Diverse Metabolic Profiles of a *Streptomyces* Strain Isolated from a Hyper-arid Environment

Mostafa E. Rateb,‡,§ Wael E. Houssen,† William T. A. Harrison,† Hai Deng,‡ Chinyere K. Okoro,‡ Juan A. Asenjo,∥ Barbara A. Andrews,∥ Alan T. Bull,∥ Michael Goodfellow,* Rainer Ebel,∥ and Marcel Jaspars*‡

†Marine Biodiscovery Centre, Department of Chemistry, University of Aberdeen, Meston Walk, Aberdeen AB24 3UE, Scotland, U.K.
‡Pharmacognosy Department, Faculty of Pharmacy, Beni-Suef University, Salah Salem Street, Beni-Suef 32514, Egypt
§Wellcome Trust Sanger Institute, Wellcome Trust Genome Campus, Hinxton, Cambridge, CB10 1SA, U.K.
∥School of Biosciences, University of Kent, Canterbury, Kent CT2 7NJ, U.K.
¶School of Biology, University of Newcastle, Newcastle upon Tyne NE1 7RU, U.K.

Supporting Information

ABSTRACT: The metabolic profile of *Streptomyces* sp. strain C34, isolated from the Chilean hyper-arid Atacama Desert soil, is dependent on the culture media used for its growth. The application of an OSMAC approach on this strain using a range of cultivation media resulted in the isolation and identification of three new compounds from the rare class of 22-membered macrolactone polyketides, named chaxalactins A–C (1–3). In addition, the known compounds deferroxamine E (4), hygromycin A (5), and 5′-dihydro-hygromycin A (6) were detected. The isolated compounds were characterized by NMR spectroscopy and accurate mass spectrometric analysis. Compounds 1–3 displayed strong activity against Gram-positive but weak activity Gram-negative strains tested.

Microbial secondary metabolites are widely considered to be one of the best reservoirs for drug discovery from natural sources.¹ Searching for natural products from terrestrial microorganisms was initiated at least six decades ago and revealed that microbes of soil origin are a prolific supply for structurally unique and bioactive secondary metabolites.² *Streptomyces* is the largest genus of the *Actinobacteria*, with over 500 species found predominantly in soil and marine ecosystems.³ To date *Streptomyces* species are responsible for about two-thirds of the clinically used antibiotics, and so chemical screening of novel *Streptomyces* isolates recovered from unusual environments for new natural products is of great interest.⁴⁻⁵ Selection of bacterial strains from the underexplored extremobiosphere⁶ provides one option, while genome mining based on PCR targeting of specific microbial genes provides an effective route for discovering new natural products.⁶ OSMAC (one strain—many compounds) is the approach that we have deployed in this study. This strategy was developed in the early 2000s by Zeeck and co-workers to maximize the productivity of a single microbe probably through turning on silent or cryptic biosynthetic genes by varying accessible cultivation parameters such as media composition, pH, temperature, oxygen supply, quality and quantity of light, bioreactor platform, or the addition of precursors or enzyme inhibitors.⁷ Using OSMAC, Zeeck’s group isolated more than 100 metabolites representing 25 structural classes from only six microorganisms.⁷ The OSMAC approach has been successfully applied to produce new secondary metabolites from single microbial strains during the last 10 years.⁸⁻¹⁰

In the past, researchers hypothesized that extremophiles would not grow or produce natural products unless complex cultivation methods were used.¹ Recently, many examples show that microbes from extreme environments can grow and produce new secondary metabolites under standard laboratory conditions.¹,¹¹ To date, most attention has been applied to marine ecosystems, especially deep sea environments, as a rich source of novel microbes and hence natural products. One of the less commonly investigated habitats is the hyper-arid or absolute deserts, which constitute large landmasses characterized by very low or zero vegetation and extremely low and unpredictable rainfall.⁵

As part of our program to investigate extremophiles as an underexplored source of new natural products, we have focused our attention on *Streptomyces* sp. strain C34 isolated from the Atacama Desert, Chile. This strain was previously shown by our group to contain a 3-amino-5-hydroxybenzoic acid (AHBA) synthase gene,⁶ and its fermentation led to the production of four new ansamycin-type polyketides.⁶ The application of the

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OSMAC approach to this strain using eight different media resulted in the production of three new 22-membered macro lactone-type polyketides, chaxalactins A—C (1–3), in addition to the known cyclic peptide deferoxamine E (4) and the known glycosides hygromycin A (5) and 5′-dihydrohygromycin A (6). The macro lactones reported in the present study were screened for antibacterial activity against a panel of Gram-positive and Gram-negative bacterial strains.

## RESULTS AND DISCUSSION

In our previous study, the taxonomically unique *Streptomyces* sp. strain C34 was induced to produce four new ansamycin-type compounds when cultivated on two different media. In this study, we have further investigated the LCMS metabolite profiles of this strain when cultured on another eight different media. Changing culture media composition not only affected the produced compounds quantitatively but also had a significant effect on activation of the biosynthetic pathways leading to the production of new metabolites from different chemical classes. Analysis of designing OSMAC approaches to this strain using eight different media (Figure 1) clearly depicted a dramatic change in the chemical profile. Cultivation on yeast extract glucose (GYE) soya flour mannitol (SFM) and potato dextrose (PD) media resulted in the production of a sole metabolite, chaxamycin A (7), which was also found as a predominant metabolite in all of the other media used. As a result, the previously reported chaxamycin A (7) is considered a useful chemical marker of this strain. Chaxamycin B (8) was produced in ISP2 and malt extract peptone (MEP) and modified Fries media and 2,16 production of chaxalactins C and D (9, 10) was exclusive to the modified ISP2 medium. The use of a defined medium led to the discovery of three new metabolites, chaxalactins A—C (1–3), belonging to the rare microbrial class of 22-membered macro lactones, in addition to the cyclic peptide deferoxamine E (4). The latter, a known siderophore, seemed to predominate when using media containing a high content of inorganic salts; that is, it was produced as a major metabolite in addition to the known glycosides hygromycin A (5) and 5′-dihydrohygromycin A (6) when using ISP3 medium (Figure 1).

HRESIMS analysis of 1 established the molecular formula as C28H40O6 thus implying nine degrees of unsaturation. The 1H and 13C NMR data (Table 1) revealed the presence of one ester carboxyl group at δC 166.6, 14 olefinic carbons, and 13 olefinic protons, consistent with the presence of six disubstituted double bonds and one trisubstituted olefinic group, three oxymethine protons, three methylene groups, and four methyl groups. This analysis brought the total number of unsaturations to eight, thus requiring the presence of one additional ring in the structure.

In the COSY spectrum, two spin systems were discernible, comprising H-2 and H-3 as well as H-5 through H-7,25 including the methyl groups H2-26 and H7-27 (Figure 2). The chain C-5—C-25 was further confirmed by HMBC correlations (Table 1). The attachment of methyl group H2-28 (δH 1.81) to the trisubstituted olefinic group was evident from its correlations to C-3, C-4, and C-5 in the HMBC spectrum. Carbonyl group C-1 (δC 166.6) was connected to C-2 on the basis of its HMBC correlations to both H-2 (δH 5.73) and H-3 (δH 7.14), while the HMBC correlation of H-21 (δH 5.32) to C-1 allowed for closing of the lactone ring in the proposed structure.

The configuration of the double bonds at C-2—C-3, C-18—C-19, and C-22—C-23 was determined to be E, while C-16—C-17 was established as Z on the basis of the values of the coupling constants. The double bond at C-4—C-5 was confirmed to be E on the basis of the NOESY correlations of H-3 to H-5 and H2-28 to both H-2 and H-6. The configuration of the remaining double bonds was determined to be E, and the relative configuration at the chiral carbons was established as C-8 R*, C-12 S*, C-13 R*, C-15 R*, and C-21 R* on the basis of X-ray crystallographic analysis of 1, while it was not possible to determine the absolute configuration. With respect to the atoms making up the ring, the conformation of the C-8—C-9 bond was found to be gauche [C-7—C-8—C-9—C-10 = −64.2(3)°], as is C-12—C-13 [C-11—C-12—C-13—C-14 = −63.8(3)°], whereas C-13—C-14 [C-12—C-13—C-14—C-15 = −171.5(2)°] (Figure 3). The torsion angle of C-16—C-17—C-18—C-19 is 170.1(3)°, indicating the conformation of C-17—C-18 to be s-trans. With respect to the side-chain junction (C-21), the ring atoms were found to be gauche [C19—C20—C21—O1 = 56.0(3)°] and the side chain was anti to C-19 [C-19—C-20—C-21—C-22 = 177.4(2)°].

In the crystal, the molecules are linked by O—H···O hydrogen bonds to generate (001) sheets (Figure 4). Atom O4 serves as both a donor and acceptor in this network. In graph-set notation, C(14) chains in [100] and C(6) chains in [010] occur. There were no other intermolecular interactions detected except van der Waals contacts.

A NOESY spectrum was acquired as a reference for the assignments of the relative configuration of the other related compounds. On this basis, it was shown that 1 was a new 22-membered macro lactone-type polyketide for which the name chaxalactin A was ascribed.

The molecular formula of 2 was determined as C28H40O6 thus having one more oxygen atom than 1. Comparison of the 1H and 13C NMR spectra of 2 and 1 (Table 1) revealed that both were closely related and that methylene protons H2-14 in 1 (δC 41.4, δH 1.59, δH 1.47) were replaced by an oxymethine proton [δC 73.2, δH 3.31] in 2. This was corroborated with the COSY correlations of H-14 to both H-13 and H-15 and the HMBC correlation of H-14 to C-12. In the 1H NMR spectrum of 2, the vicinal coupling constants between H-13—H-14 was 9.2 Hz, suggesting that H-14 and H-13 displayed an anti relationship, while the vicinal coupling constant between H-14—H-15...
was 1.4 Hz, suggesting them to adopt a gauche relationship, consistent with a C-13 $S^*$, C-14 $R^*$, and C-15 $S^*$ conformation. The close similarity of the $^{13}$C NMR data and an analogous set of NOESY correlations for the remaining part of the macrocycle indicated that 2 otherwise had the same relative configuration as 1. From this analysis, 2 was identified as a new natural product for which we suggested the name chaxalactin B.

HRESIMS analysis of 3 revealed a molecular formula of $C_{29}H_{42}O_5$, indicating 3 to be a homologue of 2. The NMR spectra of 3 and 2 (Table 1) indicated the presence of an additional methoxyl group in 3 ($\delta_C$ 56.2, $\delta_H$ 3.27) at position 15 on the basis of the HMBC correlation to C-15 ($\delta_C$ 75.4). Compounds 2 and 3 displayed similar $^{13}$C NMR data and HMBC correlations (Table 1), an analogous set of NOESY
correlations, and virtually identical coupling constants between H-13 through H-15, all of which strongly suggested that they shared the same relative conformation. On the basis of this evidence, 3 was confirmed as a new 22-membered macrolactone-type polyketide for which the name chaxalactin C was ascribed.

In Nature, microbial 22-membered macrolactones are rare. They are represented by the antibiotic pulvomycin from *Streptomyces netropsis*,19 the macrolides A82548a and yokonolide A from *Streptomyces diastatochromogenes*,20,21 the immunosuppressants ushikulides A and B from *Streptomyces sp.*,22 the antibacterial

### Table 1. NMR Spectroscopic Data for Chaxalactins A–C (1–3) (CDCl₃, 400 MHz at 298 K)

<table>
<thead>
<tr>
<th>position</th>
<th>δC, mult.</th>
<th>δH, mult. (J in Hz)</th>
<th>HMBC</th>
<th>δC, mult.</th>
<th>δH, mult. (J in Hz)</th>
<th>HMBC</th>
<th>δC, mult.</th>
<th>δH, mult. (J in Hz)</th>
<th>HMBC</th>
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<tbody>
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<td>1</td>
<td>166.6, C</td>
<td>117.5, CH 5.73, d (15.7)</td>
<td>1.4</td>
<td>166.6, C</td>
<td>117.5, CH 5.73, d (15.6)</td>
<td>1.4</td>
<td>166.6, C</td>
<td>117.2, CH 5.71, d (15.6)</td>
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<tr>
<td>2</td>
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<td>1.2,4,5,28</td>
<td>148.6, CH 7.11, d (15.6)</td>
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<tr>
<td>3</td>
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<td>132.2, C 166.8, C 166.6, C 166.6, C</td>
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<td>36.8, CH 2.42, m</td>
<td>36.8, CH 2.42, m</td>
<td>36.8, CH 2.42, m</td>
<td>36.8, CH 2.42, m</td>
<td>36.8, CH 2.42, m</td>
<td>36.8, CH 2.42, m</td>
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<tr>
<td>7</td>
<td>40.9, CH2 a 2.32, dt (3.7, 12.9)</td>
<td>40.9, CH2 a 2.30, dt (3.9, 13.1)</td>
<td>40.9, CH2 a 2.32, dt (3.8, 12.9)</td>
<td>40.9, CH2 a 2.32, dt (3.8, 12.9)</td>
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<td>40.9, CH2 a 2.32, dt (3.8, 12.9)</td>
</tr>
</tbody>
</table>

HMBC correlations are from the proton(s) stated to the indicated carbon(s). Extracted from 1D-TOCSY spectra.
difficidin compounds from Bacillus subtilis \(^{23}\), macroactin H from Bacillus sp. \(^{24}\), and the cytotoxic wortmanniactones A–D from the soil-derived fungus Talaporomyces wortmannii. \(^{25}\) Chaxalactins A–C (1–3) are structurally close to the known 24-membered polyketide macroactin family. To date, macroactins A–V have been isolated and chemically characterized from marine and soil bacterial isolates \(^{26}\) and shown to have antibacterial, antilarval, antiviral, and antitumor activities. \(^{26}\)

This rare group of compounds, produced by the taxonomically distinct Streptomyces strain C34, \(^{27}\) differed from almost all of the structurally related macroactins in that they were 22-membered rather than 24-membered macrocyclic lactones, with branching methyl groups at C-4, C-8, and C-12, and an E-but-1-ene side chain at C-21 instead of a methyl or ethyl group at the corresponding branching position in the 24-membered macroactins. They also differed from almost all 24-membered lactones in having an E configuration at C-2–C-3 instead of Z and Z instead of E at C-16–C-17 and by the sense of optical rotation, i.e., dextrorotatory instead of levorotatory.

Type I multimodular polyketide synthases (type I PKSs) should be involved in the biosynthesis of the macroclide I, and the assembly line is likely to utilize eight acetate and four propionate units without any postbiosynthetic modifications (Figure 1, SI). It is clear from the proposed biogeneses that chaxamycins and chaxalactins have different biosynthetic origins.

When tested for antibacterial activity (Table 2), compounds 1–3 displayed strong activity against Gram-positive bacteria with MIC values of <1 μg mL\(^{-1}\) against S. aureus and 3–6 μg mL\(^{-1}\) against L. monocytogenes and B. subtilis, but showed weak activity against Gram-negative strains tested. It was noticed that compound 2 showed slightly higher activity against S. aureus than compounds 1 and 3 despite the minor structural differences.

### EXPERIMENTAL SECTION

#### General Experimental Procedures.
Optical rotations were recorded on a Perkin-Elmer 343 polarimeter. UV and IR spectra were measured on a Perkin-Elmer Lambda 25 UV/vis spectrometer and a Thermo Nicolet IR 100 FT/IR spectrometer, respectively. \(^{1}\)H, \(^{13}\)C, and 2D NMR experiments were acquired on a Varian Unity INOVA 400 MHz spectrometer. High-resolution mass spectral data were obtained from a Thermo Instruments MS system (LTQ XL/LTQ Orbitrap Discovery) coupled to a Thermo Instruments HPLC system (Accela PDA detector, Accela PDA autosampler, and Accela pump).

#### Microorganism and Fermentation Conditions.
Streptomyces sp. strain C34 (EU517111) was isolated from a soil sample from Laguna de Chaxa (Salar de Atacama, Chile). \(^{27}\) The first-stage seed culture of this strain was prepared by inoculating a single colony of the organism into 50 mL of a defined medium \(^{17}\) of the following composition (g L\(^{-1}\)): KH\(_2\)PO\(_4\) (2.0), NH\(_4\)Cl (1.5), MgSO\(_4\)\(\cdot\)7H\(_2\)O (0.5), NaCl (0.5), glycerol (10), myo-inositol (0.4), monosodium 1-glutamate monohydrate (5.0), NaF (0.08%), Fe\(_2\)O\(_4\)\(\cdot\)7H\(_2\)O (0.025%), ZnSO\(_4\)\(\cdot\)7H\(_2\)O (0.01%), CoCl\(_2\)\(\cdot\)6 H\(_2\)O (0.01%), CaCO\(_3\) (0.25%), p-aminobenzoic acid (0.001%), pH 7.0. After 5 days incubation in a rotary shaker incubator at 28 °C at 150 rpm, this culture was used to inoculate the production fermentation, using 3 L of the same medium in the presence of Diaion HP20 (50 g L\(^{-1}\)) under the same conditions for 7 days. A wide range of media was used to screen the effects of simple, complex, enriched, or defined media as well as high inorganic salt content on the bacterial secondary metabolite production. The same procedure was adapted in the bacterial fermentation on GYE, SFM, PD, MEF, ISP3, and modified Fries media 1 and 2.

#### Extraction and Isolation.
The harvested fermentation broth from strain Streptomyces C34 (defined medium, 3 L) was centrifuged at 3000 rpm for 20 min, and the HP20 resin together with the cell mass were washed with distilled water and then extracted with MeOH (4 × 500 mL). The successive MeOH extracts were combined and concentrated under reduced pressure, yielding 250 mL of materials, which were fractionated with a hexane (3 × 250 mL), CH\(_2\)Cl\(_2\) (4 × 250 mL), and EtOAc (3 × 250 mL). Quick NMR analysis revealed that the CH\(_2\)Cl\(_2\) fraction had the most diverse metabolic profile. This fraction was loaded on a Sephadex LH-20 column equilibrated with MeOH–CH\(_2\)Cl\(_2\) (1:1), and two fractions were collected. The second fraction (210 mg) was further purified by reversed-phase HPLC using a gradient of MeOH in H\(_2\)O as eluent (60–100% over 60 min, 100% for 10 min) at a flow rate of 1.25 mL min\(^{-1}\). This procedure afforded the pure compounds 1 (19 mg), 2 (7 mg), 3 (11 mg), and 4 (95 mg) from the defined medium and 5 (1.4 mg) and 6 (1 mg) from ISP3 medium.

| Table 2. Antibacterial Activity of Chaxalactins A–C (1–3) |
|-----------------|-----------------|-----------------|
| strain          | average MIC (μg mL\(^{-1}\))  |
|                 | 1               | 2               | 3               |
| S. aureus       | 0.4             | 0.2             | 0.8             |
| L. monocytogenes| 6.3             | 5.0             | 6.3             |
| B. subtilis     | 3.1             | 5.0             | 6.3             |
| E. coli         | >20             | >20             | >20             |
| V. parahemolytic| 12.5            | 20              | 12.5            |

The following conditions were used: capillary voltage 45 V, capillary temperature 320 °C, auxiliary gas flow rate 10–20 arbitrary units, sheath gas flow rate 40–50 arbitrary units, spray voltage 4.5 kV, mass range 100–2000 amu (maximum resolution 30000). HPLC separations were made using a Phenomenex reversed-phase (C\(_{18}\), 250 × 10 mm, L × i.d.) column connected to an Agilent 1200 series binary pump and monitored using an Agilent photodiode array detector. Detection was carried out at 220, 230, 254, 280, and 350 nm. Diaion HP-20 was obtained from Resindion S.R.L., a subsidiary of Mitsubishi Chemical Co., Binasco, Italy.
X-ray Crystallographic Study of 1. Intensity data for 1 were collected at 120 K using a Bruker – nonius kappa CCD diffractometer (graphite-monochromated Mo Kα radiation, λ = 0.7073 Å). The structure was solved by direct methods with SHELXS-97 and the atomic model refined against |F|² with SHELXL-97. The “observed data” criterion for calculating the R(F) residuals was set as I > 2σ(I). The carbon-bound H atoms were geometrically placed (C–H = 0.95 – 1.00 Å) and refined as riding atoms with Ueq(H) = 1.2–1.5Ueq(C). The methyl groups were allowed to rotate about their C–C–bonds, but not to tip, to best fit the electron density. The O–bond H atoms were located in difference maps and refined as riding atoms in their as-found relative positions with the constraint Ueq(H) = 1.2Ueq(Carrier). Anomalous dispersion was negligible, and Friedel pairs were merged before refinement.

Crystal data for 1: C30H40O4, colorless block, 0.38 × 0.28 × 0.18 mm, Mw = 440.60, orthorhombic, P212121, (No. 19), a = 8.8119(5) Å, b = 11.8086(4) Å, c = 25.8659(15) Å, V = 2691.6(2) Å³, Z = 4, T = 120 K, ρcalc = 1.087 g cm⁻³, μ = 0.071 mm⁻¹, F(000) = 960, 2908 reflections measured (−9 ≤ h ≤ 10, −14 ≤ k ≤ 13, −31 ≤ l ≤ 31, 586° ≤ θ ≤ 52°07′). Rint = 0.068, 2908 refined reflections, 2367 with I > 2σ(I), 296 parameters, R(F) = 0.044, wR(F) = 0.094, S (goodness of fit) = 1.046, w = 1/[σ²(Fo)+0.0312P²+0.8486P], where P = (Fo²+Fc²)/3, min./max. Δρ = −0.16, +0.18 e Å⁻³. Crystallographic data (excluding structure factors) for 1 have been deposited with the Cambridge Crystallographic Data Center as supplementary publication no. CCDC 823154. Copies of the data can be obtained free of charge on application to CCDC, 12 Union Road, Cambridge CB1 2ZG, UK [fax: Int.+44(0) (1233) 366 033; e-mail: deposit@ccdc.cam.ac.uk].

Antibacterial Screening. The antibacterial activity of compounds 1–3 was evaluated against the Gram-positive strains Staphyloccocus aureus ATCC25922, Listeria monocytogenes ATCC19115, and, Bacillus subtilis NCTC2116 and the Gram-negative strains Escherichia coli ATCC25922 and Vibrio parahaemolyticus NCTC10441, using the agar diffusion method. Filter paper disks containing ampicillin (10 μg), oxolinic acid (2 μg), or oxytetracycline (30 μg) were used as positive controls. MICs against species were calculated using the method described before with minor modifications. In brief, tested strains were grown in Müller-Hinton (MH) broth⁵⁹ to early stationary phase and then diluted to an OD600 = 0.005. The assays were performed in a 96-well microtiter plate format in duplicate, with two independent cultures for each strain. All compounds were dissolved in DMSO (Sigma) and added to the cultures in wells so that the final concentration of DMSO was 10%, a concentration that did not affect the growth of any of the tested strains. The effect of different dilutions of the compounds (80–0.08 μg mL⁻¹) on the growth was assessed after 18 h incubation at 37 °C using a Labsystems iEMS reader MF plate reader at OD600. The MIC was determined as the lowest concentration showing no growth compared to the MH broth control.

ASSOCIATED CONTENT

Supporting Information. NMR spectra of 1–3, including 1H, 13C, COSY, HSQC, HMBC, and NOESY in CDCl₃, are available free of charge via the Internet at http://pubs.acs.org.

AUTHOR INFORMATION

Corresponding Author
Tel: +44 (0)1224 272895. Fax: +44 (0)1224 272921. E-mail: m.jaspers@abdn.ac.uk.

REFERENCES

