BIOTRANSFORMATION OF INDOLE ALKALOID BY TERRESTRIAL
STREPTOMYCES GW 14/587 STRAIN INTO A SELECTIVE
PHYCOTOXIC AGENT

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Methyl indole was isolated as a metabolite from the culture broth of terrestrial
Streptomyces GW 14/587 strain which was fed with indole after two days growth and then
incubated for another two, three and four days to optimize the experiment condition for highest
metabolite concentration. The structure of the isolated methyl indole was established using
spectral techniques of HPLC-MS, EI-MS, ¹H and ¹³CNMR. Also the bacterial extract showed a
high selective phycotoxic activity against Scendensmus subspicatus among the different tested
microalgae, yeast, fungi and bacteria.

INTRODUCTION

Microorganisms, in particular bacteria, have been found to have a profound effect on
the development of medical sciences. Since the discovery of penicillin in 1929, intensive
studies of mainly soil derived bacteria and fungi have shown that microorganisms are a
rich source of new biologically active secondary metabolites.¹-³ Over the past 60
years between 30,000 and 50,000 natural products have been discovered from
microorganisms. More than 10,000 of these compounds were found to be biologically active,
of which more than 8,000 are antibiotics and anti-tumor agents. Today, over 100
microbial products continue to be used clinically as antibiotics and anti-tumor agents.⁴

A large variety of indole alkaloids have been isolated from marine organisms,⁵-¹⁰ among
which is the marine Streptomycete.¹¹-¹⁴ Terrestrial Streptomycetes was also investigated
for its antibiotic contents.¹⁵,¹⁶ Indole derivatives are common secondary metabolites from
microorganisms; more than 1000 derivatives have been reported.⁴

Experiments on feeding bacteria with different compounds and the isolation of their
secondary metabolites, preferably those of biological activities, is a new trend.

Here we report on the indole feeding experiment of terrestrial Streptomycete GW
14/587 establishing the ultimate inoculation period of the fed media for the bacteria to
produce a secondary metabolite which has been isolated, purified and identified. In addition, we
report on the antibiotic effect of the extract of
indole fed bacteria on different microorganisms and micro algae.

EXPERIMENTAL

Materials
Terrestrial *Streptomyces* GW 14/587 was obtained from the collection of Alfred-Wegner Institute of Polar and Meeresforchung, Bremerhaven, Germany. *Escherichia coli* (Strain ATCC 25922), *Bacillus subtilis* (Strain Bs 1091-1), *Streptomyces viridochromogenes* (Strain Tu 57), *Staphylococcus aurens* (Strain Sau 1091-5), *Mucor miehei* (Tu 284), *Candida albicans* (HBI 101), *Chlorella vulgaris* (SAG 211-11b), *Chlorella sorokiniana* (SAG 211-8K) and *Scenedesmus subsipicus* (SAG 86-81) were all obtained from stocks maintained at the Department of Organic Chemistry, George-August-University, Gottingen, Germany.

Indole was obtained from Sigma – Aldrich corporation (Germany) and used in a concentration of 400 mg/L culture media using ethanol as a solvent.

Ehrlich’s reagent: 0.5 g of p-dimethyl amino benzaldehyde (Sigma - Aldrich corporation, Germany) was dissolved in 50 ml methanol and then 2 ml of conc. HCL was added.

Silica gel Alugra SIL G/UV254 (Macherey-Nagel GmbH and Co., Duren, Germany) pre-coated aluminum sheets were used for thin layer chromatography. Silica gel Merck 11695 (Sigma-Aldrich Corporation, Germany) was used for preparative TLC.

Equipment
$^1$H and $^{13}$C NMR spectra were measured on a varian unity 300 (300.145 MHz) spectrometer in CDCl$_3$ as internal standard.

EI-MS was recorded on a varian MAT 731 (70 eV) mass spectrometer, preparative HPLC-MS was performed using an RP18 column (Eurochrome Eurosphere RP 100-C18, 5um) using a Jasco diode array multiple wave length detector (MD-910) in a scanning range of 195-650 nm, connected to (+ve) ESI Finnigan MAT 95 A mass spectrometer. Retention times were measured on a 4 x 250 nm (Eurochrome RP18 column 60A°, 5um) with a linear acetonitrile-water azeotrope / water gradient (t= 0: 10% azeotrope, t= 25 minutes: 100% azeotrope: isocratic for another 50 minutes).

Methods
Preparation of yeast-malt extract medium$^{17}$
Malt extract (10 g), yeast extract (4 g) and anhydrous glucose (4 g) were dissolved in distilled water (1 L). Before sterilization, the pH was adjusted to 7.8 by addition of 2 N NaOH.

Fermentation and feeding
Well-grown agar cultures of *Streptomyces* GW 14/587 served to inoculate eight 1-Litre-Erlenmyer flask each containing 250 ml of yeast malt extract media. The flasks were incubated for two days at 28° with shaking at 95 rpm to grow. The cultures were then fed with 5 ml of prepared indole solution in ethanol in the two following days leaving one group of culture without feeding as a control group. Indole fed cultures were divided into three groups in respect to incubation period; one group was incubated for two days after being fed, the second group was incubated for three days and the third was incubated for four days after being fed; so as to give 4-, 5- and 6-day-old cultures, respectively. All culture flasks were taken out of the shaker and allowed to freeze and lyophilized for 12-24 hours.

Extraction$^{11}$
The fermentation broth for each group was separately filtered with the aid of celite and the resulting mycelial cake was extracted three times with ethyl acetate each with 250 ml with sonication for 15 minutes. Also the filtrate broth was extracted three times using ethyl acetate. All the ethyl acetate extract from filtrate and mycelia cake, for each group, were combined together, concentrated under reduced pressure and examined by TLC, using dichloromethane/ cyclohexane 8:2 as a solvent system and Ehrlich’s reagent as a spray reagent for spot coloring.

Separation
The concentrates of the 4, 5 and 6 days old cultures were separately subjected to preparative thin layer chromatography (PTLC) using cyclohexane / dichloromethane (1:1) as an eluting solvent system. The plates were redeveloped three times to afford a white solid
metabolite (12, 9 and 8 mg of four, five and six day-old culture concentrates, respectively). It gave a dark purplish fluorescence under UV at 254 nm, Rf= 0.60 cyclo hexane : dichloro methane; 1:1, an intense blue color when sprayed with Ehrlish’s reagent and a reddish brown color spot when dipped in freshly prepared ferric chloride solution.

RESULTS AND DISCUSSION

The structure of the metabolite obtained from four, five and six day-old culture concentrates was established as methyl indole (C₉H₉N) using different spectroscopic analyses. EI-MS showed a base peak at m/z 130.1 and a parent peak at 131.1 for M⁺ (Figure 2), while HPLC-MS (+ve) ESI showed a base peak at 132.2 for M⁺1 which appeared at Rf, 14.45 min (Figure 3). ⁱH-NMR showed peaks at δ=8.05 ppm (s, br, 1H, H-N), δ=7.65 (d, J=7.5 Hz, 1H, H-4), δ=7.39 (d, J=7.5 Hz, 1H, H-7), δ=7.24-7.06 (m, 2H, H-5 and H-6), δ=6.95 (s, 1H, H-2) and at δ=2.30 (s, 3H, CH₃) (Figure 4). ¹³C-NMR showed nine peaks at δ ppm 136.22 C-7a, δ 128.22 C-4a, δ 121.81 C-2, δ 121.51 C-4, δ 119.05 C-5, δ 118.78 ppm C-6, δ 111.69 C-3, δ 110.89 C-7 and at δ 9.66 CH₃ (Figure 5). APT ¹³C-NMR shows the quaternary carbons C-2, C-4a and C-7a appear down the spectrum base line (Figure 6).

This data was confirmed by comparison with data from AntiBase¹⁹ using molecular weight and the substructures determined from the ¹H-NMR data.²⁰ Although methyl indole has been previously synthesized as the known compound Skatole,²¹ this is the first report of its isolation from the Streptomycete strain GW 14/587 as a result of biotransformation of the indole base fed into the growth media. It was reported that²² acriflavine treatment of Streptomyces generated a bald mutant that produced two carbazole derivatives, where DNA intercalated the acriflavine dye by mutagenesis. The mechanism of Streptomycete strain GW 14/587 to synthesize methyl indole from the indole fed in the media is still under investigation; however mutagenesis could be a possible mechanism.

Fig. 1: Work-up of the strain GW 14/587

Antimicrobial activity

Antibacterial, antifungal and antimicroalgal (phycotoxic) activities were estimated by the agar diffusion method using paper discs of 8 mm diameter. Each paper disc impregnated with 50 µg of tested culture extract was placed on agar media suspended with test microorganisms. Inhibition zones were observed after incubation at 27° for 24 hours for yeast and 48 hours for fungi, and microalgae and at 37° for 24 hours for bacteria.¹⁸
Fig. 2: EI-MS spectrum of methyl indole.
Fig. 3: HPLC-MS (+ve) ESI spectrum of methyl indole.
Fig. 4: $^1$H-NMR spectrum of methyl indole.
Fig. 5: $^{13}$C-NMR spectrum of methyl indole.
Fig. 6: APT $^{13}$C-NMR spectrum of methyl indole.
Table 1: Antimicrobial activities of *Streptomycete* strain GW 14/587 (Parental and indole-fed) culture extracts compared to germanomycine.

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Diameters of inhibition zones in mm.

(EC= *Escherichia coli*; BS= *Bacillus subtilis*; SV= *Streptomyces viridochromogenes*; SA= *Staphylococcus aurens*; MM= *Mucor miehei*; CA= *Candida albicans*; CV= *Chlorella vulgaris*; CS= *Chlorella sorokiniana*; SS= *Scenedesmus subspicatus*)

The high yield of methyl indole produced by the four–days old culture crude extract (12%) was encouraging to test it for antimicrobial activity (Table 1). Feeding *Streptomycete* strain GW 14/587 with indole has changed the antibiotic pattern of the parent strain. As shown in Table 1 the crude extract of the culture fed with indole in its malt media showed in addition to the antibacterial activity of the parent strain against *Bacillus subtilis* and *Streptomyces viridochromogenes*, a moderate activity against *Escherichia coli* and *Staphylococcus aurens*. The parent culture showed moderate activity against the yeast *Candida albicans*, while the fed indole culture extract showed no activity. It was also found that the fed culture extract has a remarkably stronger selective antimicroalgal activity against *Scenedesmus subspicatus* than the parent crude extract which showed the same moderate phycotoxic activity against the three tested microalgae *Chlorella vulgaris*, *Chlorella sorokiniana* and *Scenedesmus subspicatus*. Both cultures showed no activity against the fungus *Mucor miehei*.

**Conclusion**

Terrestrial *Streptomycete* strain GW 14/587 showed the ability to synthesize methyl indole from indole fed into its growth media. The biotransformation of indole resulted in a strong active antimicroalgal agent against *Scenedesmus subspicatus*.

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**REFERENCES**


