The impact of Schiff bases on antibiotic production by *Streptomyces hygroscopicus*

Slavica B. Ilić · Sandra S. Konstantinović · Dragiša S. Savić · Vlada B. Veljković · G. Gojgić-Cvijović

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**Abstract** A media consisting of isatin-Schiff bases (isatin-3-thiosemicarbazone, isatin-3-semicarbazone, and isatin-3-phenylhydrazone) was developed to maximize the production of antibiotics Hexaene H-85 and Azalomycine B by *Streptomyces hygroscopicus*. The media isatin-3-thiosemicarbazone resulted in the maximum antibiotics concentration of 372 μg cm⁻³ for Hexaene H-85 and 118 μg cm⁻³ for Azalomycine B. The impact of modified media on soil morphology also was investigated.

**Keywords** *Streptomyces hygroscopicus* · Schiff base · Antibiotic production · Morphology

**Introduction**

The genus Actinomyces is an important group of microbes due to their ability to produce commercially valuable secondary metabolites (Abbas and Edwards, 1990; Vučetić *et al.*, 1994; Okami and Hotta, 1988; Prosser and Tough, 1991). The actinomycete *Streptomyces hygroscopicus* produces a range of polyene antibiotics compounds depending on environmental and nutritional conditions (Vučetić *et al.*, 1994; Karadžić *et al.*, 1991). To make the production of the antibiotic feasible, it is necessary to develop the optimum production, which includes among the other...
conditions, formation of chemically defined media. There have been some
investigations about different nitrogen and carbon sources on growth and production
(Abbas and Edwards, 1990; Lee et al., 1997; de Queiroz Sousa et al., 2001; Tripathi
et al., 2004), but no data are available about the influence of Schiff base. In the
present study, an extensive study has been made on the isatin-Schiff bases as a
nitrogen source in chemically defined media on antibiotic production by Strepto-
myces hygroscopicus as well as on soil morphology.

Materials and methods

Organism, media, and growth condition

A strain Streptomyces hygroscopicus was isolated from a soil sample from
Vojvodina, Serbia (Vučetić et al., 1994; Karadžić et al., 1991). Streptomyces
hygroscopicus was maintained as spore and mycelia suspensions in sterile glycerol
(20% [wt/vol]), which were prepared from speculated colonies grown at 30°C on L
agar that contained the following (in g l⁻¹): tryptone (Difco Laboratories), 5; yeast
extract (Lab-M), 5; NaCl, 5; glucose, 1; agar, 10 (pH 7.2). Suspensions were stored
at −20°C until required. Liquid cultures were grown in starch–yeast extract (SY)
broth that contained the following (in g l⁻¹): soluble starch, 15; yeast extract
(Difco), 1; K₂HPO₄ · 7H₂O, 1; NaCl, 3 (final pH adjusted to 7.2). Flasks (250 ml)
that contained 50 ml of this media were inoculated with 0.1 ml of spore suspension
and incubated at 30°C with shaking at 200 rpm. The fermentation media were
inoculated with 5% (v/v) of a preculture after 48 h growth and incubated at 30°C for
240 h under the standard condition of aeration and agitation (200 rpm). The
fermentation basal media has the following composition (g/l): glucose 15, CaCO₃ 3,
NaCl 3, MgSO₄ 0.5, (NH₄)₂HPO₄ 0.5, K₂HPO₄ 0.5, soya bean 1.0. The fermentation
modified media has the follow composition (g/l): glucose 15, CaCO₃ 3, NaCl 3,
MgSO₄ 0.5, (NH₄)₂HPO₄ 0.5, K₂HPO₄ 0.5, L-tryptophan 0.5, Schiff base 0.5.

After fermentation, the antibiotics of the broth were determined by extraction
with n-butanol and ethyl acetate. The results were obtained by measuring
absorbance at λ_max = 364 nm (Hexaene H-85) and λ_max = 252 nm (Azalomycine)
with Perkin-Elmer Lambda 15 UV/VIS spectrophotometer (Vučetić et al., 1994;
Karadžić et al., 1991). Growth was determined by measuring dry weights of cells.
The broth was centrifuged at 4000 rpm for 15 min to separate the mycelial biomass.
After that biomass was dried at 105°C to constant weight and weighed.

General methods of preparation of Schiff bases

Equimolar amounts of isatin and thiosemicarbazide, semicarbazide, and phen-
ethylhydrazine were dissolved in 95% ethanol. The solutions were heated under reflux
for 1 h. The products were filtered, washed with ethanol, and dried in vacuum over
CaCl₂ (Konstantinović et al., 2007). The structures of Schiff bases are given in
Fig. 1.
Methods

Microanalysis for carbon, hydrogen, and nitrogen was performed by using a Carlo Erba 1106 microanalyzer. The chloride content was determined potentiometrically. The melting points were determined by using Thomas–Hoover melting point apparatus and are uncorrected. FTIR spectra were recorded using a Michaelson Bomen MB-series spectrophotometer, using KBr pellet (1 mg/100 mg) technique. The electronic spectra were recorded on a Perkin/Elmer Lambda 15 UV/VIS spectrophotometer using 10^{-3} mol dm^{-3} solutions in DMF. 1H NMR spectra were obtained in DMSO solution with a Gemini-200 “HF NMR” spectrometer.

Isatin-3-thiosemicarbazone (ITC)

Yield 91.1%, Color Yellow. m.p. 239–241°C. IR (KBr, cm^{-1}): 3470, 3304 ν(NH2), 3239, 3132 ν(NH), 1710 ν(C=O), 1585 ν(C=N), 1250 ν(C=S). UV/VIS (DMF, λ (nm/ε · 10^{3} (mol^{-1} dm^{3} cm)): 349/0.946 π → π*, 366/1.325 π → π* 1H NMR (DMSO, δ, ppm) 6.9–7.7 (m, 4H, Ar), 8.69, 9.05 (s, 2H, NH2), 11.21 (2, 1H, NH), 12.47 (s, 1H, NH). Analysis: Found: 49.05%C, 3.75%H, 25.30%N, 14.51%S; Calculated: 49.08%C, 3.70%H, 25.32%N, 14.56%S.
Isatin-3-semicarbazone (ISC)

Yield 90.5%, Color Yellow. m.p. 239°C. IR (KBr, cm$^{-1}$): 3467, 3301 ν(NH$_2$), 3237, 3126 ν(NH), 1704, 1686 ν(C=O), 1595 ν(C=N). UV/VIS (DMF, ν(cm$^{-1}$/ε · 10$^3$)(mol$^{-1}$ dm$^3$ cm): 321.8/3.121 π → π*, 271.8/2.662 π → π*. $^1$H NMR (DMSO, δ, ppm) 6.02–7.94 (m, 4H, Ar), 8.34, 9.02 (s, 2H, NH$_2$), 11.21 (2, 1H, NH), 12.42 (s, 1H, NH). Analysis: Found: 52.92%C, 3.95%H, 27.45%N; Calculated: 52.94%C, 3.92%H, 27.45%N.

Isatin-3-phenylhydrazone (IPH)

Yield 47.89%, Color orange, m.p. 249°C. IR (KBr, cm$^{-1}$): 3326, 3161 ν(NH), 1686 ν(C=O), 1597 ν(C=N). UV/VIS (DMF, ν(cm$^{-1}$/ε · 10$^3$)(mol$^{-1}$ dm$^3$ cm): 398.5/2.260 π → π*, 258.5/1.625 π → π*, 207.5/2.914 π → π*. $^1$H NMR (DMSO, δ, ppm) 6.91–7.57 (m, 4H, Ar), 11.00 (2, 1H, NH), 11.00 (s) (2, 1H, NH), 12.32 (s, 1H, NH). Analysis: Found: 70.86%C, 4.62%H, 17.70%N; Calculated: 70.89%C, 4.64%H, 17.72%N.

Results and discussion

Influence of Schiff bases production of Hexaene H-85 and Azalomycine B

To improve production of Hexaene H-85 and Azalomycine B by Streptomyces hygroscopicus, part of soya beans (0.5%) in basal medium was replaced with isatin Schiff bases (ITC, ISC, and IPH) as a nitrogen source. The maximum concentration of Hexaene H-85 and Azalomycine B (Fig. 2), pH and dry biomass, achieved during the fermentation in basal and modified media are given in Table 1.

Change of pH values

Considering all media, as it can be seen, pH increases until the third or fourth day. The basal medium possesses the highest pH 9.3, whereas the maximum values of pH in tested media is in the range 8.1–8.4 (Fig. 2a).

Glucose utilization

As shown in Fig. 2b, Schiff bases do not have any impact on glucose utilization during the fermentation. In the control medium, the glucose utilization is finished by the third day, whereas media with Schiff bases possess a small amount of unused glucose.

Dry biomass

As shown in Table 1, the addition of Schiff bases to media slightly increases the growth of production soil. The maximum concentration (9.6 g dm$^{-3}$) of dry
Biomass is reached by the fourth day of fermentation in medium with ITC. The values are lower for media with ISC and IPH (9.3 g dm$^{-3}$ and 9.1 g dm$^{-3}$, respectively). The maximum concentration of dry biomass in basal medium is reached by the third day and its value is 8.9 g dm$^{-3}$.

Production of Hexaene H-85

The addition of Schiff bases is stimulated the production of Hexaene H-85, and the values are higher than basal medium. Maximum concentration of antibiotic is reached by the third day in basal medium and by third and fourth days in modified media (Table 1). The maximum concentration of Hexaene H-85 in medium with ITC is 372 µg cm$^{-3}$, which is for 63% higher compared with basal medium.
The media with other ISC and IPH also stimulated the production of this antibiotic for 32% and 52%, respectively, compared with the basal medium, but the values are lower than medium with ITC (293 μg cm⁻³ and 329 μg cm⁻³, respectively; Fig. 3c).

Production of Azalomycine B

The addition of Schiff bases also stimulated the production of Azalomycine B (Table 1). The highest concentration is achieved on the fourth day of fermentation. Compared to the basal medium, ITC increases the concentration of antibiotic two times, whereas ISC and IPH increase the production of the same antibiotic by 85% and 57%, respectively (Fig. 3d).

The mechanism of action of tested Schiff bases was not examined in this work, but there is no doubt that those compounds can be used as a carbon source for antibiotic production. In this study, we used those compounds as a nitrogen source, because there is a similarity between L-tryptophan, an amino acid already used as a nitrogen source in a basal medium, and used Schiff bases. There is a probably a connection between the structure of Schiff bases and their impact on antibiotic production. The ITC has the highest influence on antibiotic production, and yet the only difference compared with ISC is in C=S group, which ITC possesses and it is known that biological activity of Schiff bases is due to C=N group and C=S group if compound contained it.

Impact of Schiff bases on strain morphology

During fermentation, the nutrient media with isatin Schiff bases, as a nitrogen source, the strain is in the form of pellets, and little of single, free filaments (Table 2). The morphology of S. hygroscopicus is shown in Fig. 3.

### Table 1

| Nitrogen source | \( k_{\text{max}} \) \( \text{d}^{-1} \) | \( X_{\text{max}} \) \( \text{g dm}^{-3} \) | Hexaene H-85 | \( C_{\text{max}}^H \) \( \mu\text{g cm}^{-3} \) | \( y_{\text{max}}^H \) \( \mu\text{g g} \text{s.b} \) | Azalomycine B | \( C_{\text{max}}^A \) \( \mu\text{g cm}^{-3} \) | \( y_{\text{max}}^A \) \( \mu\text{g g} \text{s.b} \) |
|-----------------|-----------------|-----------------|-------------|-----------------|-----------------|--------------|-----------------|-----------------|
| SB              | 0.97            | 8.9             | 212         | 23.82           | 56              | 6.29        |
| SB + ITC        | 1.04            | 9.6             | 372         | 38.75           | 118             | 12.29       |
| SB + ISC        | 1.01            | 9.3             | 293         | 31.50           | 92              | 9.89        |
| SB + IPH        | 1.03            | 9.1             | 329         | 36.15           | 106             | 11.64       |

SB soya bean

(212 μg cm⁻³).
Fig. 3  Morphology of *S. hygroscopicus* in basal medium and media with Schiff bases: a ITC, b ISC, and c IPH
### Table 2  Impact of Schiff bases on morphology *S. hygroscopicus* and production of antibiotics

| Nitrogen source | Strain morphology                                      | Yield of antibiotics | \(Y_H^{\max}\) | \(Y_A^{\max}\) |
|-----------------|--------------------------------------------------------|----------------------|-----------------|-----------------|
| ITC             | Pellets, single, weakly branched filaments              | 38.75                | 12.29           |
| ISC             | Pellets, single, weakly branched filaments              | 31.50                | 9.89            |
| IPH             | Pellet, a little of single filaments                    | 36.15                | 11.64           |

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