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A Novel Isolate (S15) of *Streptomyces griseobrunneus* Produces 1-Dodecanol

Serap Çetinkaya[1]

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Abstract
One-dodecanol was identified to be the predominant secondary metabolite of a novel isolate (S15) of *Streptomyces griseobrunneus*. For its demonstration, secondary metabolite extracts were electrophoresed by sodium dodecyl sulphate–polyacrylamide gel electrophoresis (SDS–PAGE). A yellowish unique band was then cut out from the gel and its metabolite content was eluted in *n*-butanol. GC–MS analysis indicated that more than 93% of the of the elution material were 1-dodecanol. The compound was further characterized by FTIR and ^{13}C NMR analyses. Dendrogram built on the basis of 16S rRNA gene sequence indicated that the isolate S15 was a member of *Streptomyces griseobrunneus*.

Introduction

The search of new natural products often involves the isolation of Actinomycetes [1]. *Streptomyces*, a large genus of Actinomycetes, comprises filamentous soil bacteria with complex morphology and produces a wide variety of bioactive secondary metabolites [2]. Although their life cycle is similar to that of fungi, they are eubacteria because they have no nucleus, and their genomes have a high GC (about 70%) content [3, 4]. Their spore form is an essential part of their lifecycle, unlike *Bacillus* [5]. Chromosomal DNA of *Streptomyces* members ranges between 8 and 9 Mb that containing approximately eight thousand protein-coding genes [6].

In liquid media, *Streptomyces* tend to form aggregates [7]. Thus, the cells residing in the centre are unable to reach food [8]. This growth property results in a mixture of spore- and vegetative life, and to the simultaneous production of primary and secondary metabolites [9].

Secondary metabolites are not essential for the producing organism. They are often composed of tens of different substances. Whilst crude preparations can produce combinatorial biological effects, each metabolite can exert diverse biological activities in pure form. Secondary metabolite production is usually controlled by a series of operons and by a set of single genes clustered together [10].

Members of *Streptomyces* synthesize industrially very important secondary metabolite molecules, including red-, yellow-, and dark coloured pigments [11]. They also produce antibacterial enzymes [12].

One-dodecanol or lauryl alcohol ($C_{12}H_2O_6$) is a fatty alcohol (Fig. 1). It is insoluble in water and colourless in liquid form. It has an unpleasant oil odour at high concentrations, but it emits a delicate floral scent at low concentrations [13, 14]. In blue crab processing it can be obtained as a volatile by-product [15]. It is mostly prepared from palm kernel- or coconut-oil. In pure form the range of its technological use is rather broad. It can be found in lubricants and in the formation of monolithic polymers, in detergent and soaps, in shampoo, body wash- and shaving gel, and in hair dyes. Though less frequently, it is also used in the production of emulsifying and wetting agents, and in fragrances. Its use in food has been approved to be safe [16, 17].

In the study, a solid growth medium, prepared with agar in glass pots (approximately 25 cm in diameter), was preferred to prevent the aggregation of growing cells and to provide the filaments of *S. griseobrunneus* with a spongy growth environment. This study was the first in demonstrating the presence of 1-dodecanol as a secondary metabolite in bacteria. Sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS–PAGE) was used for the partitioning of the

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✉ Serap Çetinkaya
serapcetinkaya2012@gmail.com

[1] Department of Molecular Biology and Genetics, Science Faculty, Sivas Cumhuriyet University, Sivas, Turkey

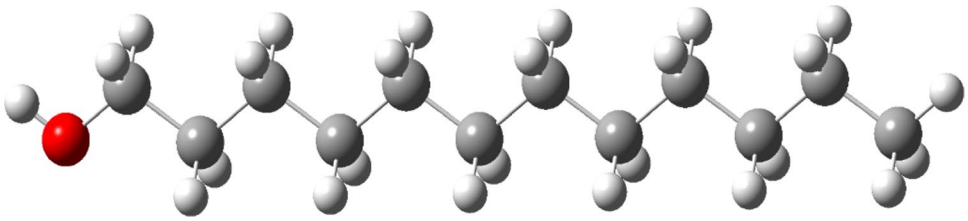

Fig. 1 Structure of 1-dodecanol

metabolites. A unique and loose band was cut out from the gel and its content was eluted in *n*-butanol. More than 93% of the elution material was 1-dodecanol. This finding was obtained by GC–MS and the molecule was characterized by FTIR and ^{13}C NMR analyses.

Materials and Methods

Soil Sample Collection

White soil samples were collected from Gemerek (Sivas, Turkey). This soil has been used by the local coiffeurs as an artisanal cosmetic.

Isolation of *Streptomyces*

Humid white soil, 10 g, was homogenized in 90 ml NaCl (0.85%) for 2 h at room temperature. Purification of the isolate was performed as described [18]. Pure isolates were then stored in 20% glycerol at −80 °C.

Typing

Identification by Mass Spectrometry

Typing on the basis of partial proteomics was achieved by MALDI-TOFF MS (Bruker IVD MALDI Biotyper, Sivas Cumhuriyet University Hospital). This analysis indicated that the isolate S15 belonged to genus *Streptomyces*.

Identification by 16S rRNA Gene Sequencing

Genomic DNA was prepared as described by Yavuz et al. [19]. Approximately 1400 bases were sequenced using the oligo-primers 27F (5′-AGAGTTTGATCMTGGCTCAG-3′) and 1492R (5′-TACGGYTACCTTGTTACGACTT-3′) [20]. Nucleotide sequence was deposited in GenBank (Accession No.: MW077440). The isolate S15 belonged to the species *S. griseobrunneus*. The nucleotide data were also analysed using Mafft software [21, 22] and a dendrogram was produced (Fig. 2).

Secondary Metabolite Preparation

S15 cells in glycerol stocks were first activated overnight in 50 ml LB at 37 °C. For the production of secondary metabolites, a solid growth medium was prepared (100 ml LB including 0.15% agar. A small aliquot of the overnight culture, 100 µl, was spread onto the solid medium in a glass container, 25 cm in diameter (data not shown). Incubation was continued until the colour of the solid medium turned into dark purple. This usually took 7 days. Secondary metabolite extraction was carried out by pouring an equal volume of *n*-butanol onto the solid culture. The solid medium was then meshed in this organic solvent. Liquid phase was recovered by centrifugation at 5000 rpm and butanol extract was obtained by evaporating the solvent at 70 °C [23, 24].

Thin Layer Chromatography (TLC)

A qualitative assessment of the secondary metabolite extract was achieved by TLC (silica gel 60, Merck). The mobile phase consisted of chloroform and methanol (10:1, v/v). Bands were visualised on an ultraviolet light box and their retention factors (R_f) were estimated [18].

SDS–PAGE (Sodium Dodecyl Sulphate–Polyacrylamide Gel Electrophoresis)

The butanol extract was electrophoresed in a 10% acrylamide gel [30% acrylamide: bisacrylamide, 1.5 M Tris–HCl, pH 8.8, 10% SDS, 10% ammonium persulphate (APS)] for 2 h at 70 V. After the electrophoresis, a yellowish band visible to naked eye was cut out from the gel and its contents was eluted in *n*-butanol overnight at 4 °C. Elution material was dried and the success of recovery was checked by SDS–PAGE.

Identification of the Elution Material

The elution sample was first analysed by gas chromatography–mass spectrometry (GC–MS, Shimadzu, Model: GCMS—QP 2010 ULTRA, Research Centre Laboratories of Kastamonu University, Turkey). Further characterisation involved Fourier Transform Infrared Spectroscopy (FTIR, Bruker, Tensor II, Research Centre Laboratories of Sivas Cumhuriyet University, Turkey) and ^{13}CNMR

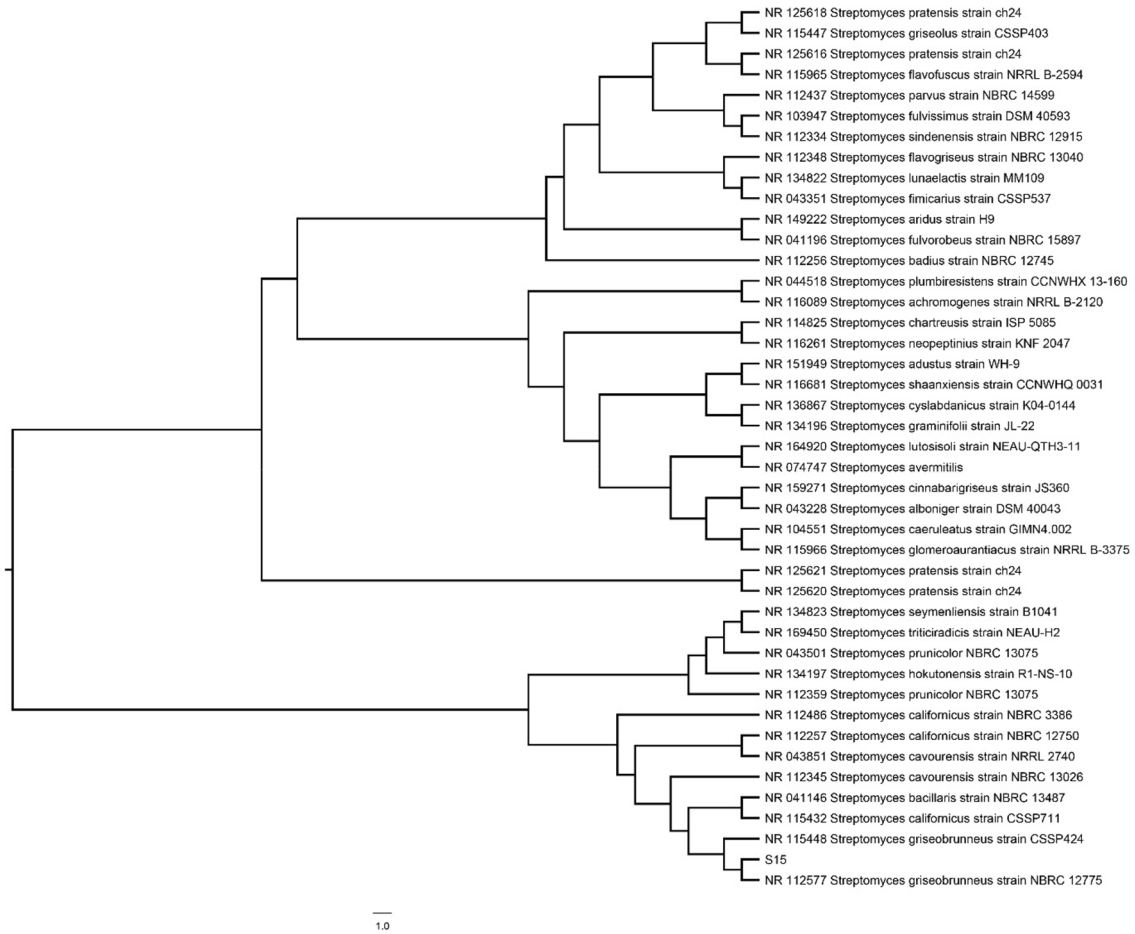

Fig. 2 Dendrogram of *Streptomyces griseobrunneus* including S15 isolate

(nuclear magnetic resonance, Bruker UltraShield 600 MHz, Research Centre Laboratories of Inonu University, IBTAM, Turkey).

Results

Identification of S15

The isolate was first identified by mass spectrometry (MALDI-TOF). This approach allowed the typing only at genus level. The dendrogram (Fig. 2) produced using 16S rRNA gene sequence homology indicated that the isolate S15 was a member of *S. griseobrunneus*. This identification appeared to be unambiguous because S15 was located, at the bottom part of the dendrogram, between two strains of *S. griseobrunneus*: NR115448, strain CSSP424 and NR112577, strain NBRC12775. S15 could grow between 30 and 40 °C at pH7 and its colonies assumed a purple colour on agar media (data not shown).

Thin Layer Chromatography

The image of the TLC showed one distinct and quite loose band. Its retention factor value was estimated to be 0.3 (D1, indicated by an arrow in Fig. 3).

Resolution, Purification, and Characterisation of 1-Dodecanol

Total secondary metabolite extracts produced a unique band both on the TLC plate and in the acrylamide gel. Efficiency of the recovery was also checked by SDS–PAGE (D1 band, Fig. 4). In a 10% polyacrylamide gel, 1-dodecanol had a mobility rate approximately equal to that of the 10 kDa marker protein band.

Most of the elution material (%93.32) was identified by GC–MS to be 1-dodecanol (Table 1). The second most abundant compound (%4.83) was indicated to be 2-propenamide, 2-methyl-*N*-phenyl-. These findings could explain the existence of the unique and loose band obtained by both TLC (Fig. 4) and SDS–PAGE (Fig. 5).

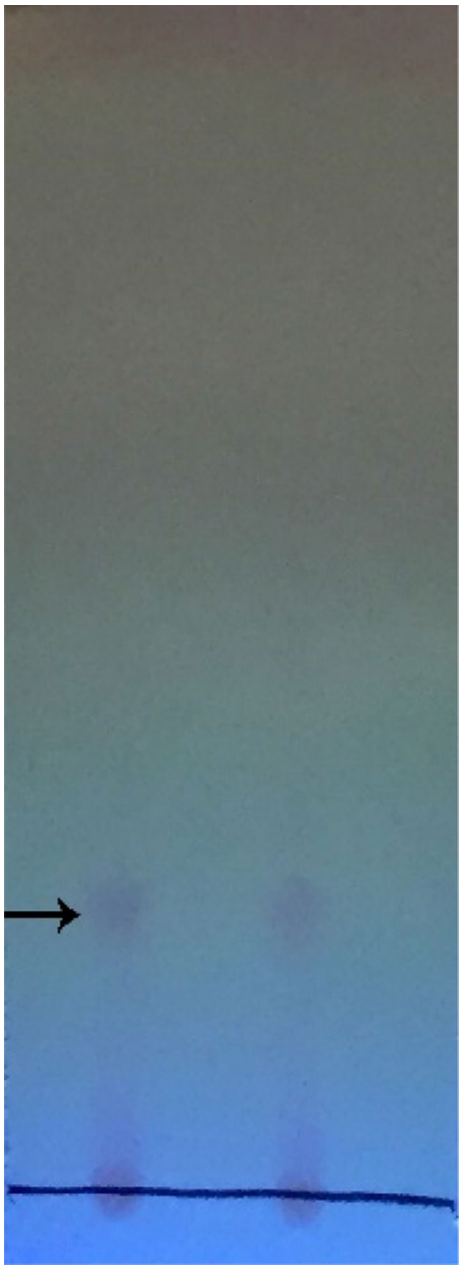

Fig. 3 Thin layer chromatography of the total extract (S15)

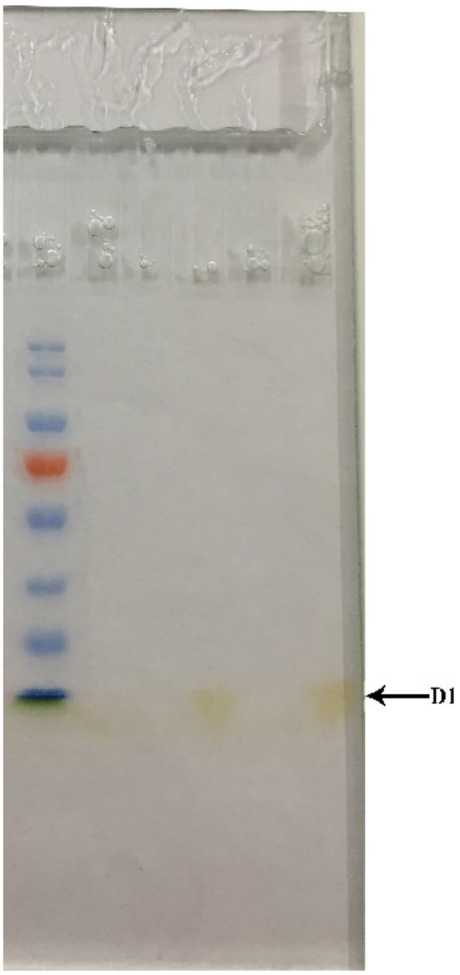

Fig. 4 The image of the secondary metabolites in the polyacrylamide gel. From left to right: A commercial protein marker (PageRuler™ prestained protein ladder, 10–180 kDa, ThermoFisher), recovered elution material from D1 band

Table 1 Two predominant compounds of the D1 band (GC–MS analysis)

Peak	Retention time	Name of the compound	Peak (%)
1	29.194	1-Dodecanol	93.32
2	28.979	2-Propenamide, 2-methyl-*N*-phenyl-	4.83

The two compounds also had closer retention times in GC–MS (29.194 and 28.979, Table 1). The findings, overall, could suggest that chemical compounds having closer retention times in GC–MS, also tend to run closely in lower resolution systems, such TLC and SDS–PAGE. Moreover, the spectroscopic screening of the two elution compounds produced the same absorbance maximum at 190 nm (see supplementary Fig. S1).

Structural Analysis of the Elution Material

Interpretation of FTIR spectra (*see supplementary Fig. S2*): *v* max. (cm^{-1}): 3346–3127 (OH stretch), 2921 (aliphatic CH asymmetric. stretch), 2872 (aliphatic CH symmetric stretch), 1287 (OH bending). OH stretch bands are generally seen at 3600 cm^{-1}. In the spectra obtained they appeared to be flattened and found with low frequency because of the hydrogen bonding. Alkyl groups also

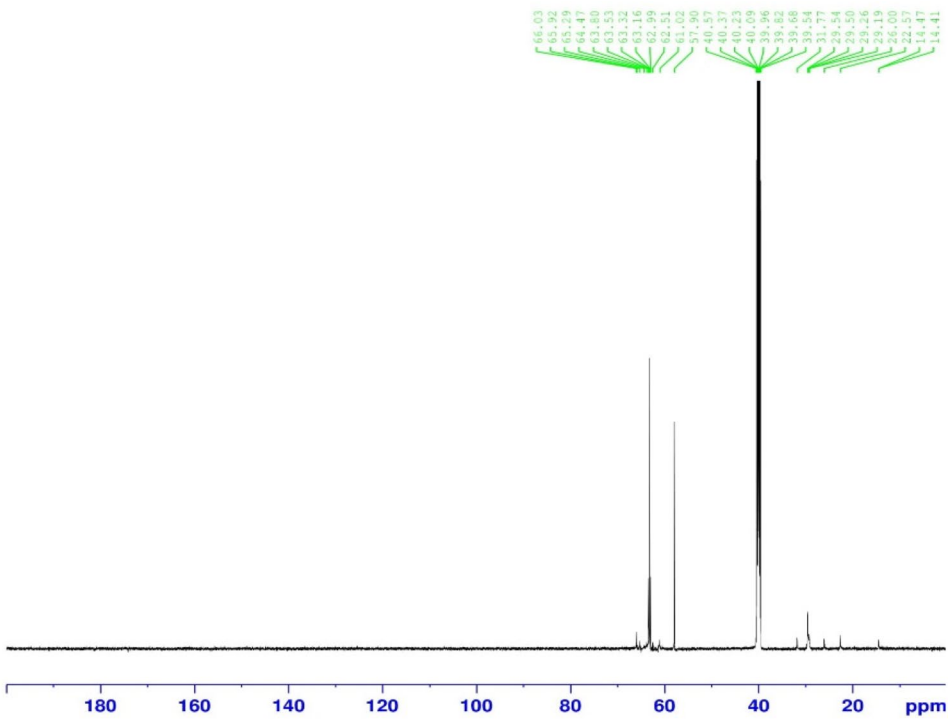

Fig. 5 ^{13}C NMR spectra of 1-dodecanol

produced stretches and bending vibrations at expected regions.

Interpretation of ^{13}*CNMR* [*600 MHz*, (*D6*) *DMSO*] (*Fig. 5*): $\delta = 62.51$ (1-dodecanol C1), $\delta = 31.77$ (1-dodecanol C2), $\delta = 26.00$ (1-dodecanol C3), $\delta = 29.50$ (1-dodecanol C4), $\delta = 29.26$ (1-dodecanol C5), $\delta = 31.77$ (1-dodecanol C6), $\delta = 22.57$ (1-dodecanol C7), $\delta = 14.41$ and 14.47 (1-dodecanol C8).

The above carbon NMR spectra of the elution material were found to be the same with those of the 1-dodecanol samples found in the literature [25]. Thus, the ^{13}C NMR spectra of the eluted material confirmed that 1-dodecanol was the predominant seconder metabolite.

Discussion

In clinical microbiology, mass spectrometry (MALDI-TOF) have often been used for the rapid identification of pathogen microorganisms. Therefore, the software used is rather specific to the comparison of the pathogens. Nevertheless, as in this case, environmental isolates could also be identified in these systems at least at genus level. 16S rRNA gene sequence homology confirmed this finding and further identified the isolate S15 at species level.

The single band visualized by ultraviolet light on the TLC plate was visible to naked eye in the polyacrylamide gel (Fig. 4). The protein marker run along with the extract sample indicated that 1-dodecanol migrated at approximately the same rate with the last, 10 kDa, protein marker band. Although the yellowish colour appeared to be rather faint, this appearance could be quite misleading because the elution material was more than enough to carry out all of the analytical studies. In future studies a quantification of the elution yield will be made possible by HPLC, using a commercial 1-dodecanol along with the elution sample.

This work indicated that an electrophoresis system (SDS–PAGE) frequently used in molecular biology could also be a versatile tool for the resolution of chemical compounds. The approach could be refined by adjusting the system's parameters to the chemistry of the compounds and its capacity could be improved by increasing the size of the instrumentation.

Author Contributions SC conceived and designed research. SC conducted experiments. SC contributed new reagents or analytical tools. SC analysed data. SC wrote the manuscript.

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Compliance with Ethical Standards

Conflict of interest Serap Çetinkaya declares that she has no conflict of interest.

Ethics statement This article does not contain any studies with human participants or animals performed by any of the authors.

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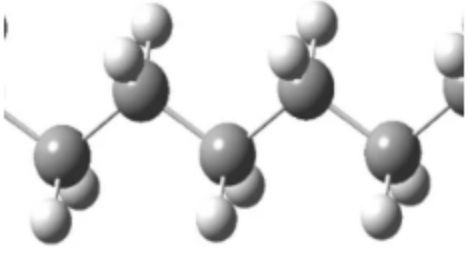

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