCGCCGGGCAGGTGTG TCGgcgttcggagtcagcggcaccaacgcacat |
| | Reverse $ACP_{complete}$ | TAGCCGTCGCGAAGAGCGCTGCTCGCTTCCCGGGCGGGAGTCCC GCTGTCgaggccgcgggccaggaaatccgccagttg |
| | Reverse $ACP_{SacI}$ | GCCTGCTGGAGCTGGTTGCGCAGCCCGACCGCGGTCAGCGAGTC GAAGCCGAgctccttgagcgcgcgggcggggtcgac |
| pMU3-DEBS 1-TE-URA3-$Las_{M7}$ | Forward KS | AGCAGGCGGCACCGGCGACCACGGCCCCCGTCGACGAGCCGATC GCGATCggcatggcctgccggttgcccggtggc |
| | Forward KS-AT linker | GGGAGTGGTCGCCCGCCGCGGACGGGGTGCGCCGGGCAGGTGTG TCGgccttcggcatcagcggcaccaacgcccac |
| | Reverse $ACP_{complete}$ | TAGCCGTCGCGAAGAGCGCTGCTCGCTTCCCGGGCGGGAGTCCC GCTGTCgagttcggcccgcaggtggcgtaccaccgc |
| | Reverse $ACP_{SacI}$ | GCCTGCTGGAGCTGGTTGCGCAGCCCGACCGCGGTCAGCGAGTC GAAGCCGAggtcccggaaggcacgcgtcggctcgac |
| pMU3-DEBS 1-TE-URA3-$Lkm_{M1}$ | Forward KS | AGCAGGCGGCACCGGCGACCACGGCCCCCGTCGACGAGCCGATC GCGATCcgtcgccatggccgtacggctgcccggcggg |
| | Forward AT | GGGAGTGGTCGCCCGCCGCGGACGGGGTGCGCCGGGCAGGTGTG TCGtcgaagaggcaccacgcgacgtccgggccgcc |
| | Reverse $ACP_{complete}$ | TAGCCGTCGCGAAGAGCGCTGCTCGCTTCCCGGGCGGGAGTCCC GCTGTCgagttcggcctccaggcgcagggcgacggc |
| | Reverse $ACP_{SacI}$ | GCCTGCTGGAGCTGGTTGCGCAGCCCGACCGCGGTCAGCGAGTC GAAGCCGAgttccgtgaacgcccggtccgcggggac |
| Sequencing of pMU3-DEBS 1-TE-URA3 | Forward complete module | cggagcgcgagaaagcgctg |
| | Forward KS-AT linker | tcggtgaagtcgaacatcgg |
| | Reverse complete and *Sac*I-fused module | gccgggctggggcacggcccg |

[a]Sequence homologous to pMU3-DEBS 1-TE-URA3 is in all caps, while that in lower case represents sequence homologous to the genomic/cosmid DNA from the various actinomycete strains used during this study.

**Table S3.** Sources of DNA used in this work.

| Module | Source of DNA |
|---|---|
| Amph M1 and M11 | *Streptomyces nodosus* strain ATCC 14899 (Prof. P. Caffrey, University College Dublin, Ireland) |
| Bfm M4 and M9 | *Kitasatospora setae* DSM 43861 strain from the DSMZ (Germany) |
| Hba M4 and M9 | *Streptomyces varsoviensis* strain DSM 40346 from the DSMZ (Germany) |
| Las M7 | *Streptomyces lasaliensis* strain NRRL 3382 (Prof. P.F. Leadlay, University of Cambridge, United Kingdom) |
| Lkm M1 | Cosmid 14F1 (Prof. K. Arakawa, Hiroshima University, Japan) |
| Nys M1 and M11 | *Streptomyces noursei* strain DSM 40635 from the DSMZ (Germany) |
| Med M7a | *Streptomyces blastmyceticus* strain 40029 from the DSMZ (Germany) |
| Olm M5 | *Streptomyces avermitilis* strain ATTC 31267 from the DSMZ (Germany) |

**Table S4.** Possible products resulting from exchanging module 2 of DEBS 1-TE for modules containing A2 and B2 KRs.

| Catalytic events (Introduced module) | Product | Retention time (min)[a] | |
|---|---|---|---|
| | | R = Et (**a**) | R = Me (**b**) |
| **B2:** Epimerisation + B-type reduction |  | 14.9 | 13.9 |
| **A1:** No epimerisation, A-type reduction |  | 15.3 | 14.3 |
| **B1:** No epimerisation, B-type reduction |  | 15.6 | 14.6 |
| **A2:** Epimerisation + A-type reduction |  | 15.8 | 14.8 |
| No reduction |  | 14.1 | 13.1 |

[a]We observed a dependence of the retention time on product yields, with low quantities of lactone leading to variation of as much as 0.1 min. However, these discrepancies were never large enough to preclude conclusive assignment of stereochemistry.

**A**

```
DEBS_M2     TGAEQAAPAT TAPVDEPIAI VGMACRLPGE VDSPERLWEL
Amph_M11    VLEVAGPVAT GGTDDEPIAI IGMSCRYPGG VSSPEQLWDL
Bfm_M4      GDARRQLREA EQARHEPIAI VSMACRYPGG ADTPELLWDL
Bfm_M9      RDTRQRLREA EARSSEPIAI VAMSCRFPGG VRDPEELWDL
Hba_M4      GEAKRRLRDA ENARREPIAI VSMGCRYPGG ADTPERLWDL
Hba_M9      QNTRRRLADA ETRAHEPIAV VSMACRFPGG VRTPDDLWEL
Las_M7      SRVRRQLQET EAASREPIAI IGMACRLPGG VDSPEGLWEL
Lkm_M1      PDPRRREEAA GHTPDEPIAV VAMAVRLPGG VRTPEQFWEL
Nys_M1      RRARRRIGEL ESKDNEPIAI VGMGCRLPGG VNSPESLWDL
Nys_M11     VLEVAGPVAT GGADDEPIAI IGMACRFPGG VSSPEQLWDL
Med_M7a     RQARRRLREV EDRHQEPIAI VAMSCRYPGG VRTPEDLWRL
Olm_M5      VRQHEDEVAP TADHDDPIAI VGMACRYPGG VRGPEDLWDL
```

**B**

```
DEBS_M2     --GEIELADG VREWSPAADG VRRAGVSAFG VSGTNAHVII
Amph_M11    -PGTVRLLGE NTDW-PQTGR PRRAAVSSFG ISGTNAHVIL
Bfm_M4      --GAVELLTE ARAW-DDTGR PRRAAVSAFG VSGTNAHLIV
Bfm_M9      --GAVELLTE ARDW-PAVDR PRRAAVSSFG MSGTNAHVVL
Hba_M4      -AGAVRVLSR SEPW-PETGR PRRAGVSAFG VSGTNAHLIL
Hba_M9      -AGAVELLTD ARPW-PETGR PRRAGVSSFG MSGTNAHLVL
Las_M7      -SGAVTLLTE PVDW-PDSDR PRRAGVSAFG ISGTNAHVIL
Lkm_M1      -RGEVRLLSE PVPW-AGAAR PRRAGVSSFG ISGTNAHVVL
Nys_M1      -AGSVRLLTE GQQW-PETGR PRRAAVSSFG ISGTNAHALL
Nys_M11     -AGTVRLLTQ ARAW-PETGR PRRAAVSSFG ISGTNAHVLL
Med_M7a     SVGAVSLLTE TTPW-PETGR PRRAAVSSFG FSGTNAHTIL
Olm_M5      -AGTVGLLTE NREW-PHTGK PRRVGVSAFG VSGTNAHVVL
```

**C**

```
DEBS_M2     TLVFDHPNA SAVAGFLDAE LGTEVRGEAP SALAGLDALE
Amph_M11    TMVFDYPNP AALAGFLHSE LADVHS--AG AVAVTAGAPV
Bfm_M4      TVVFDYSSA TALAGRLETG LLGAADQPAA GRPAAVRRPP
Bfm_M9      TLIFDHPSA TALAREIRAE LLG--RPAAD PAPAGPDTAA
Hba_M4      TVVFDYSSA TALARHLGAL LLGDAST-GE GAPLAAGALP
Hba_M9      SLIFDYPSA AALSRHIGTE LMGGDAPPTA PAPSAEPAAA
Las_M7      TLVFDHPTP EAVVRHLRAE LGLEGDGAPD PVFDELDGLE
Lkm_M1      TLVYDHPHA RAVALRLEAE LFGGARPEPA AGVAGSGSG-
Nys_M1      TMVFDHPNC AALAAFLKTT ALGVPG-AAP QQHAATGTPA
Nys_M11     TMVFDYPNP AALAAYLHGE LAGARSAAAG AAAVPTGAP-
Med_M7a     TLIFDYPTP AVLARHLRAE LAGGQLATAA PLPTAAALAD
Olm_M5      TMVFDHPTI AELADFLARG LTPEAAVPAE PATVVRVDQD
```

**D**

```
DEBS_M2     PKAVRATTPF KELGFDSLAA VRLRNLLNAA TGLRLPSTLV
Amph_M11    AEDLTDQRAF RDVGFDSLTA VGLRNRLASV TGLTLPSTMV
Las_M7      LEAVEPTRAF RDLGFDSLMA VELRNRIGAA TGLRLAPTLV
Lkm_M1      AERVPADRAF TELGFDSLAS VELRNRLTAA TGLRLPTTLV
Olm_M5      ARSVDPARAL KELGFDSLTA VELRNRLSTA TGLRLPATMV
```

**Fig. S2** Location of splice sites for introducing the heterologous modules into the DEBS 1-TE (indicated with arrows). (A) Junction retained to exchange the module while preserving intact the intermodular linker region. In purple, the highly conserved **PIAI** motif at the beginning of the KS.[8] (B) Junction retained to exchange the module while preserving the ACP/KS intermodular interface (underlined *Hind*III restriction site).[9] The conserved **GTNAH** motif used by others to swap AT domains[10] is also indicated. (C) Junction retained to exchange the module while keeping the entire ACP domain intact. In green, the conserved **L** corresponds to the end of the last $\alpha$-helix of the ACP. (D) Junction retained to exchange the module by generating a chimeric protein $ACP_X$/DEBS $ACP_6$, adjacent to a well-conserved **GFDSL** motif (blue). The underlined *Sac*I restriction site was used for the construction of the original DEBS 1-TE system.[11]

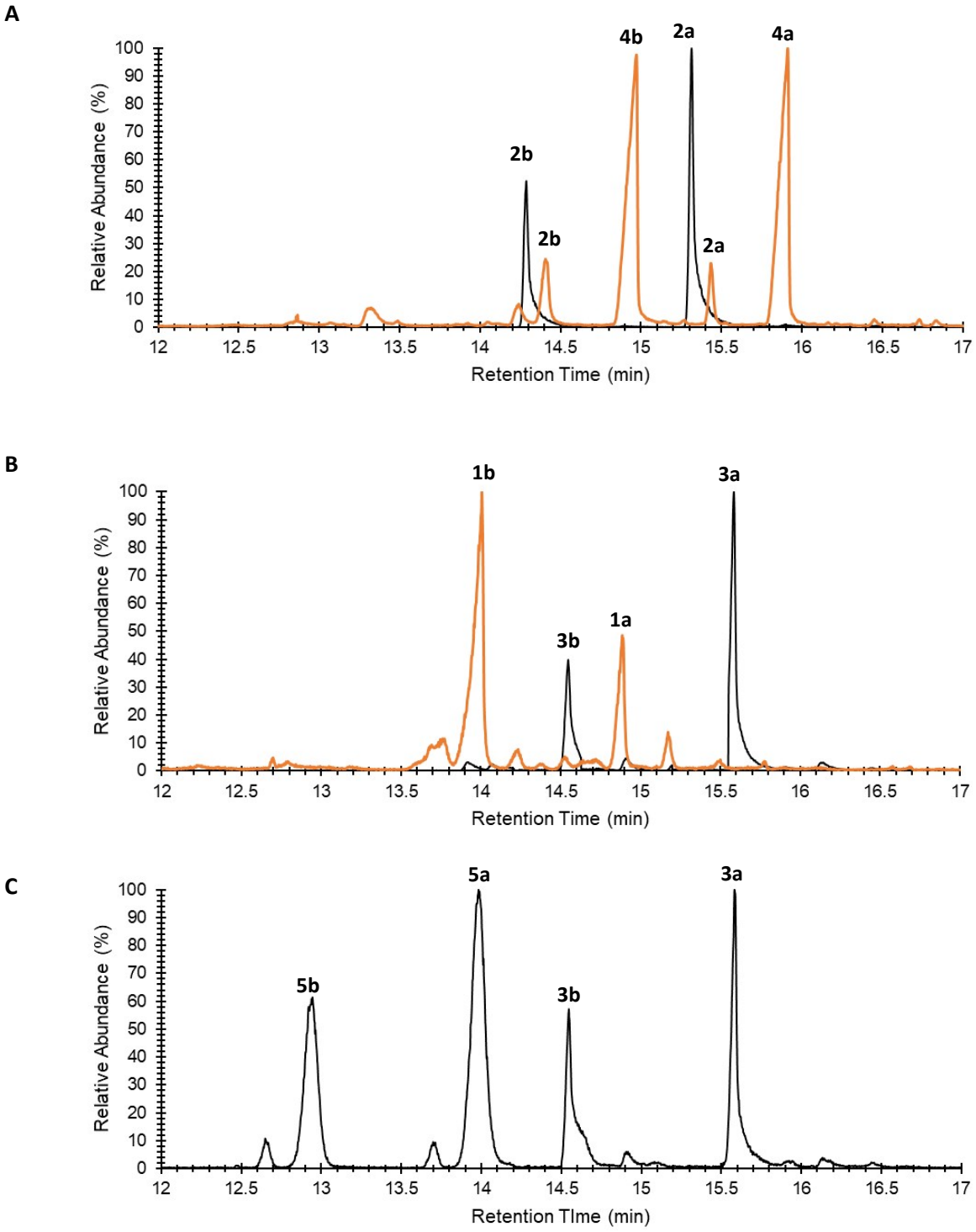
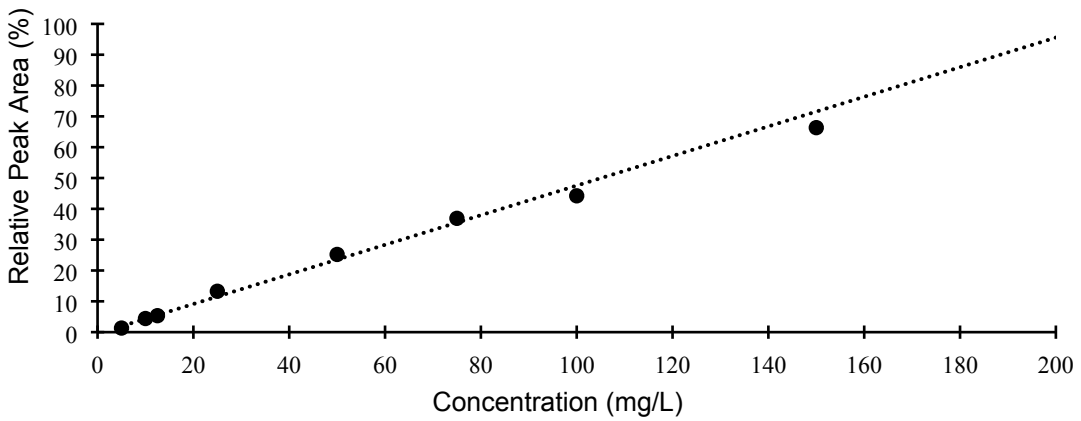



**Fig. S3** GC-MS identification of the lactones. (A) Analysis of previously-characterised strain JC2/pJLK25[2] (black) producing **2a** and **2b** superimposed with JC2/Amph M11-A (orange). As discussed in the main text, we observed some variation of retention time with lactone yield (as much as 0.1 min), explaining why peaks **2a** and **2b** in the two samples do not strictly superimpose. (B) Analysis of previously-chacterised strain JC2/pJLK30[2] (black) giving rise to **3a** and **3b** and traces of **1a**, **1b** (black), superimposed with JC2/Lkm M1-A (orange). (C) Analysis of previously-characterised strain JC2/pJLK35[2] giving rise to lactones **3a**, **3b**, **5a** and **5b**. The identities of lactones **1a**, **2a** and **3a** were confirmed by comparison to the chromatographic behaviour of synthetic standards, while those of **4a** and **4b** (shown here in (A), JC2/Amph$_{M11}$-A) were assigned by elimination.



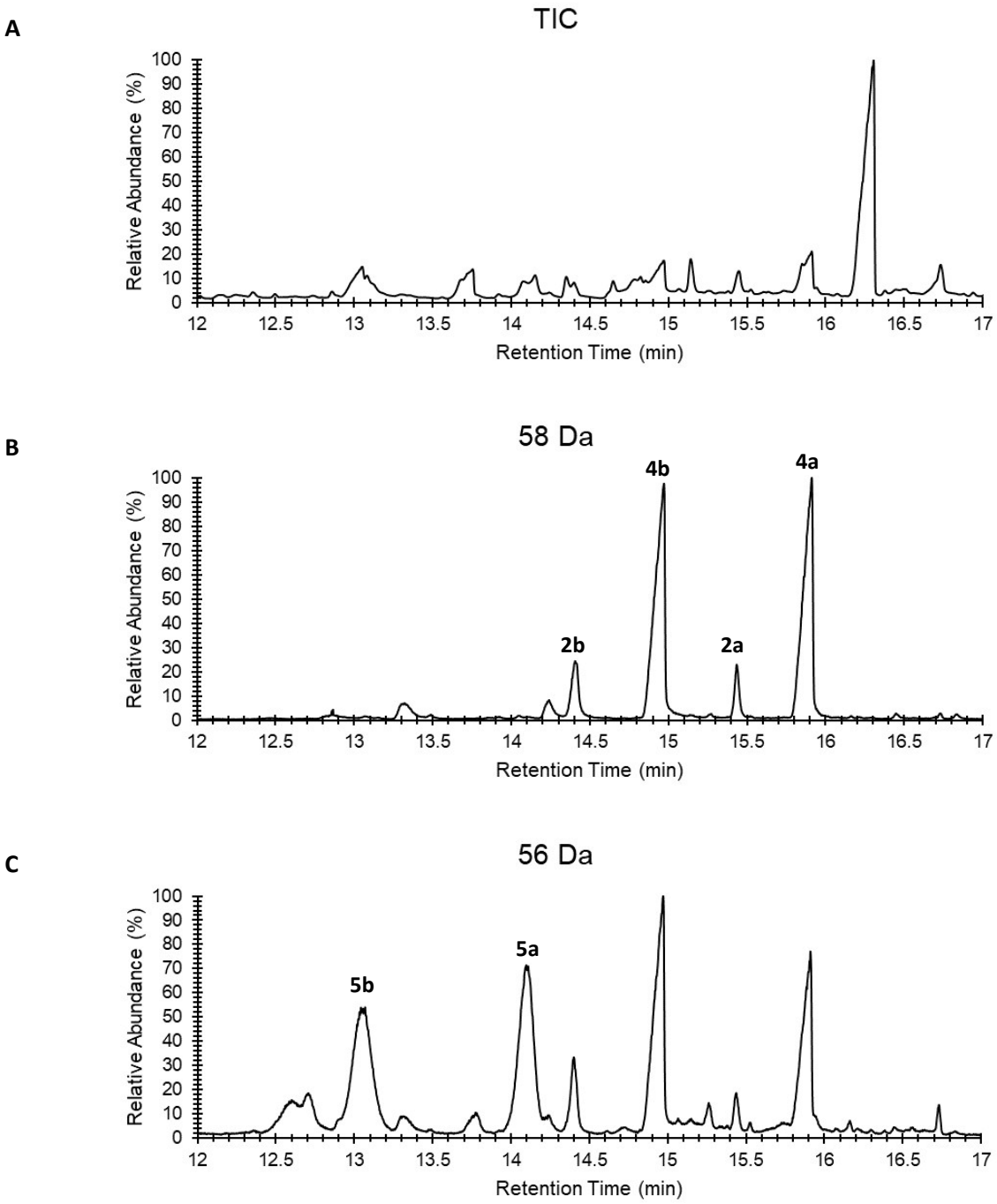

Data used to generate the calibration curve:

| Concentration (mg/L) | Calculated peak area | Relative peak area (%) |
|---|---|---|
| 200 | 56653020 | 100 |
| 150 | 37583022 | 66.3 |
| 100 | 25055348 | 44.2 |
| 75 | 20915773 | 36.9 |
| 50 | 14281548 | 25.2 |
| 25 | 7514826 | 13.2 |
| 12.5 | 3026725 | 5.3 |
| 10 | 2505535 | 4.4 |
| 5 | 751482 | 1.3 |

**Fig. S4** Calibration of lactone yields using known quantities of 2.5:1 mixture of synthetic standards of reduced lactones **1a** and **3a**. Peak areas were calculated from extracted ion chromatograms (*m/z* = 58). This analysis yielded the following equation which was used to determine lactone yields in the biological samples: $y = 0.4802x - 0.4732$ ($R^2 = 0.9925$).

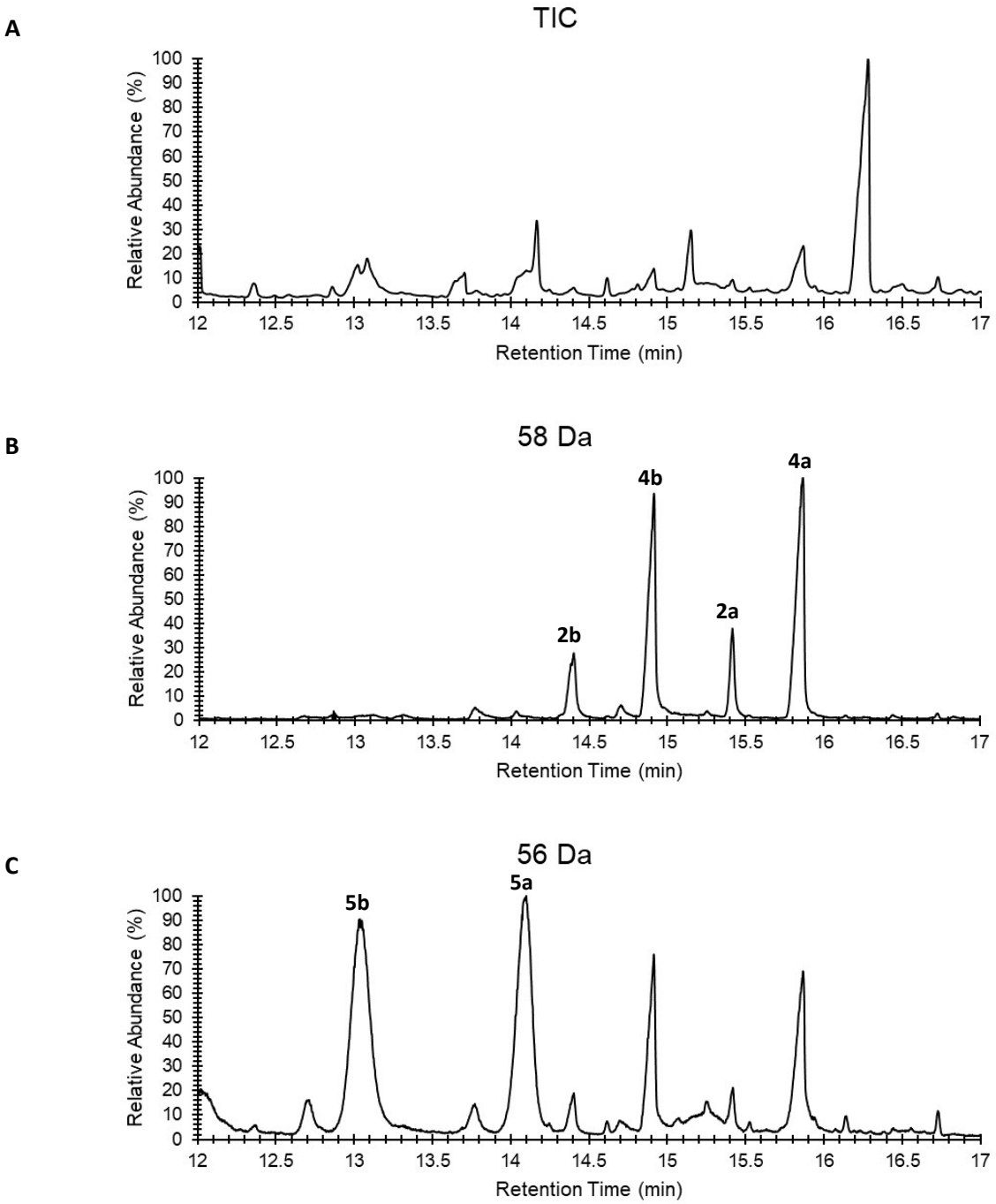
**3. Triketide lactone GC-MS data**

**Amph$_{M11}$-A:**



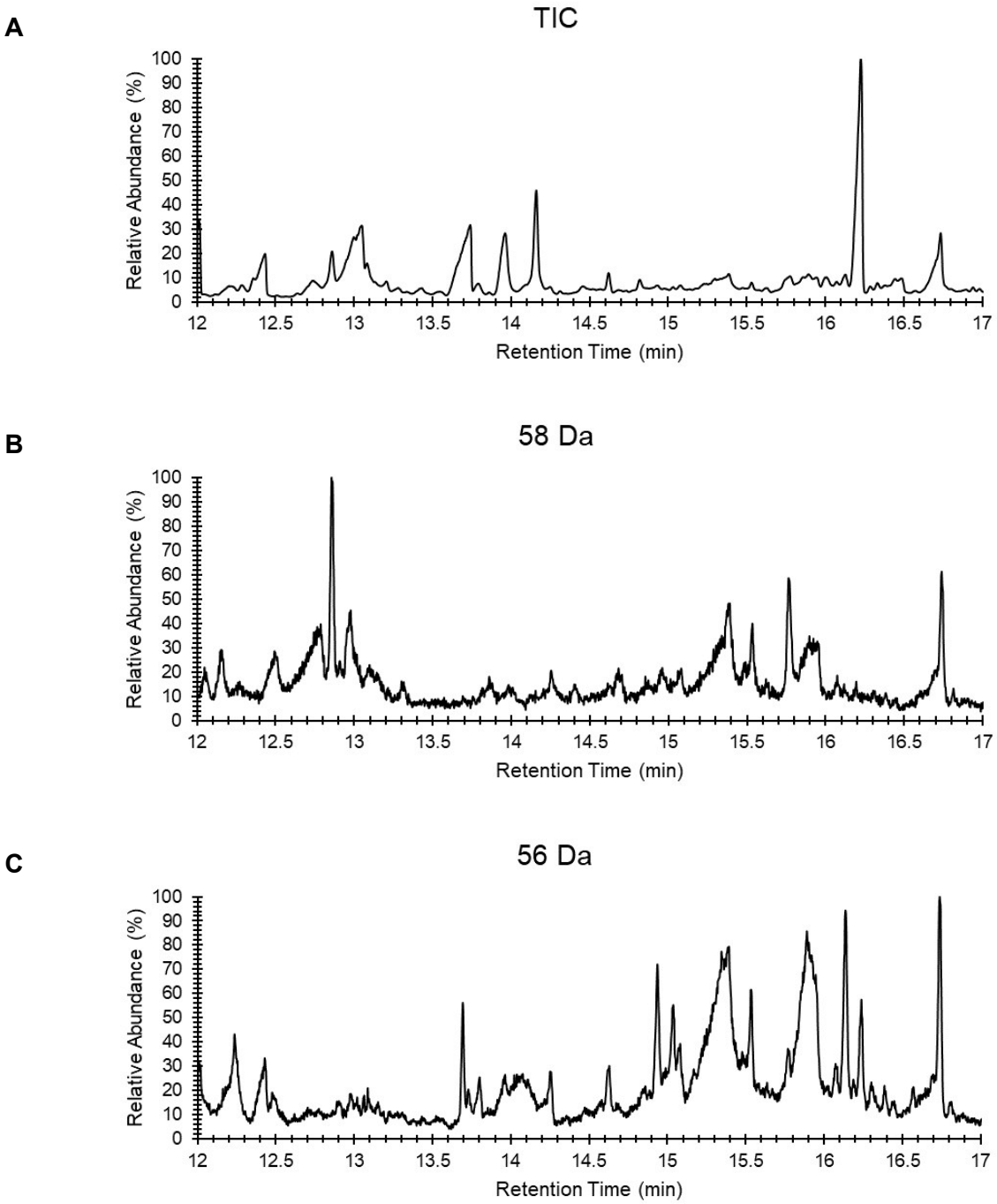

GC-MS separation and identification of triketide lactones from fermentation of *Sac. erythraea* JC2, containing pMU3-DEBS 1-TE-URA3-Amph$_{M11}$-A. (A) Total ion chromatogram (TIC). (B) Extracted ion chromatogram (*m/z* = 58 Da). The 58 Da fragment was used systematically for identification/quantification of the reduced lactones, as it is not generated by the ketolactones **5a**/**5b** and is therefore diagnostic. (C) Extracted ion chromatogram (*m/z* = 56 Da). The 56 Da fragment allowed identification/quantification of the ketolactones **5a**/**5b**, although peaks corresponding to the reduced lactones were also observed.

**Amph$_{M11}$-B:**




GC-MS separation and identification of triketide lactones from fermentation of *Sac. erythraea* JC2, containing pMU3-DEBS 1-TE-URA3-Amph$_{M11}$-B. (A) Total ion chromatogram (TIC). (B) Extracted ion chromatogram (*m/z* = 58 Da]). (C) Extracted ion chromatogram (*m/z* = 56 Da).

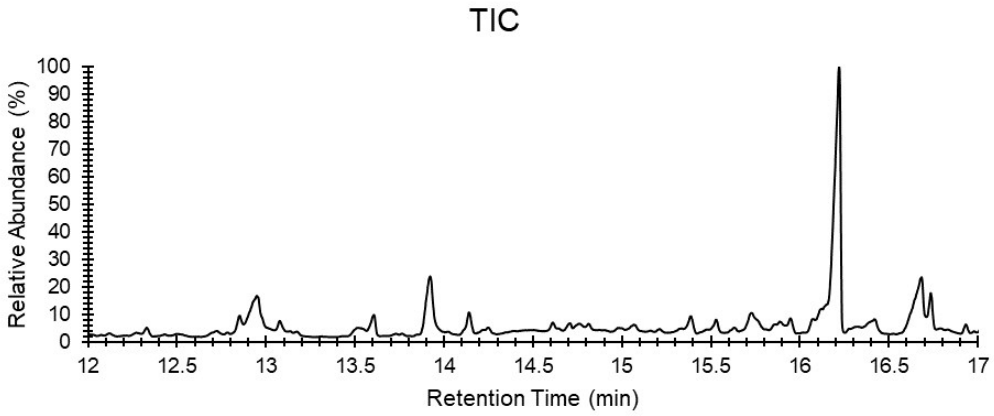
**Amph$_{M11}$-C:**



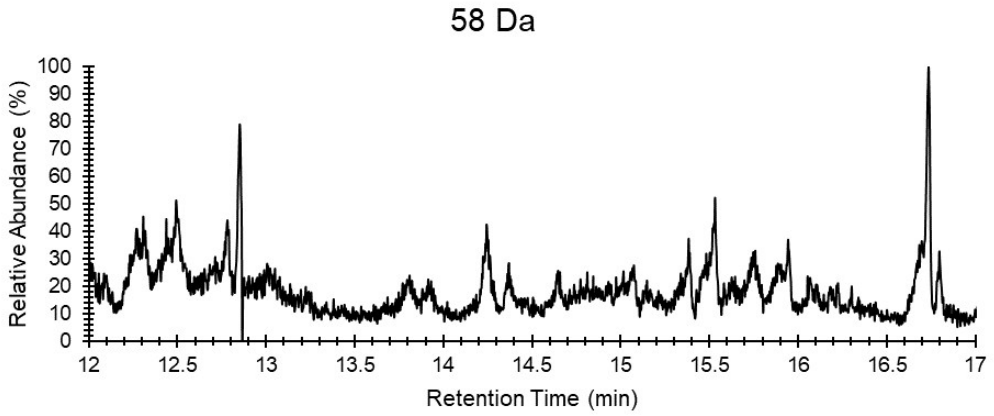

GC-MS separation and identification of triketide lactones from fermentation of *Sac. erythraea* JC2, containing pMU3-DEBS 1-TE-URA3-Amph$_{M11}$-C. (A) Total ion chromatogram (TIC). (B) Extracted ion chromatogram (*m/z* = 58 Da). (C) Extracted ion chromatogram (*m/z* = 56 Da).

**Amph$_{M11}$-D:**

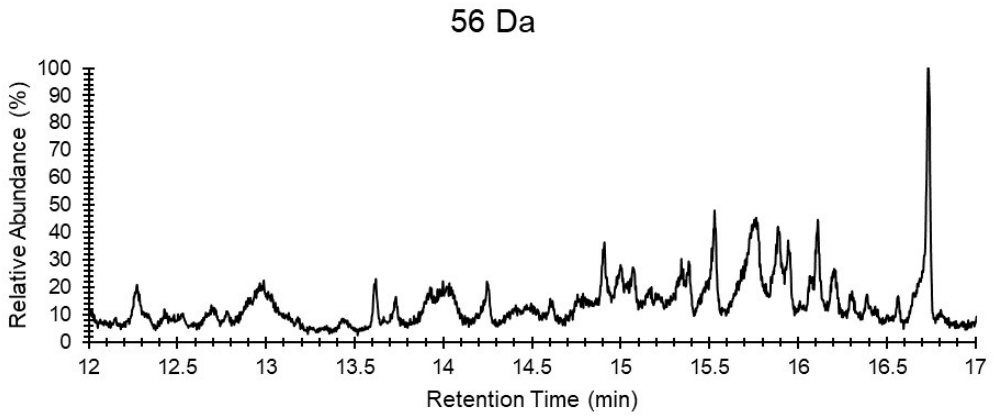
**A**




**B**

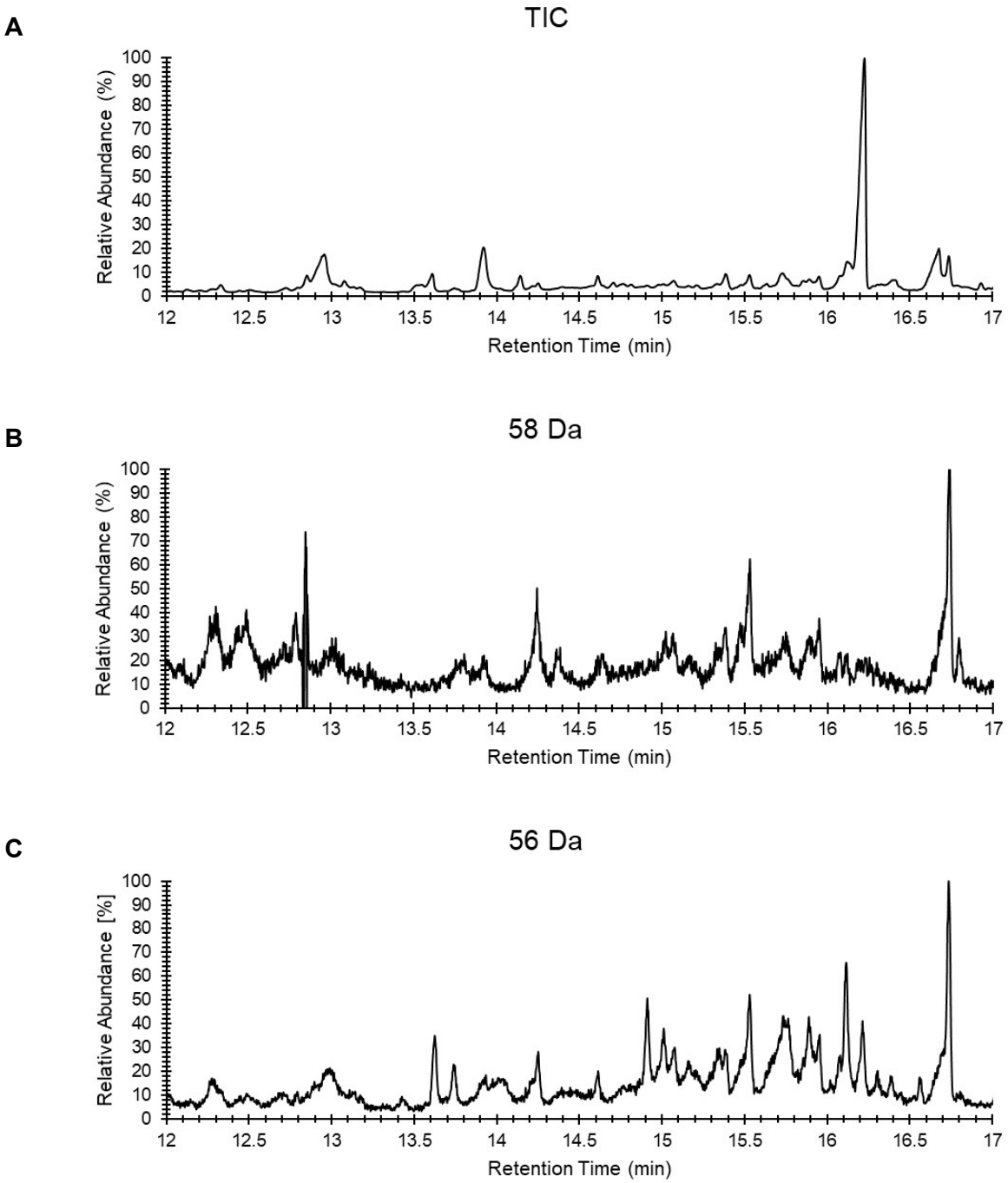



**C**



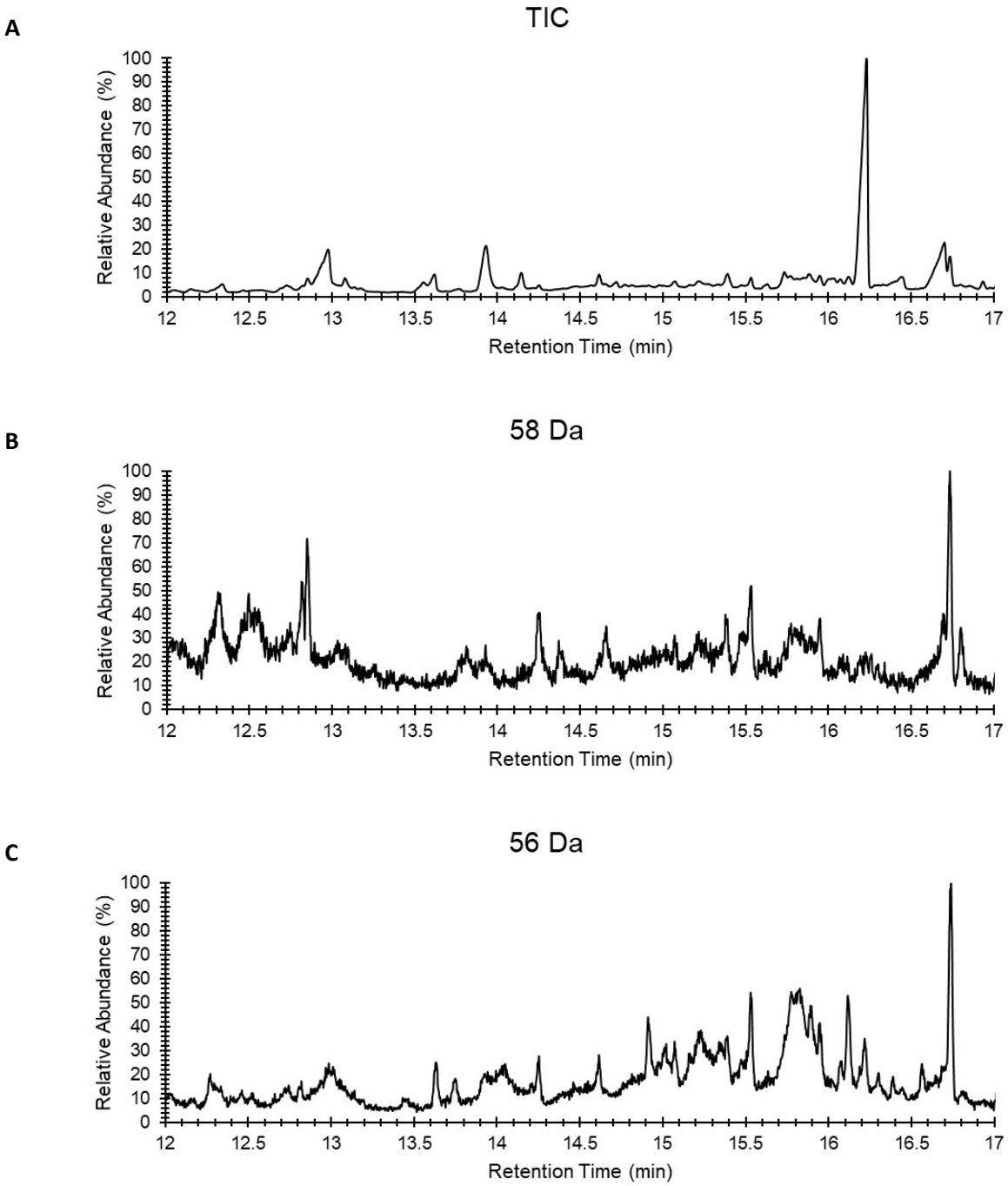

GC-MS separation and identification of triketide lactones from fermentation of *Sac. erythraea* JC2, containing pMU3-DEBS 1-TE-URA3-Amph$_{M11}$-D. (A) Total ion chromatogram (TIC). (B) Extracted ion chromatogram (*m/z* = 58 Da). (C) Extracted ion chromatogram (*m/z* = 56 Da).

**$Bfm_{M4}$-A:**



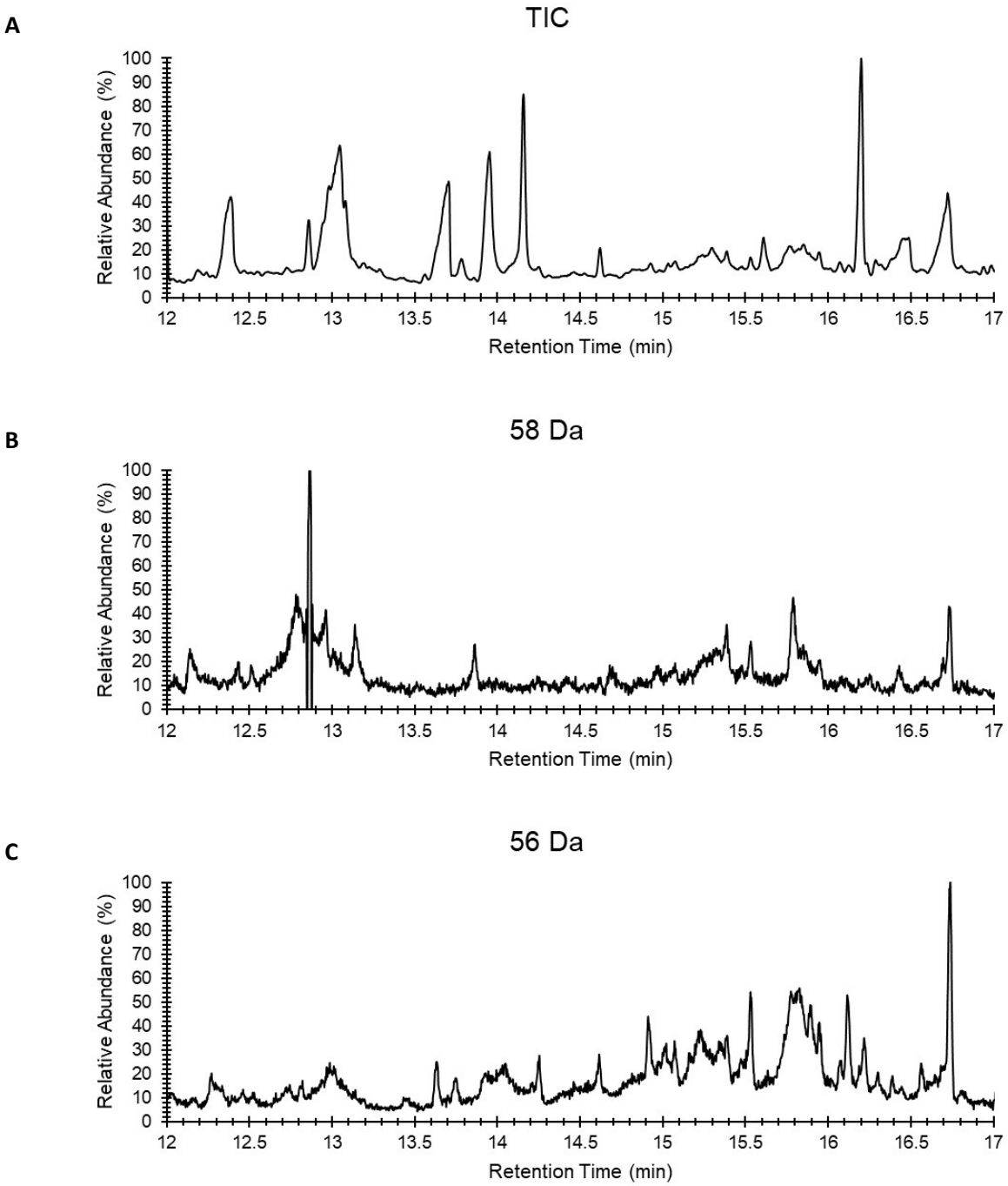

GC-MS separation and identification of triketide lactones from fermentation of *Sac. erythraea* JC2, containing pMU3-DEBS 1-TE-URA3-$Bfm_{M4}$-A. (A) Total ion chromatogram (TIC). (B) Extracted ion chromatogram (*m/z* = 58 Da). (C) Extracted ion chromatogram (*m/z* = 56 Da).

**$Bfm_{M4}$-B:**



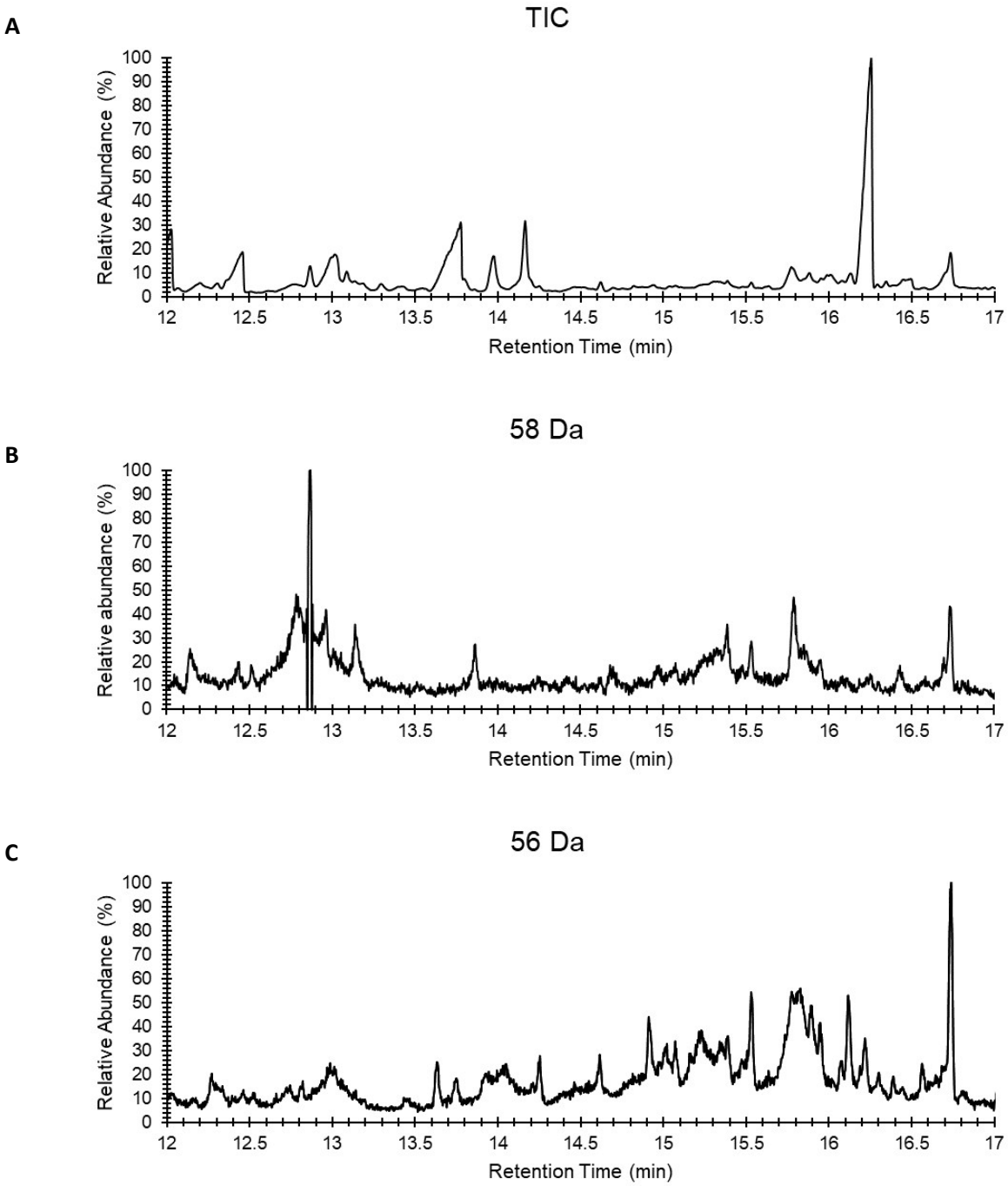

GC-MS separation and identification of triketide lactones from fermentation of *Sac. erythraea* JC2, containing pMU3-DEBS 1-TE-URA3-$Bfm_{M4}$-B. (A) Total ion chromatogram (TIC). (B) Extracted ion chromatogram (*m/z* = 58 Da). (C) Extracted ion chromatogram (*m/z* = 56 Da).

**$Bfm_{M9}$-A:**



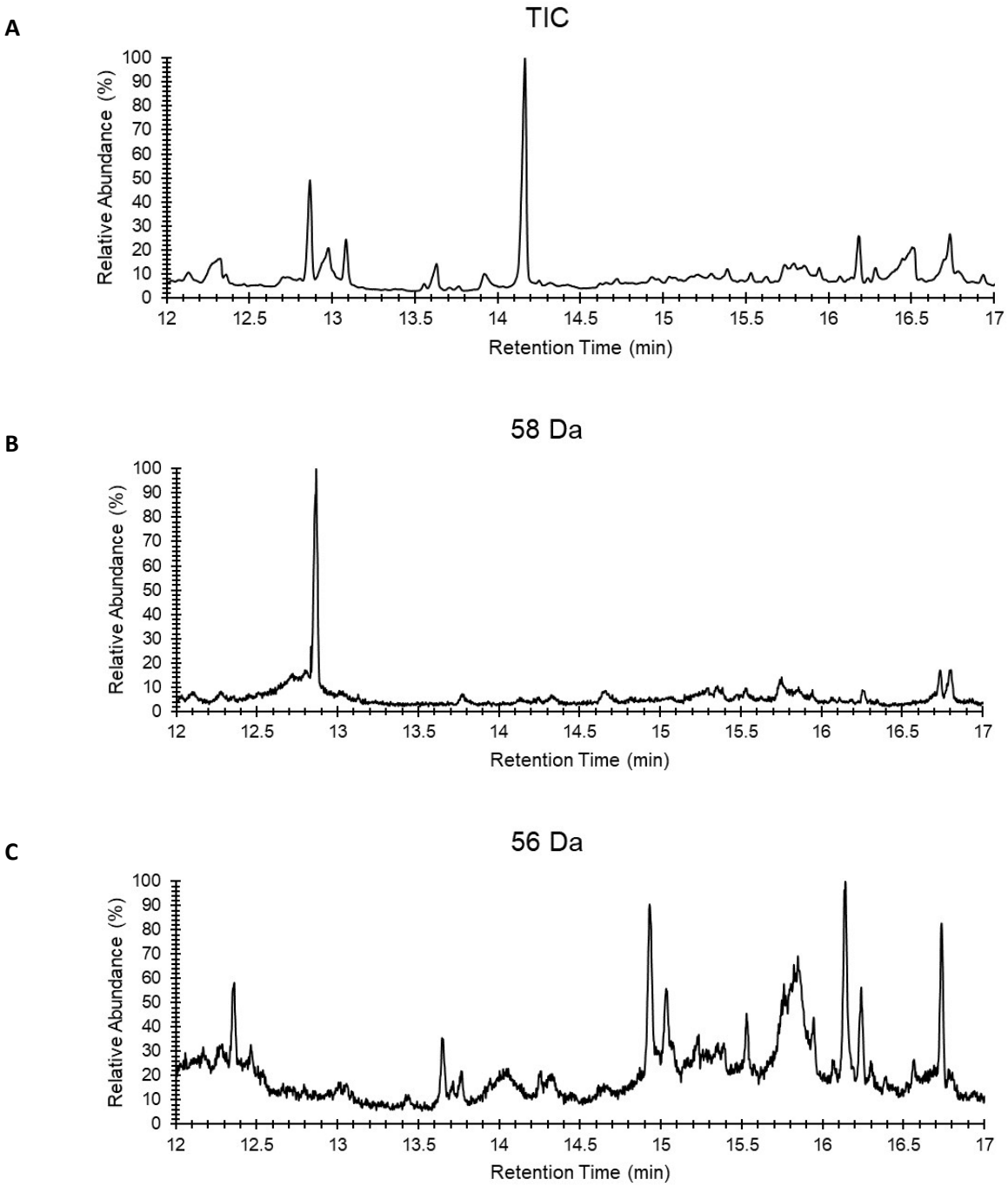

GC-MS separation and identification of triketide lactones from fermentation of *Sac. erythraea* JC2, containing pMU3-DEBS 1-TE-URA3-$Bfm_{M9}$-A. (A) Total ion chromatogram (TIC). (B) Extracted ion chromatogram (*m/z* = 58 Da). (C) Extracted ion chromatogram (*m/z* = 56 Da).

**Bfm$_{M9}$-B:**



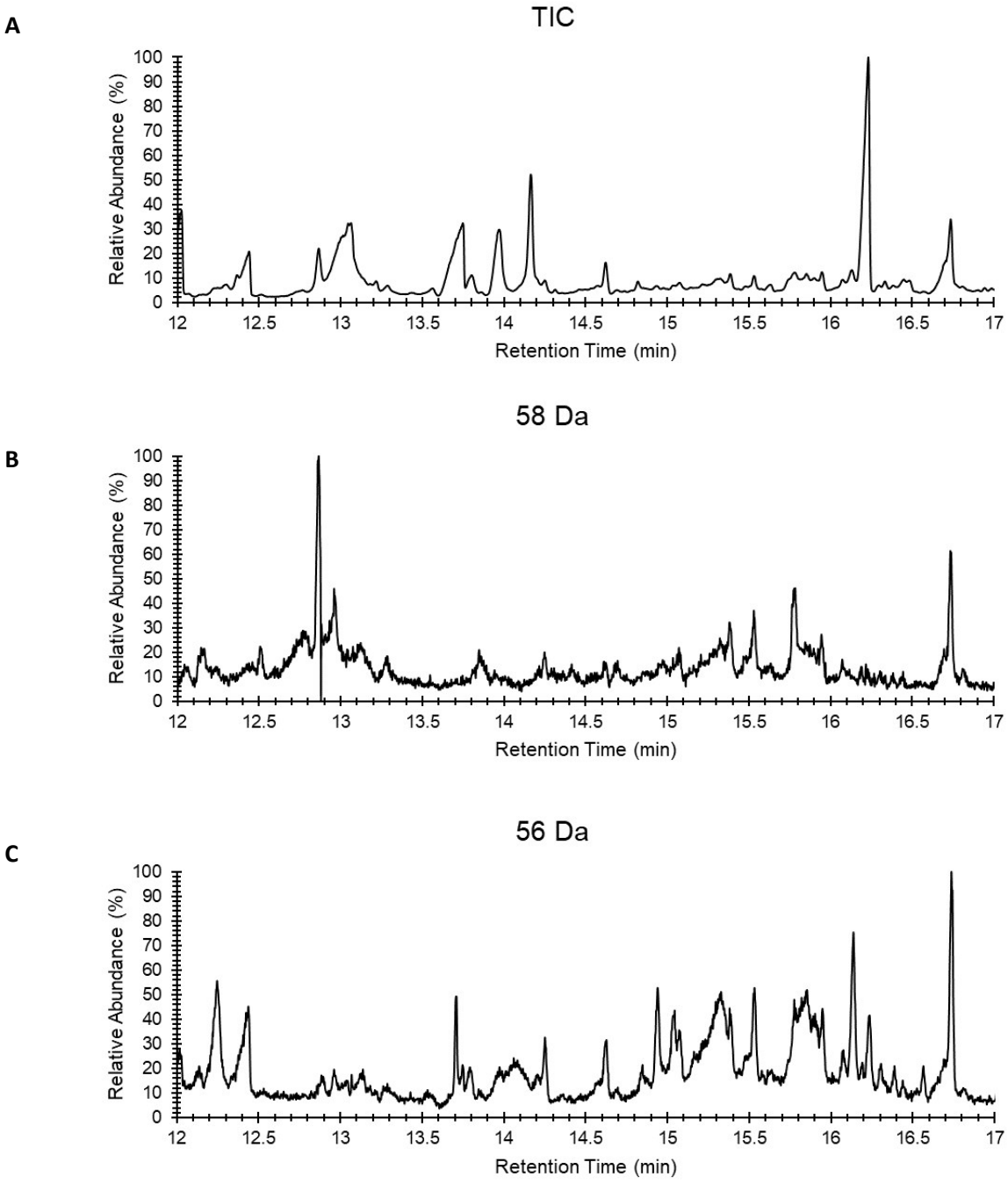

GC-MS separation and identification of triketide lactones from fermentation of *Sac. erythraea* JC2, containing pMU3-DEBS 1-TE-URA3-Bfm$_{M9}$-B. (A) Total ion chromatogram (TIC). (B) Extracted ion chromatogram (*m/z* = 58 Da). (C) Extracted ion chromatogram (*m/z* = 56 Da).

**$Hba_{M4}$-A:**



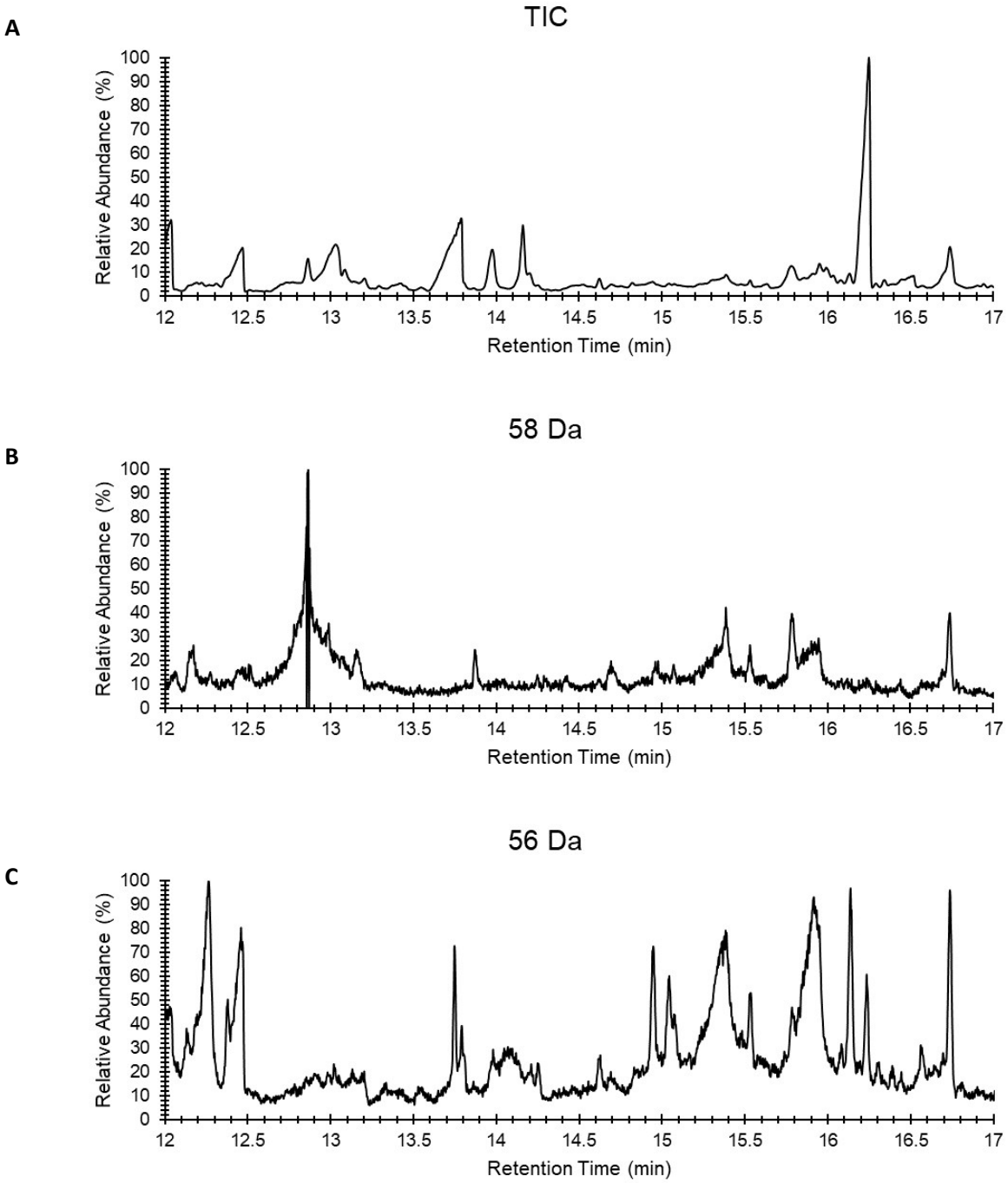

GC-MS separation and identification of triketide lactones from fermentation of *Sac. erythraea* JC2, containing pMU3-DEBS 1-TE-URA3-$Hba_{M4}$-A. (A) Total ion chromatogram (TIC). (B) Extracted ion chromatogram (*m/z* = 58 Da). (C) Extracted ion chromatogram (*m/z* = 56 Da).

**$Hba_{M4}$-B:**



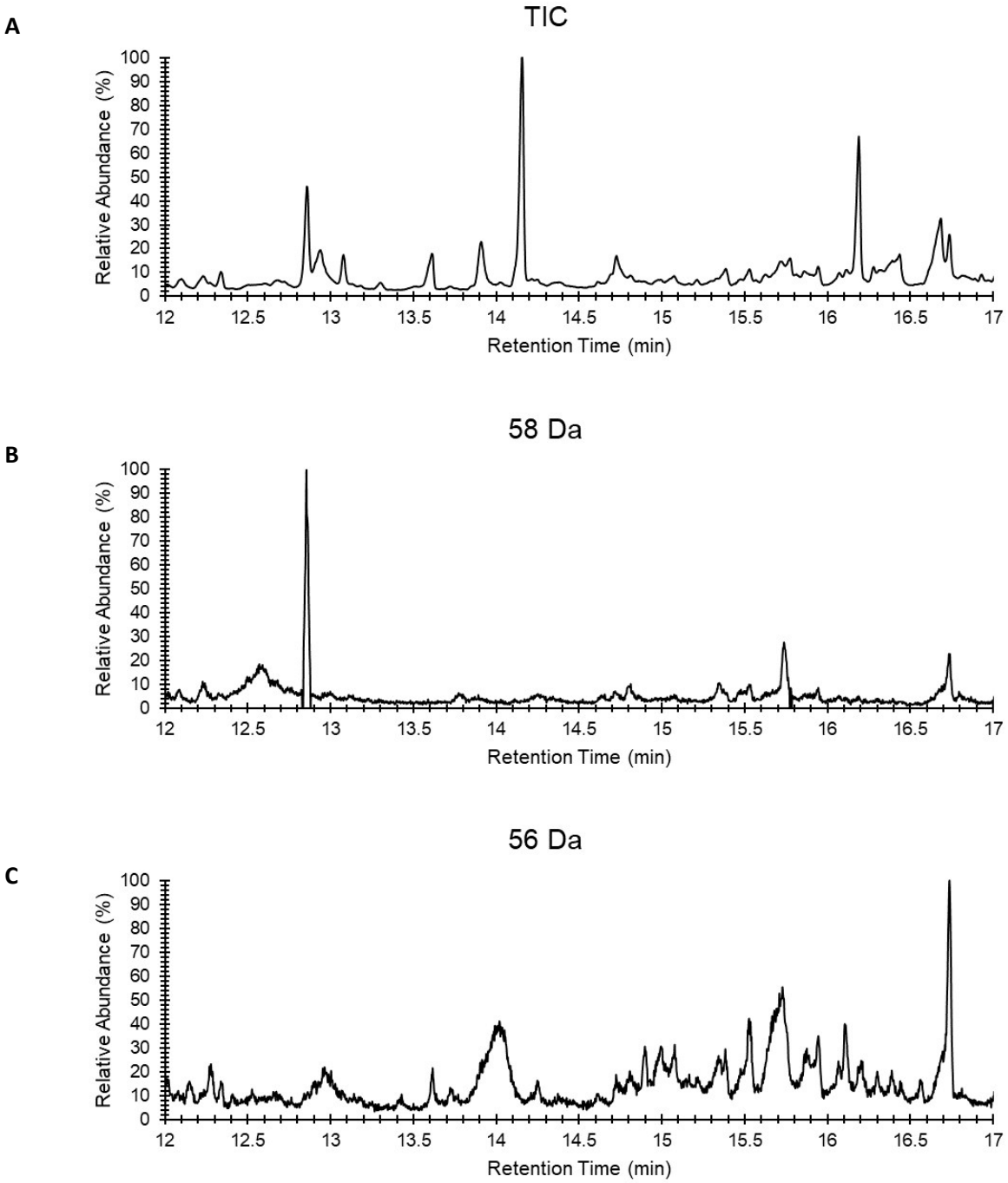

GC-MS separation and identification of triketide lactones from fermentation of *Sac. erythraea* JC2, containing pMU3-DEBS 1-TE-URA3-$Hba_{M4}$-B. (A) Total ion chromatogram (TIC). (B) Extracted ion chromatogram (*m/z* = 58 Da). (C) Extracted ion chromatogram (*m/z* = 56 Da).

**$Hba_{M9}$-A:**



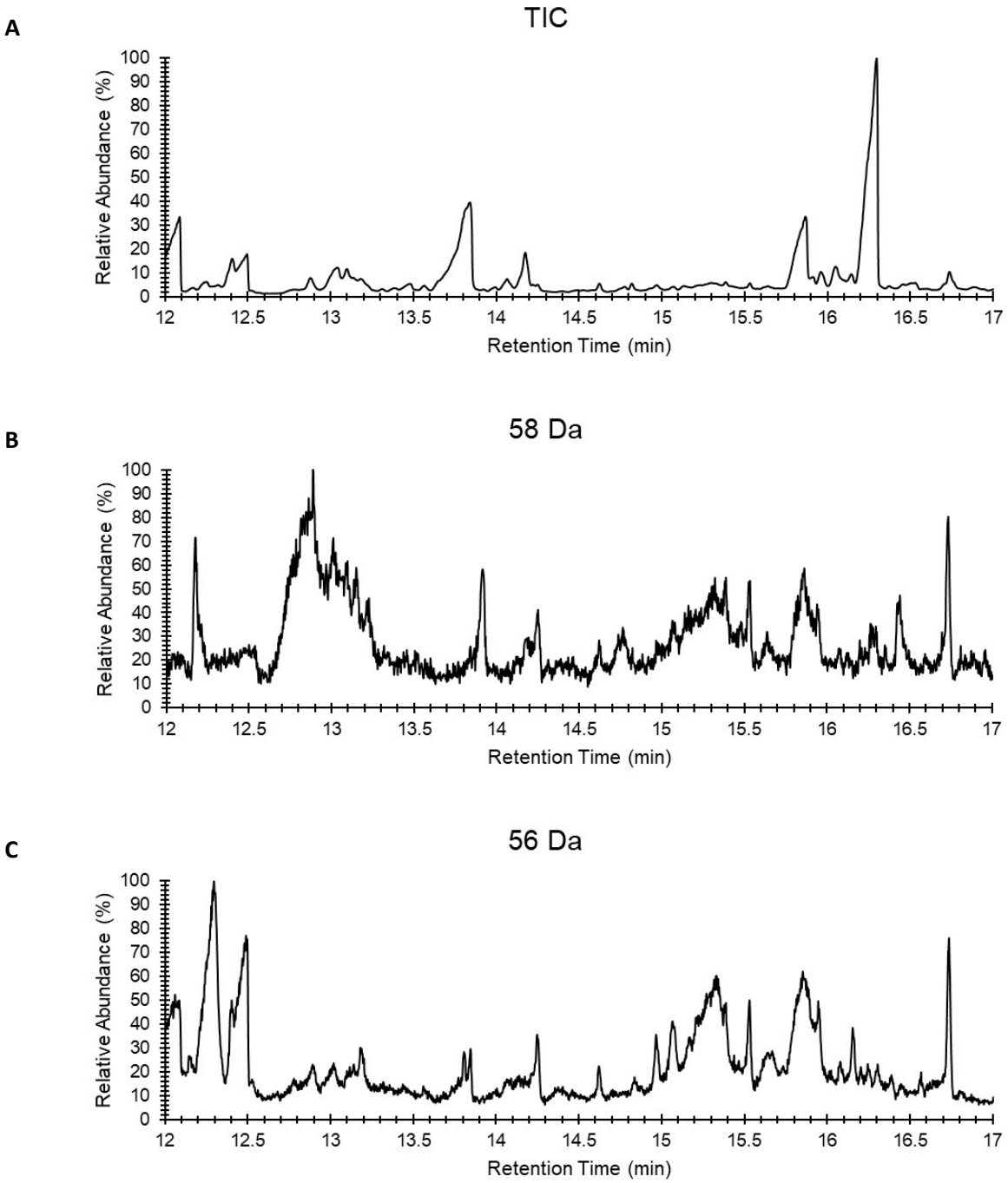

GC-MS separation and identification of triketide lactones from fermentation of *Sac. erythraea* JC2, containing pMU3-DEBS 1-TE-URA3-$Hba_{M9}$-A. (A) Total ion chromatogram (TIC). (B) Extracted ion chromatogram (*m/z* = 58 Da). (C) Extracted ion chromatogram (*m/z* = 56 Da).

**$Hba_{M9}$-B:**



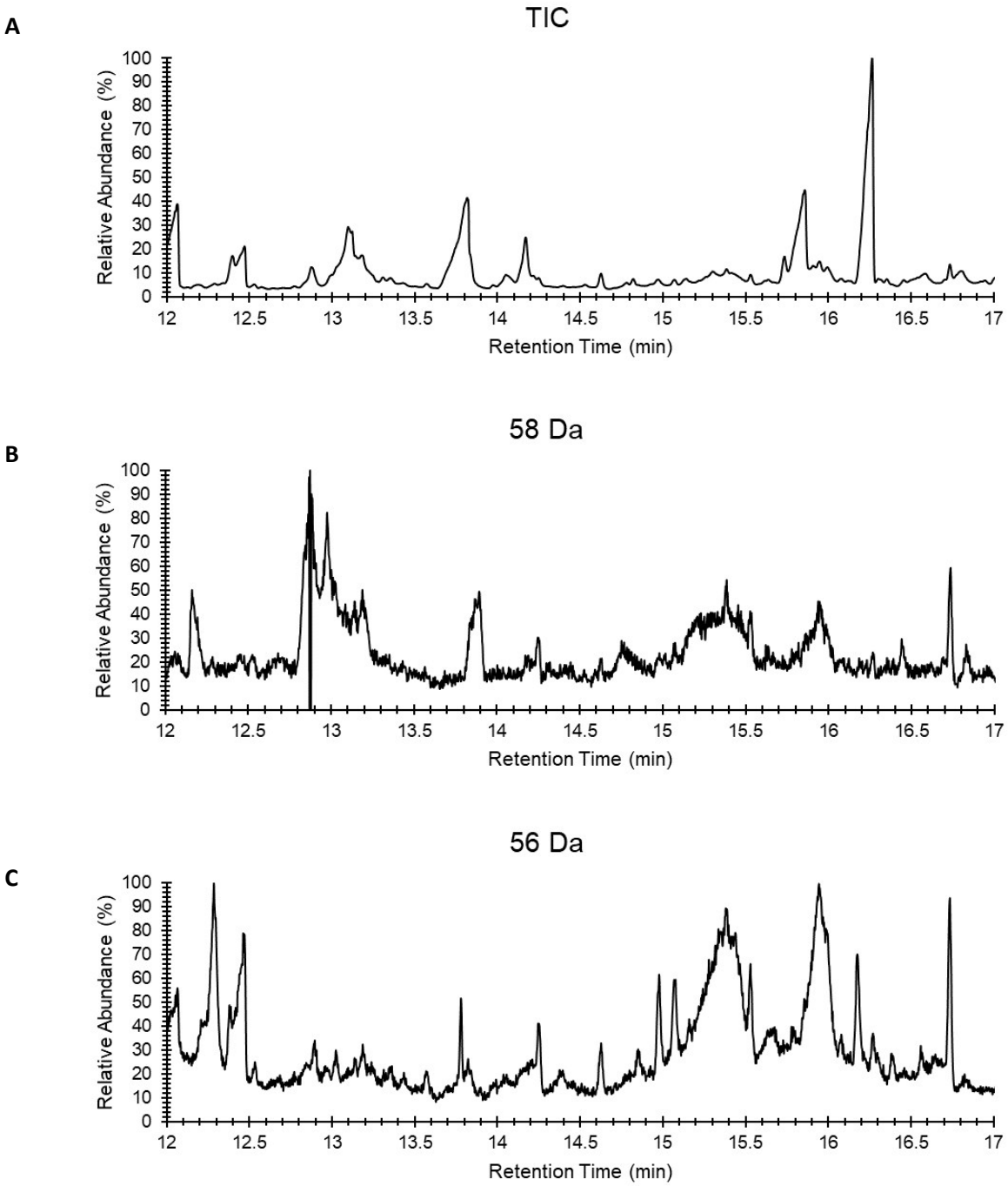

GC-MS separation and identification of triketide lactones from fermentation of *Sac. erythraea* JC2, containing pMU3-DEBS 1-TE-URA3-$Hba_{M9}$-B. (A) Total ion chromatogram (TIC). (B) Extracted ion chromatogram (*m/z* = 58 Da). (C) Extracted ion chromatogram (*m/z* = 56 Da).

**$Las_{M7}$-A:**



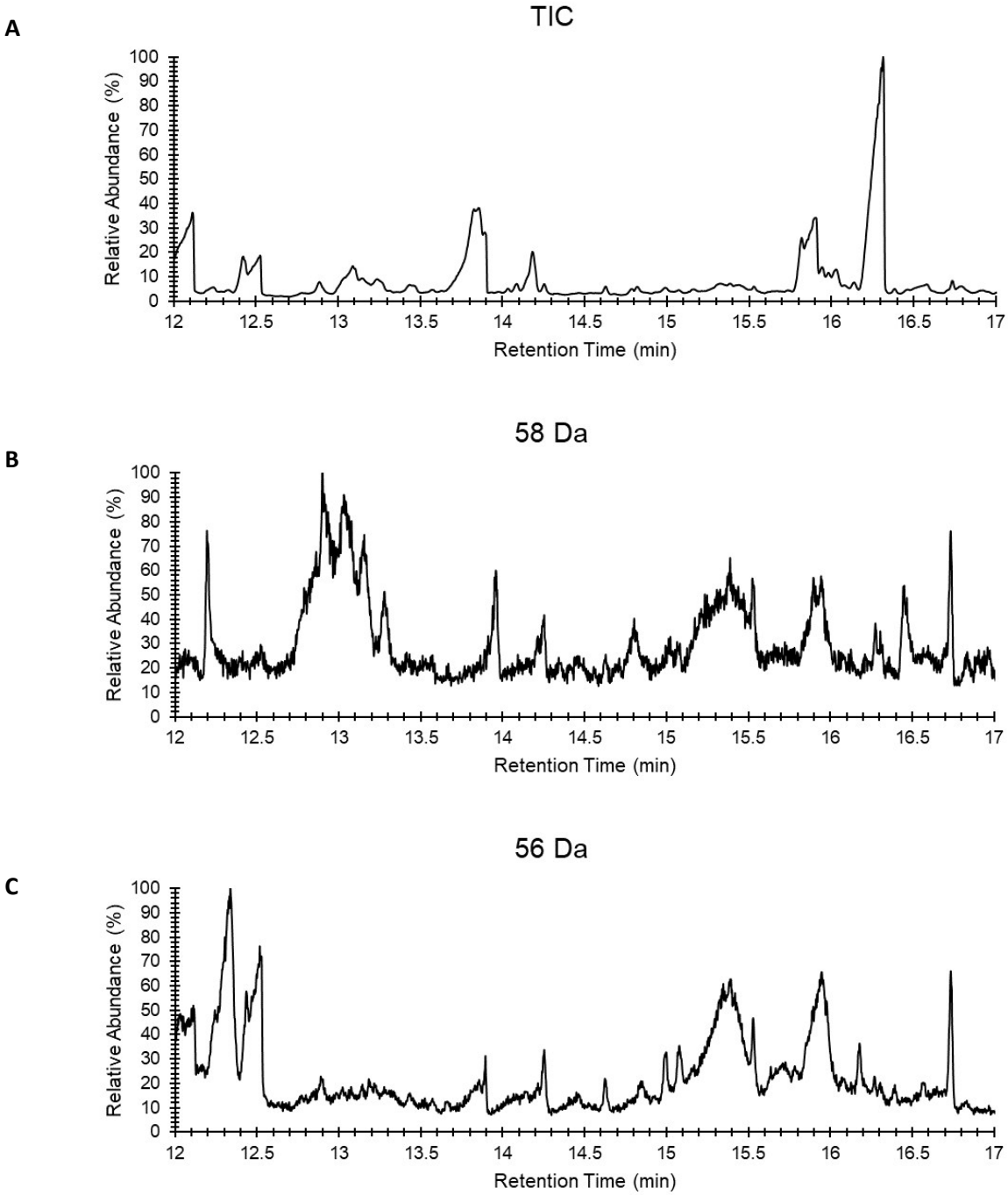

GC-MS separation and identification of triketide lactones from fermentation of *Sac. erythraea* JC2, containing pMU3-DEBS 1-TE-URA3-$Las_{M7}$-A. (A) Total ion chromatogram (TIC). (B) Extracted ion chromatogram (*m/z* = 58 Da). (C) Extracted ion chromatogram (*m/z* = 56 Da).

**$Las_{M7}$-B:**



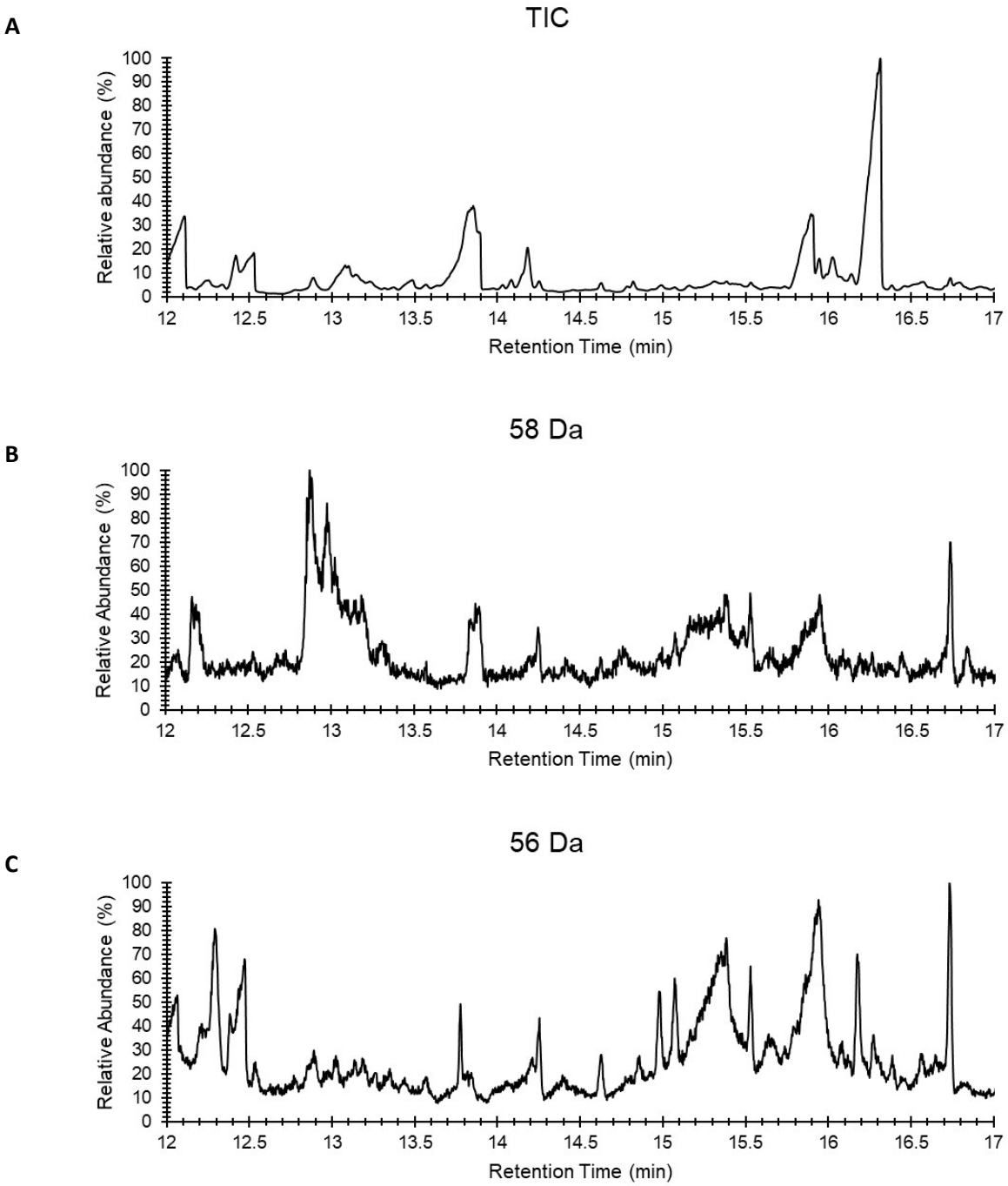

GC-MS separation and identification of triketide lactones from fermentation of *Sac. erythraea* JC2, containing pMU3-DEBS 1-TE-URA3-$Las_{M7}$-B. (A) Total ion chromatogram (TIC). (B) Extracted ion chromatogram (*m/z* = 58 Da). (C) Extracted ion chromatogram (*m/z* = 56 Da).

**$Las_{M7}$-C:**



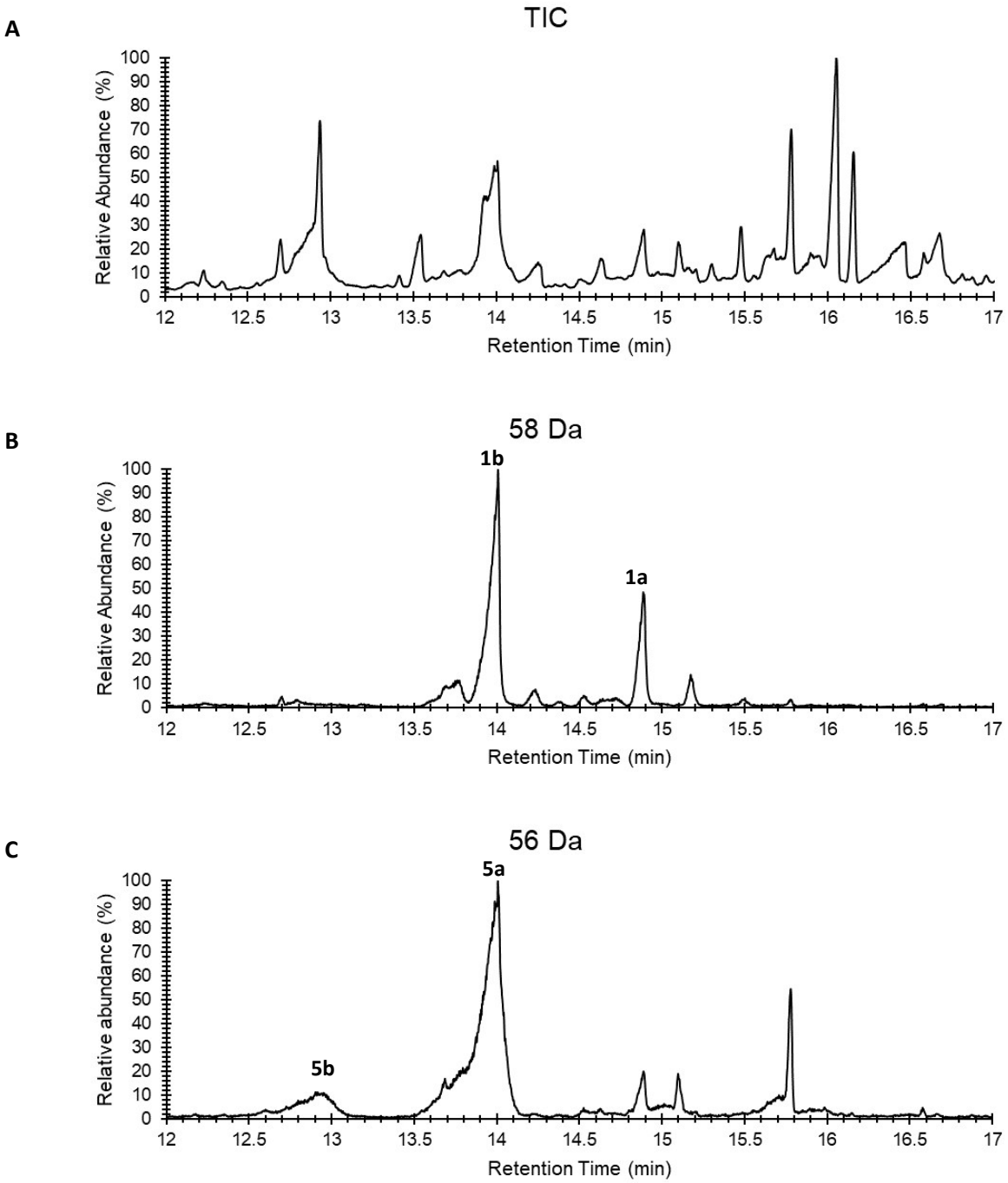

GC-MS separation and identification of triketide lactones from fermentation of *Sac. erythraea* JC2, containing pMU3-DEBS 1-TE-URA3-$Las_{M7}$-C. (A) Total ion chromatogram (TIC). (B) Extracted ion chromatogram (*m/z* = 58 Da). (C) Extracted ion chromatogram (*m/z* = 56 Da).

**$Las_{M7}$-D:**



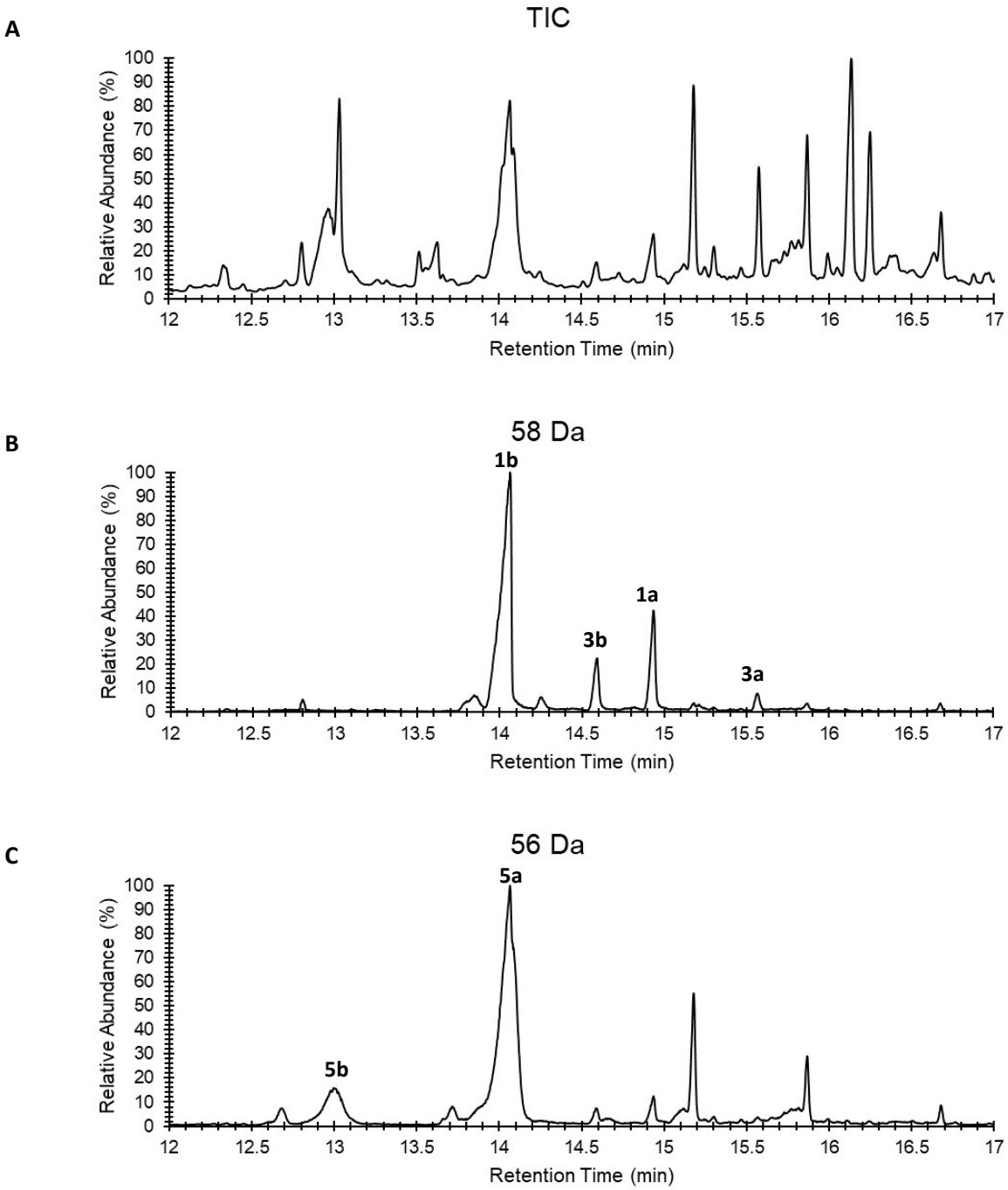

GC-MS separation and identification of triketide lactones from fermentation of *Sac. erythraea* JC2, containing pMU3-DEBS 1-TE-URA3-$Las_{M7}$-D. (A) Total ion chromatogram (TIC). (B) Extracted ion chromatogram (*m/z* = 58 Da). (C) Extracted ion chromatogram (*m/z* = 56 Da).

**Lkm$_{M1}$-A:**



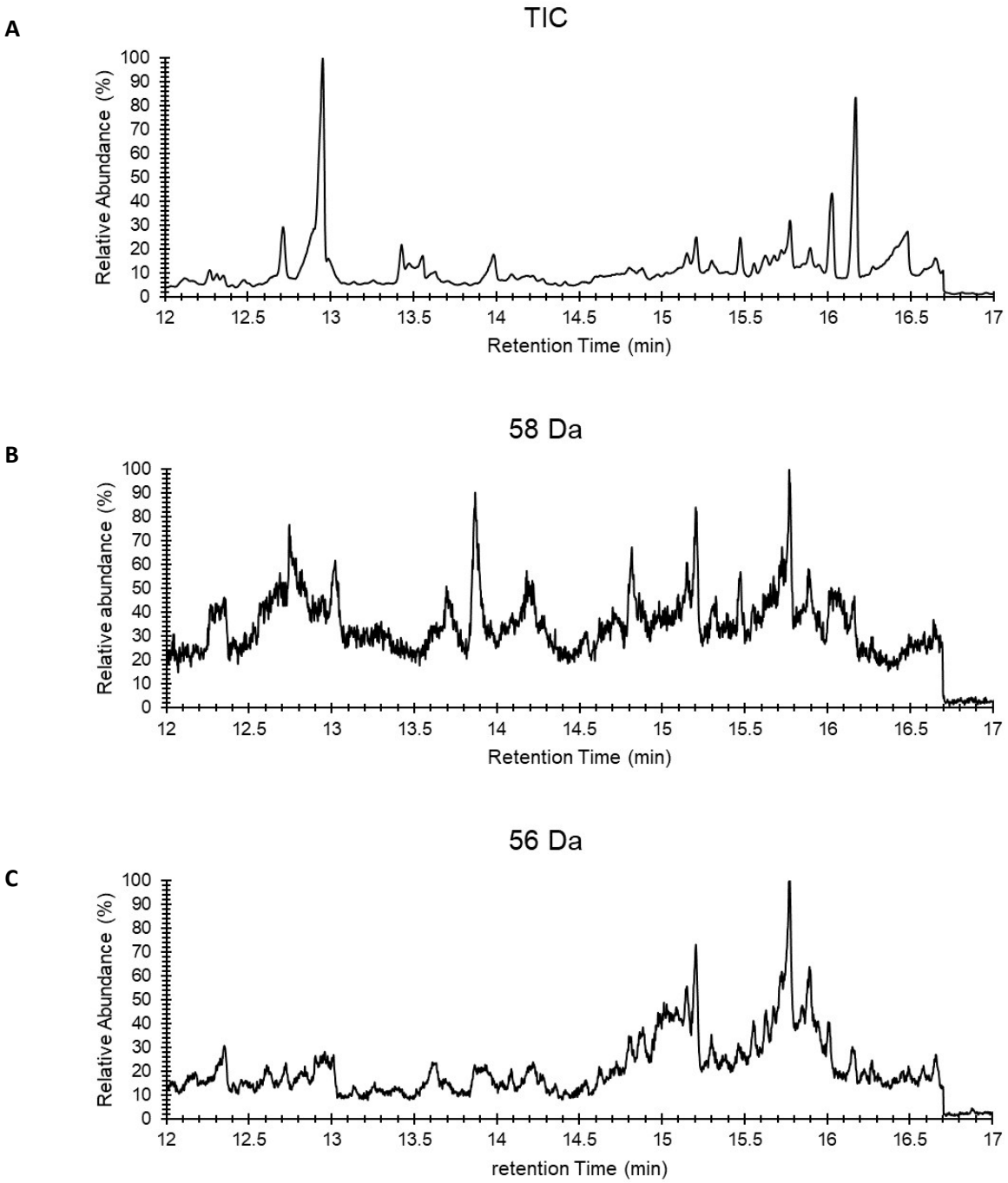

GC-MS separation and identification of triketide lactones from fermentation of *Sac. erythraea* JC2, containing pMU3-DEBS 1-TE-URA3-Lkm$_{M1}$-A. (A) Total ion chromatogram (TIC). (B) Extracted ion chromatogram (*m/z* = 58 Da). (C) Extracted ion chromatogram (*m/z* = 56 Da).

**Lkm$_{M1}$-B:**



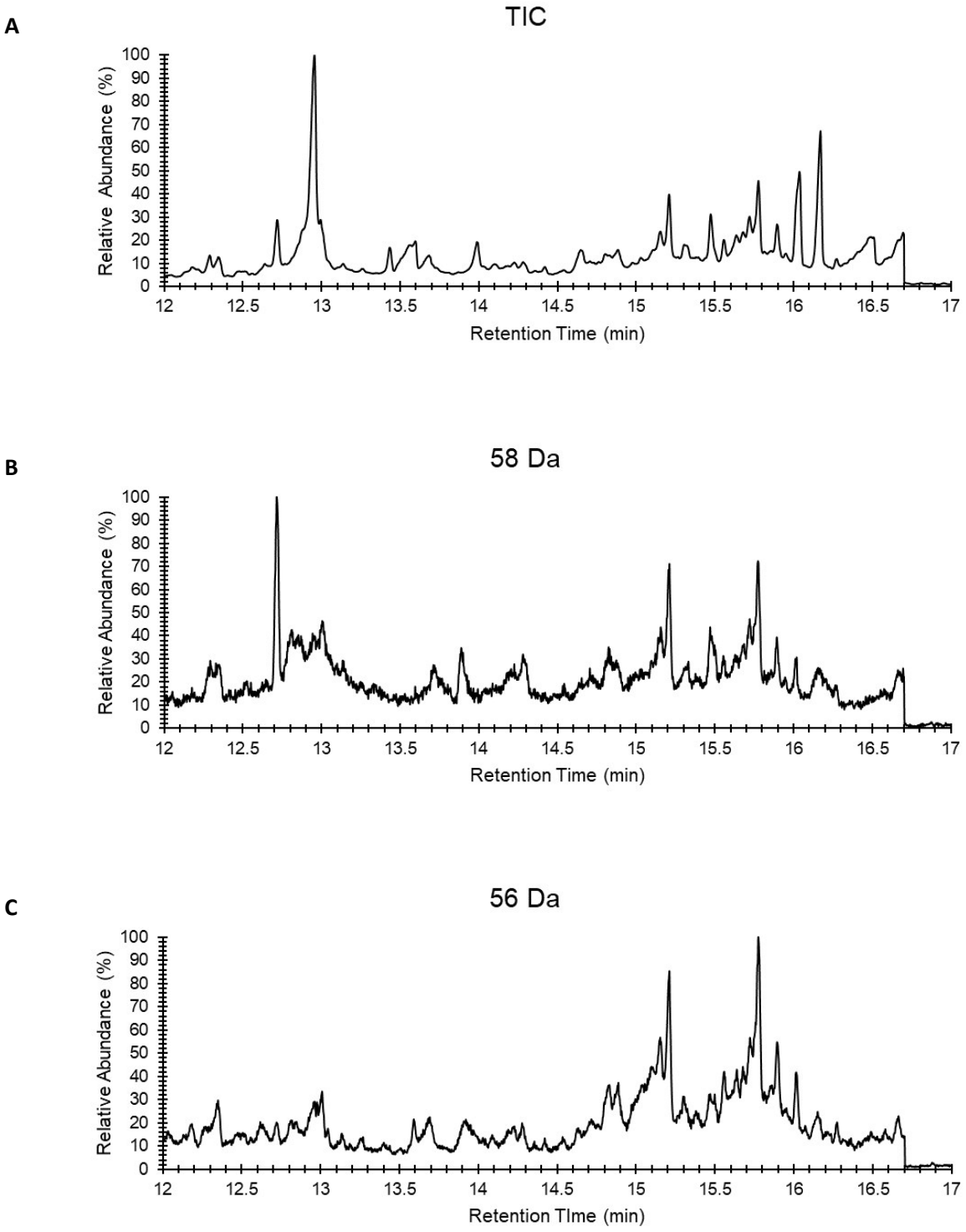

GC-MS separation and identification of triketide lactones from fermentation of *Sac. erythraea* JC2, containing pMU3-DEBS 1-TE-URA3-Lkm$_{M1}$-B. (A) Total ion chromatogram (TIC). (B) Extracted ion chromatogram (*m/z* = 58 Da). (C) Extracted ion chromatogram (*m/z* = 56 Da).

**Lkm$_{M1}$-C:**



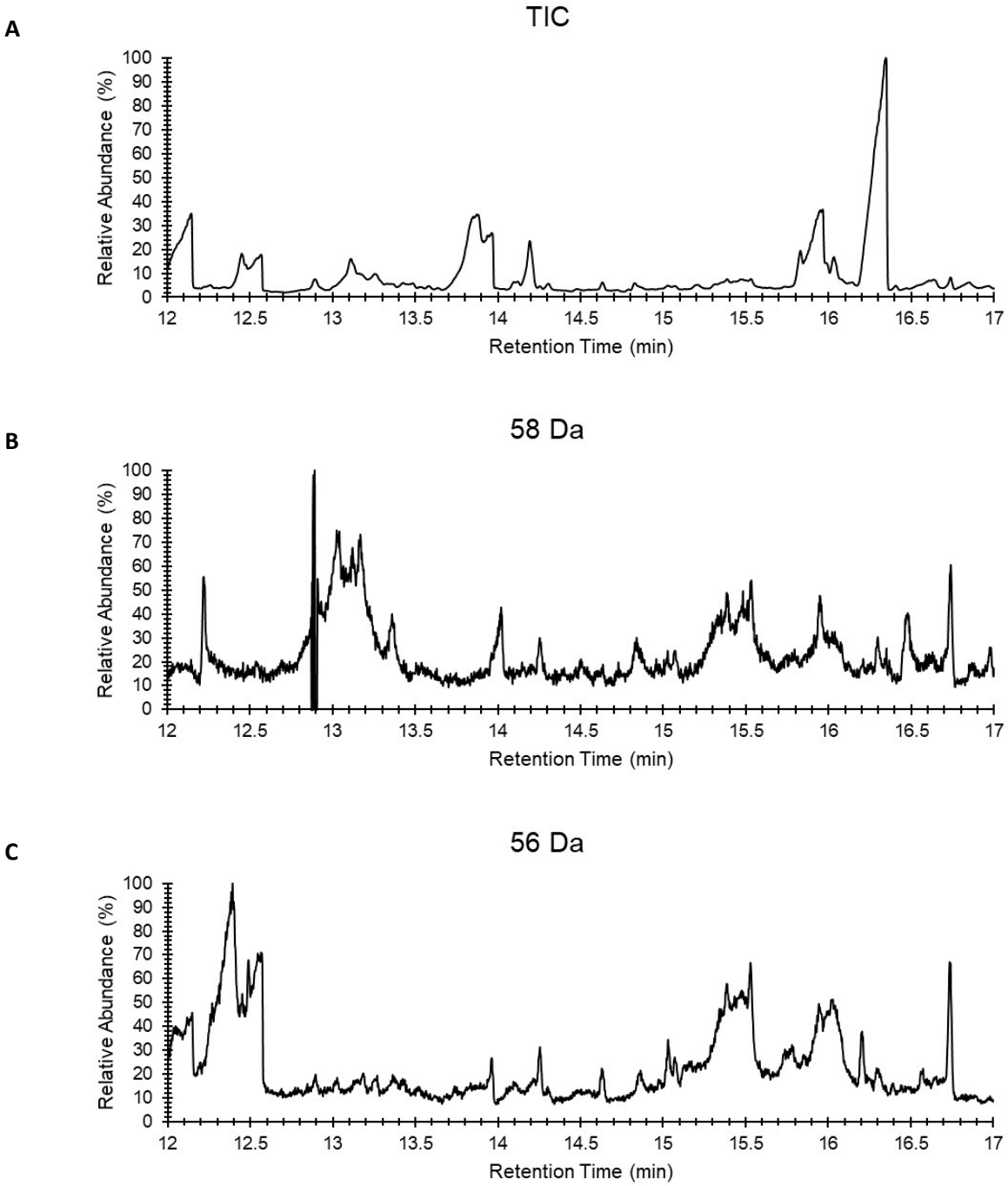

GC-MS separation and identification of triketide lactones from fermentation of *Sac. erythraea* JC2, containing pMU3-DEBS 1-TE-URA3-Lkm$_{M1}$-C. (A) Total ion chromatogram (TIC). (B) Extracted ion chromatogram (*m/z* = 58 Da). (C) Extracted ion chromatogram (*m/z* = 56 Da).

**Lkm$_{M1}$-D:**



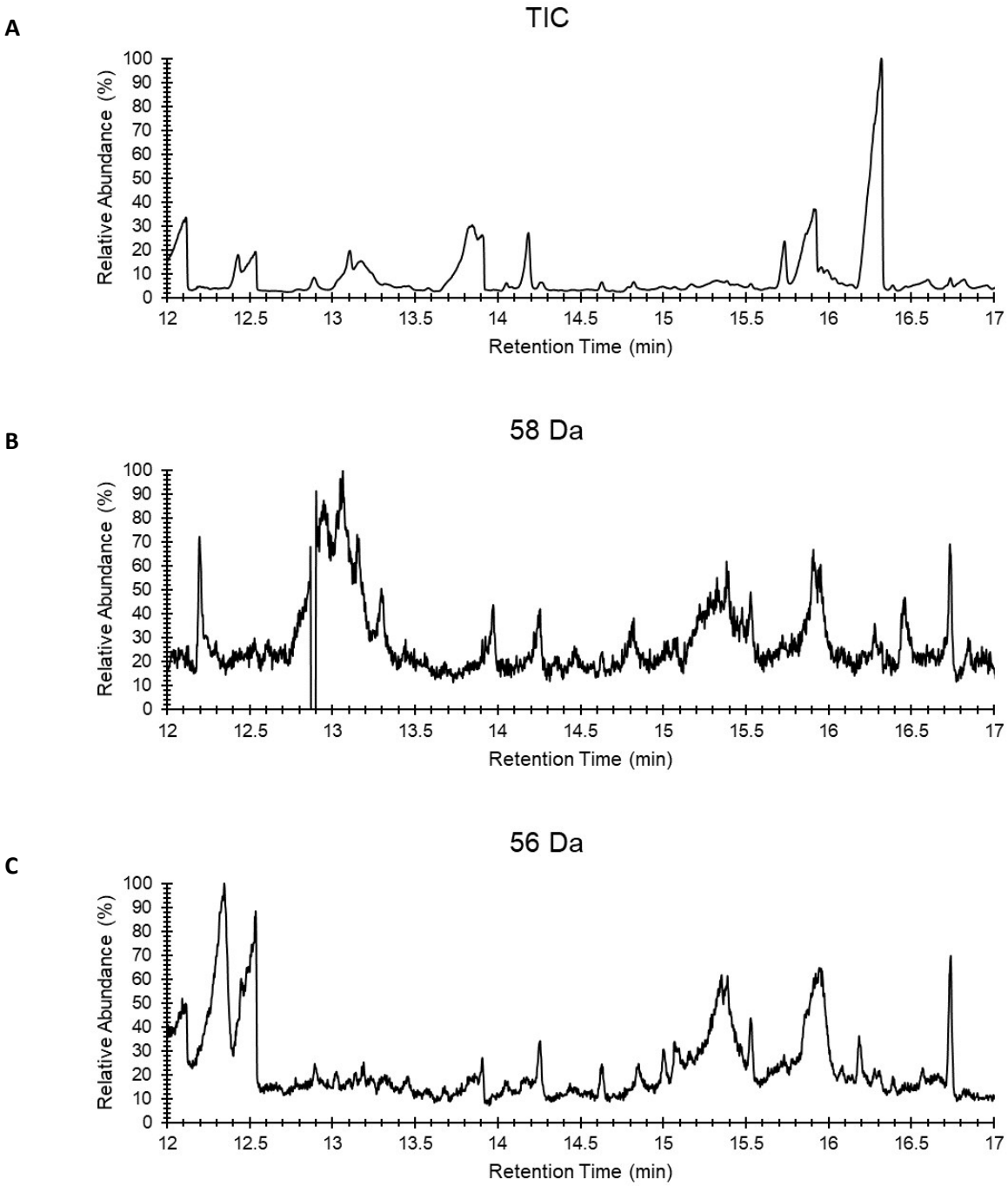

GC-MS separation and identification of triketide lactones from fermentation of *Sac. erythraea* JC2, containing pMU3-DEBS 1-TE-URA3-Lkm$_{M1}$-D. (A) Total ion chromatogram (TIC). (B) Extracted ion chromatogram (*m/z* = 58 Da). (C) Extracted ion chromatogram (*m/z* = 56 Da).

**Med$_{M7a}$-A:**



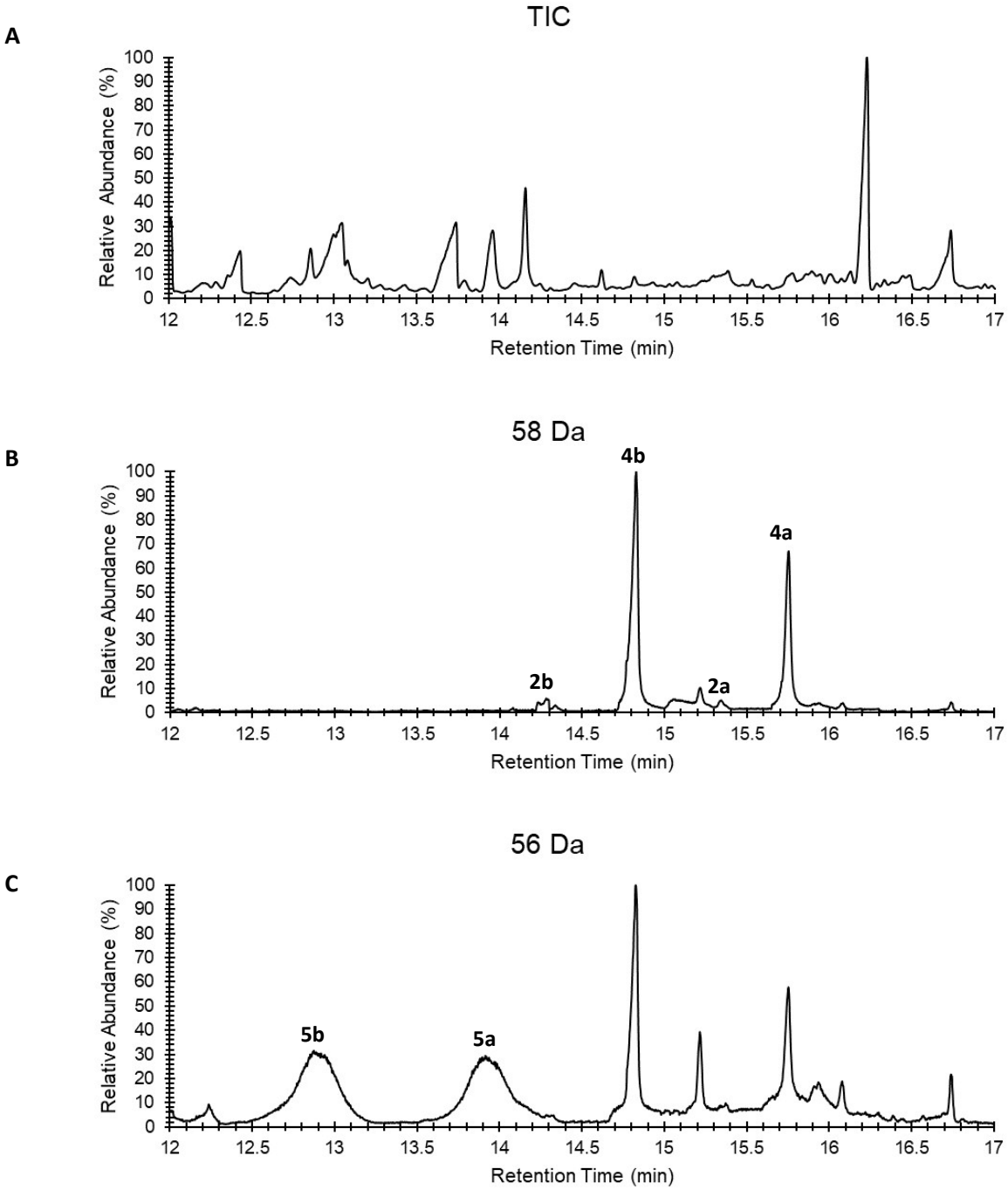

GC-MS separation and identification of triketide lactones from fermentation of *Sac. erythraea* JC2, containing pMU3-DEBS 1-TE-URA3-Med$_{M7a}$-A. (A) Total ion chromatogram (TIC). (B) Extracted ion chromatogram (*m/z* = 58 Da). (C) Extracted ion chromatogram (*m/z* = 56 Da).

**Med$_{M7a}$-B:**



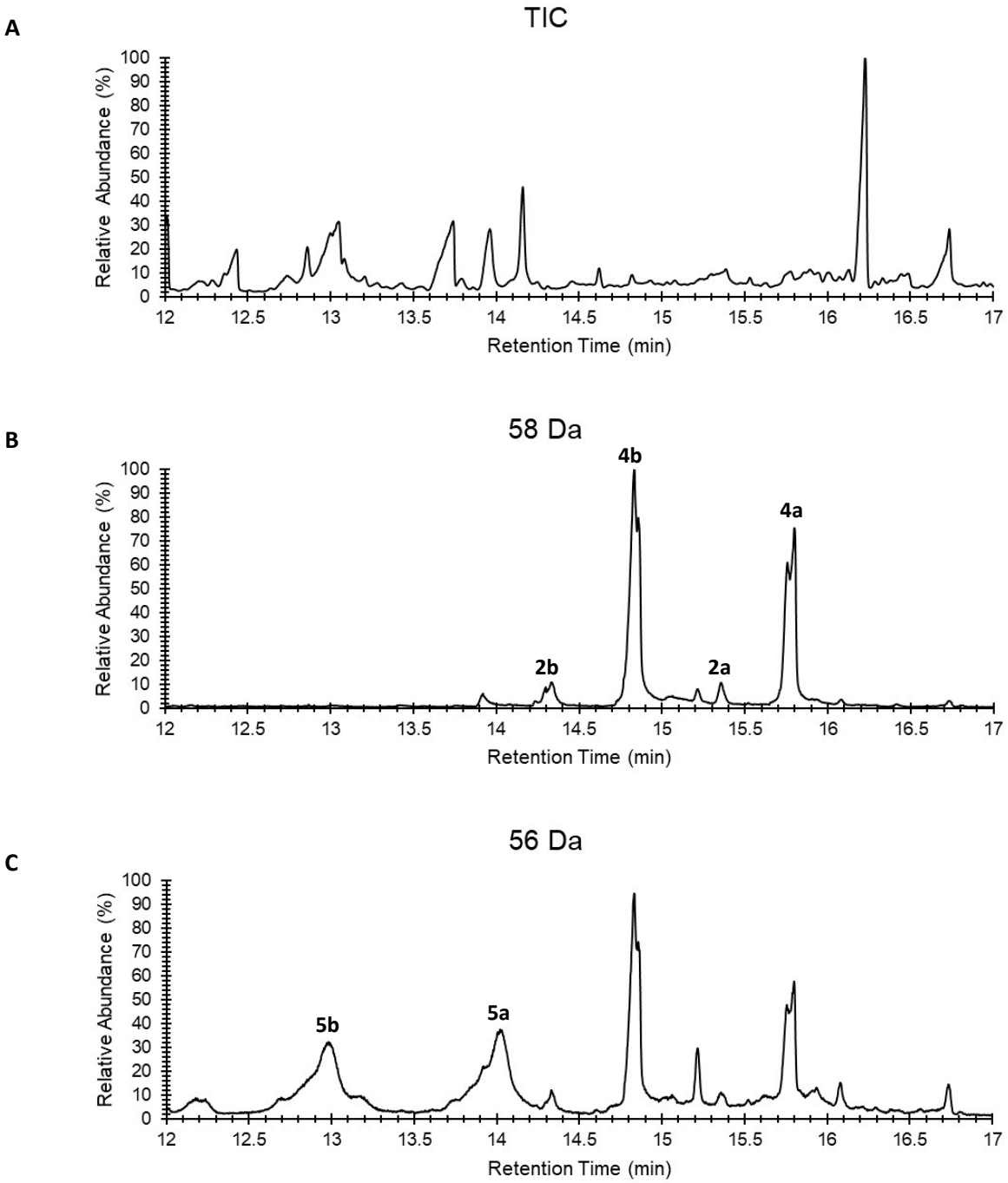

GC-MS separation and identification of triketide lactones from fermentation of *Sac. erythraea* JC2, containing pMU3-DEBS 1-TE-URA3- Med$_{M7a}$-B. (A) Total ion chromatogram (TIC). (B) Extracted ion chromatogram (*m/z* = 58 Da). (C) Extracted ion chromatogram (*m/z* = 56 Da).

**$Nys_{M1}$-A:**



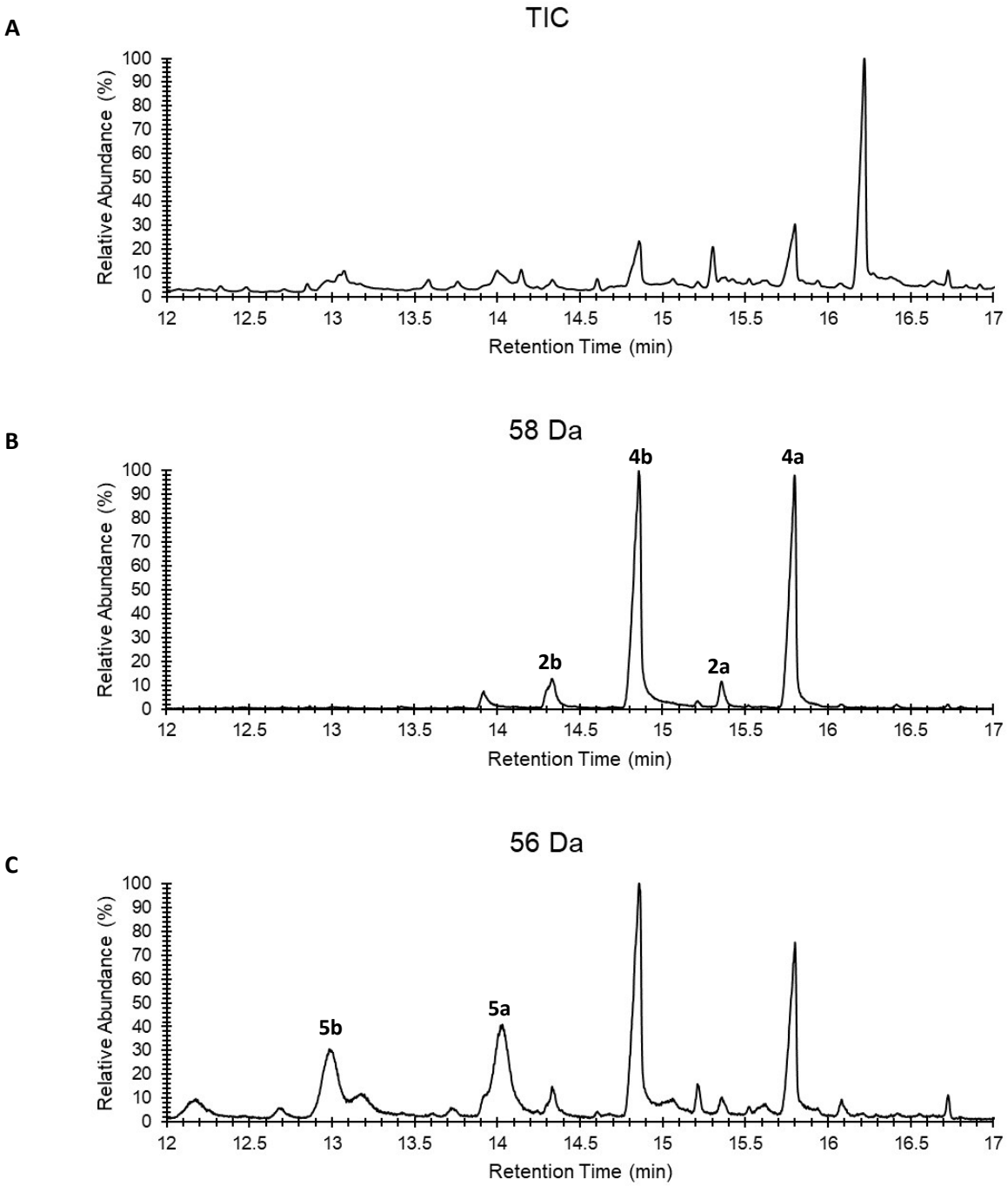

GC-MS separation and identification of triketide lactones from fermentation of *Sac. erythraea* JC2, containing pMU3-DEBS 1-TE-URA3- $Nys_{M1}$-A. (A) Total ion chromatogram (TIC). (B) Extracted ion chromatogram (*m/z* = 58 Da). (C) Extracted ion chromatogram (*m/z* = 56 Da).

**$Nys_{M1}$-B:**



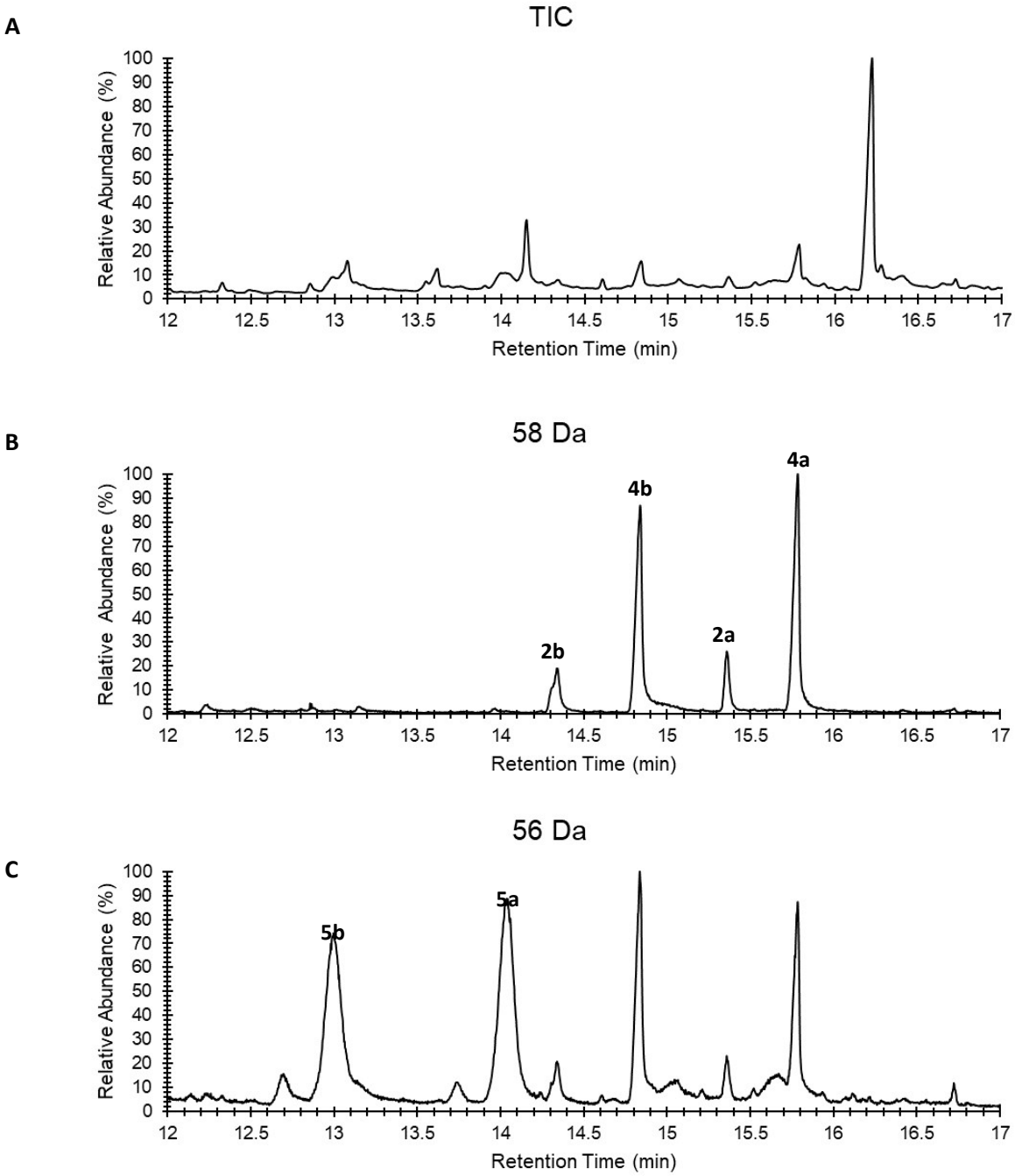

GC-MS separation and identification of triketide lactones from fermentation of *Sac. erythraea* JC2, containing pMU3-DEBS 1-TE-URA3- $Nys_{M1}$-B. (A) Total ion chromatogram (TIC). (B) Extracted ion chromatogram (*m/z* = 58 Da). (C) Extracted ion chromatogram (*m/z* = 56 Da).

**$Nys_{M11}$-A:**



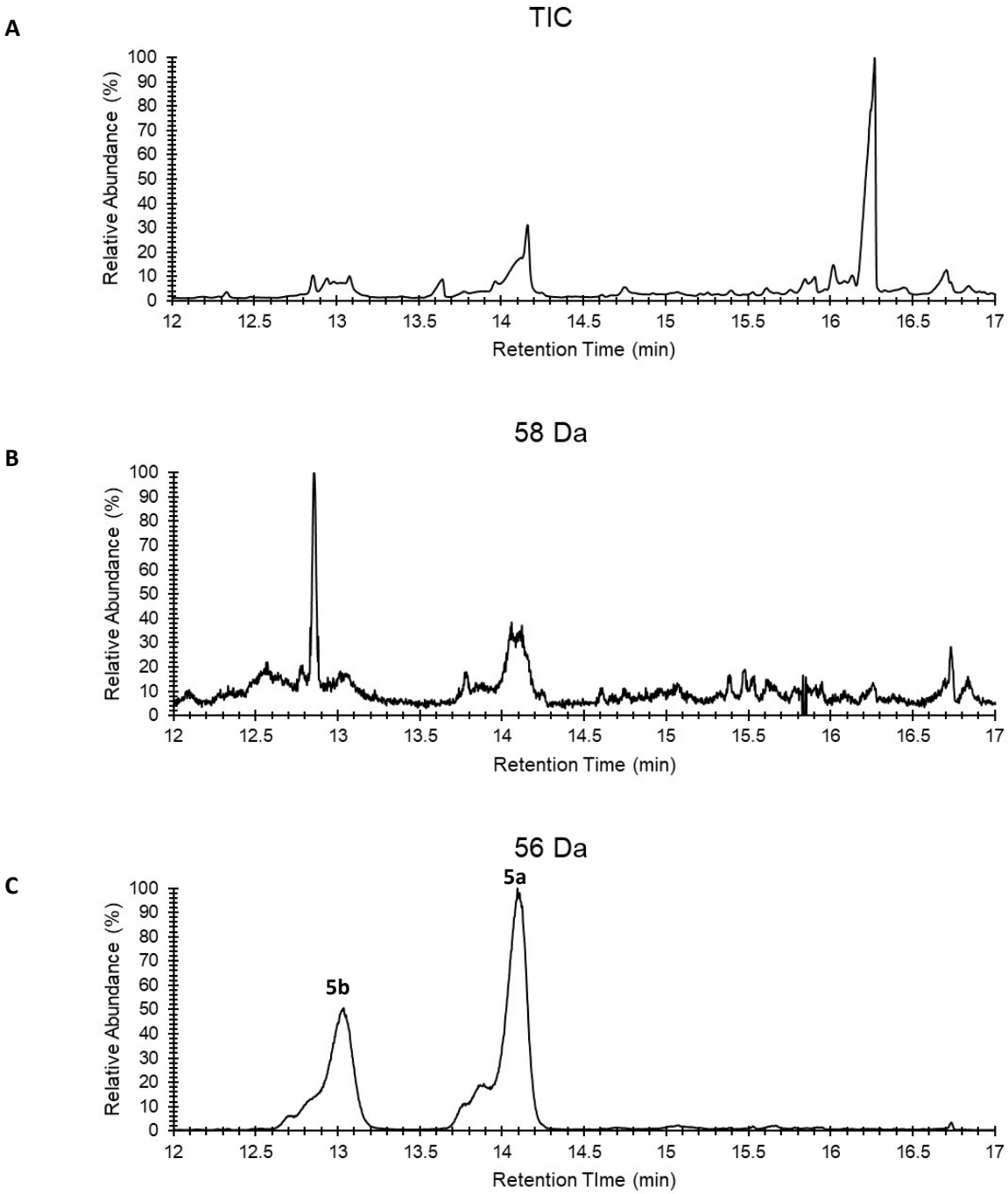

GC-MS separation and identification of triketide lactones from fermentation of *Sac. erythraea* JC2, containing pMU3-DEBS 1-TE-URA3- $Nys_{M11}$-A. (A) Total ion chromatogram (TIC). (B) Extracted ion chromatogram (*m/z* = 58 Da). (C) Extracted ion chromatogram (*m/z* = 56 Da).

**$Nys_{M11}$-B:**



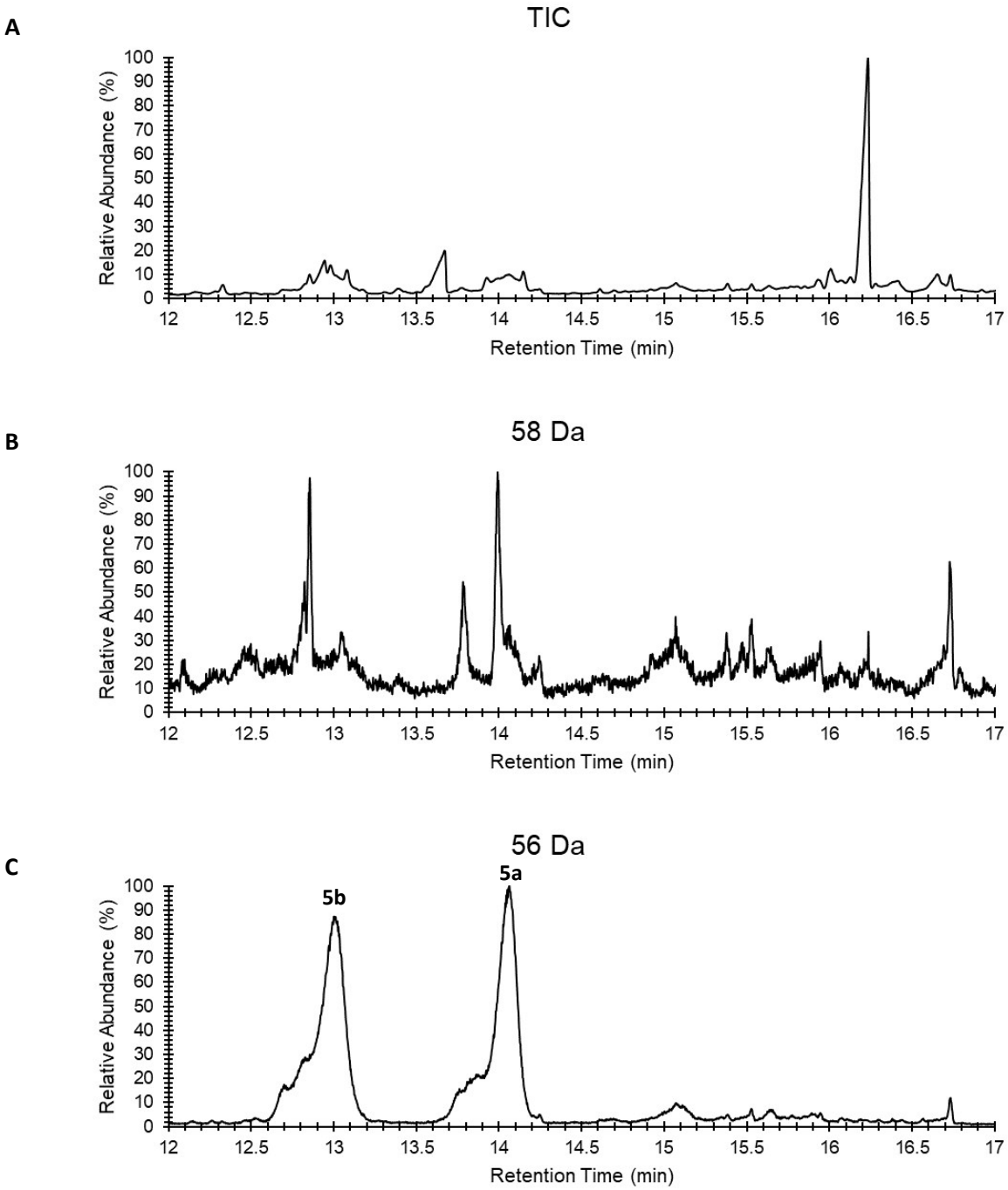

GC-MS separation and identification of triketide lactones from fermentation of *Sac. erythraea* JC2, containing pMU3-DEBS 1-TE-URA3- $Nys_{M11}$-B. (A) Total ion chromatogram (TIC). (B) Extracted ion chromatogram (*m/z* = 58 Da). (C) Extracted ion chromatogram (*m/z* = 56 Da).

**Olm$_{M5}$-A:**



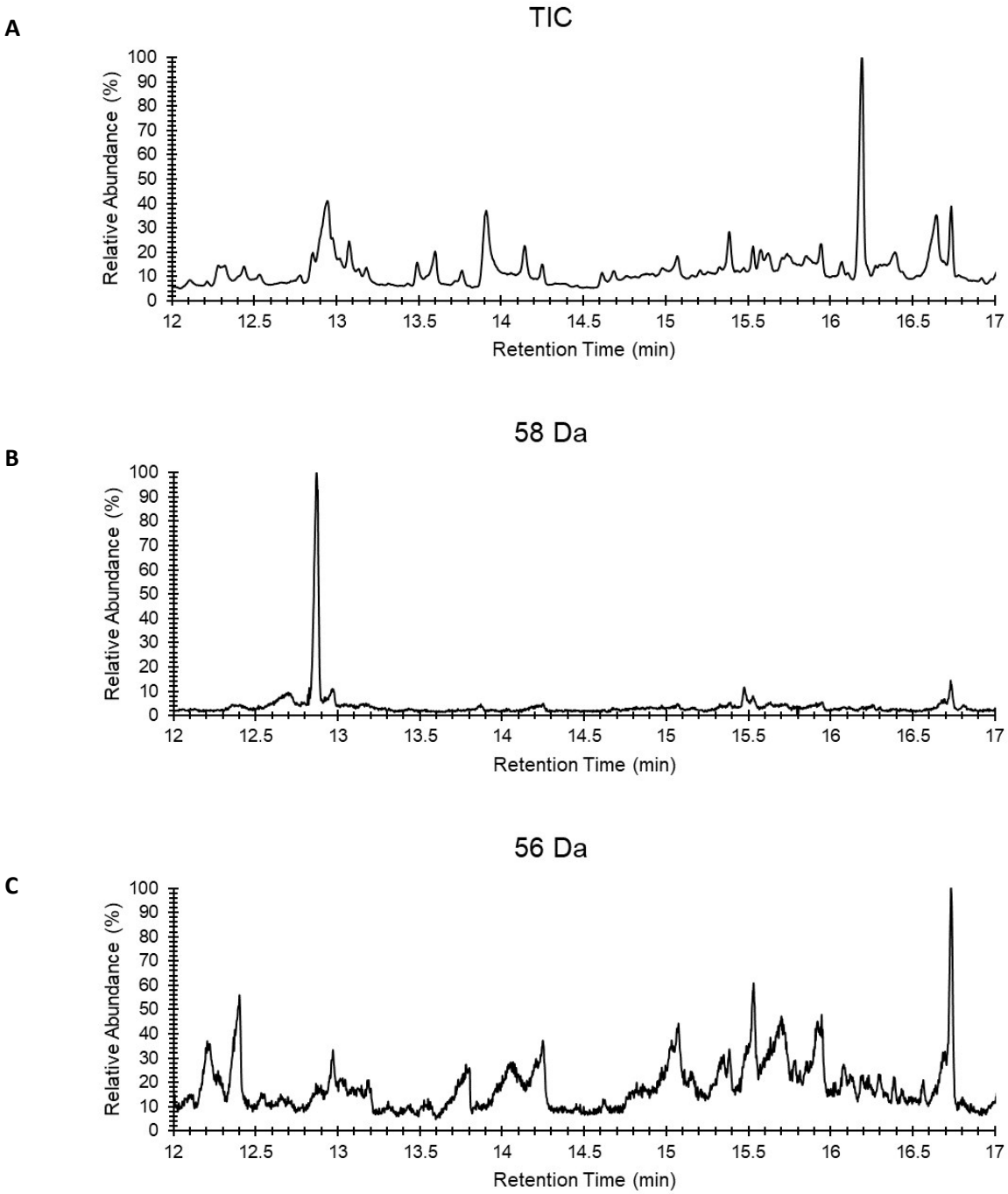

GC-MS separation and identification of triketide lactones from fermentation of *Sac. erythraea* JC2, containing pMU3-DEBS 1-TE-URA3- Olm$_{M5}$-A. (A) Total ion chromatogram (TIC). (B) Extracted ion chromatogram (*m/z* = 58 Da). (C) Extracted ion chromatogram (*m/z* = 56 Da).

**Olm$_{M5}$-B:**



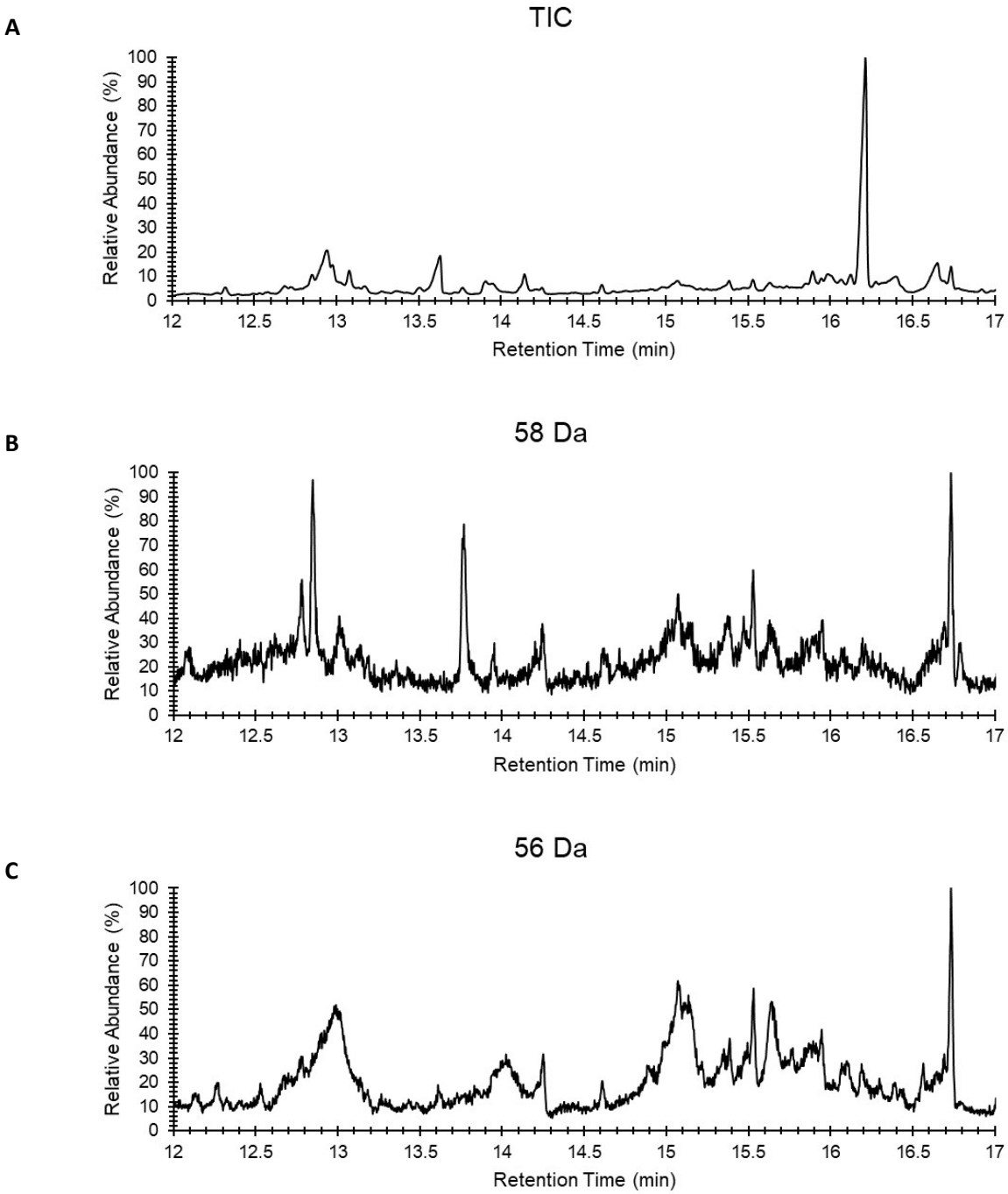

GC-MS separation and identification of triketide lactones from fermentation of *Sac. erythraea* JC2, containing pMU3-DEBS 1-TE-URA3- Olm$_{M5}$-B. (A) Total ion chromatogram (TIC). (B) Extracted ion chromatogram (*m/z* = 58 Da). (C) Extracted ion chromatogram (*m/z* = 56 Da).

**Olm$_{M5}$-C:**




GC-MS separation and identification of triketide lactones from fermentation of *Sac. erythraea* JC2, containing pMU3-DEBS 1-TE-URA3- Olm$_{M5}$-C. (A) Total ion chromatogram (TIC). (B) Extracted ion chromatogram (*m/z* = 58 Da). (C) Extracted ion chromatogram (*m/z* = 56 Da).

**Olm$_{M5}$-D:**




GC-MS separation and identification of triketide lactones from fermentation of *Sac. erythraea* JC2, containing pMU3-DEBS 1-TE-URA3- Olm$_{M5}$-D. (A) Total ion chromatogram (TIC). (B) Extracted ion chromatogram (*m/z* = 58 Da). (C) Extracted ion chromatogram (*m/z* = 56 Da).

Peak areas for the chromatograms shown:

| | Lactone product | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| **Construct** | **1a** | **1b** | **2a** | **2b** | **3a** | **3b** | **4a** | **4b** | **5a** | **5b** |
| $Amph_{M11}$-A | | | 3870717 | 5116032 | | | 26191115 | 25083510 | 17839138 | 16905214 |
| $Amph_{M11}$-B | | | 6952497 | 3369644 | | | 14877348 | 12647122 | 25663243 | 22060377 |
| $Nys_{M1}$-A | | | nq | nq | | | 4334385 | 7632632 | 8608377 | 8261861 |
| $Nys_{M1}$-B | | | nq | nq | | | 933964 | 1660804 | 4625551 | 4252341 |
| $Nys_{M11}$-A | | | 1027298 | 1898647 | | | 13487590 | 10867466 | 6544640 | 3347253 |
| $Nys_{M11}$-B | | | 193686 | 1557642 | | | 7408472 | 6557642 | 8232459 | 7926235 |
| $Olm_{M5}$-A | | | nd | nd | | | nd | nd | 36330627 | 51743140 |
| $Olm_{M5}$-B | | | nd | nd | | | nd | nd | 23801927 | 22205200 |
| $Lkm_{M1}$-A | 1228182 | 3684001 | | | nd | nd | | | 368401 | nq |
| $Lkm_{M1}$-B | 5194822 | 15863262 | | | nq | nq | | | 25863262 | nq |

nq: no quantification possible; nd: not detected

**Example yield calculation for $Amph_{M11}$-A, 4a/4b products:**

In each case, 1 mL of growth medium was extracted and analysed.
Equation (from **Fig. S4**): relative peak area = 0.4802(conc. (mg/L)) – 0.4732

Total peak area corresponding to 4a + 4b = 26191115 + 25083510 = 51274625
Conversion to relative peak area: (51274625/56653020)(100%) = 90.5% (where 56653020 represents 200 mg/L, 100% relative peak area)
Solving for concentration:
Conc. (mg/L) = (relative peak area + 0.4732)/0.4802
Conc. (mg/L $Amph_{M11}$-A) = (90.5 + 0.4732)/0.4802 = 189.4
As the extracts analysed were 5× more concentrated than the growth samples, the final concentration was obtained by dividing by 5.
This gave 37.9 mg/L for this particular sample.

**Note: the values given in Table 2 in the main text represent the average yield from three biological replicates.**

## 4. References


1 C. J. Rowe, J. Cortés, S. Gaisser, J. Staunton and P. F. Leadlay, *Gene*, 1998, **216**, 215–223.

2 T. Annaval, C. Paris, P. F. Leadlay, C. Jacob and K. J. Weissman, *ChemBioChem*, 2015, **16**, 1357–1364.

3 N. Bonneaud, O. Ozier-Kalogeropoulos, G. Y. Li, M. Labouesse, L. Minvielle-Sebastia and F. Lacroute, *Yeast*, 1991, **7**, 609–615.

4 D. Gietz, A. St Jean, R. A. Woods and R. H. Schiestl, *Nucleic Acids Res.*, 1992, **20**, 1425.

5 L. Kellenberger, I. S. Galloway, G. Sauter, G. Böhm, U. Hanefeld, J. Cortés, J. Staunton and P. F. Leadlay, *ChemBioChem*, 2008, **9**, 2740–2749.

6 M. Grenson, *Eur. J. Biochem.*, 1969, **11**, 249–260.

7 P. Mazodier, R. Petter and C. Thompson, *J. Bacteriol.*, 1989, **171**, 3583–3585.

8 R. S. Gokhale, S. Y. Tsuji, D. E. Cane and C. Khosla, *Science*, 1999, **284**, 482–485.

9 A. Ranganathan, M. Timoney, M. Bycroft, J. Cortés, I. P. Thomas, B. Wilkinson, L. Kellenberger, U. Hanefeld, I. S. Galloway, J. Staunton and P. F. Leadlay, *Chem. Biol.*, 1999, **6**, 731–741.

10 S. Yuzawa, K. Deng, G. Wang, E. E. K. Baidoo, T. R. Northen, P. D. Adams, L. Katz and J. D. Keasling, *ACS Synth. Biol.*, 2017, **6**, 139–147.

11 J. Cortés, K. E. Wiesmann, G. A. Roberts, M. J. Brown, J. Staunton and P. F. Leadlay, *Science*, 1995, **268**, 1487–1489.