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MEDICINAL CHEMISTRY RESEARCH

ORIGINAL RESEARCH

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

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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

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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 *Streptomyces hygroscopicus* as well as on soil morphology.

Materials and methods

Organism, media, and growth condition

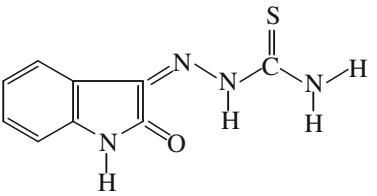
A strain *Streptomyces hygroscopicus* was isolated from a soil sample from Vojvodina, Serbia (Vučetić *et al.*, 1994; Karadžić *et al.*, 1991). *Streptomyces hygroscopicus* was maintained as spore and mycelia suspensions in sterile glycerol (20% [wt/vol]), which were prepared from speculated colonies 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; $K_2HPO4 \cdot 7H_20$, 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, $CaCO_3$ 3, NaCl 3, $MgSO_4$ 0.5, $(NH_4)_2HPO_4$ 0.5, K_2HPO_4 0.5, soya bean 1.0. The fermentation modified media has the follow composition (g/l): glucose 15, $CaCO_3$ 3, NaCl 3, $MgSO_4$ 0.5, $(NH_4)_2HPO_4$ 0.5, K_2HPO_4 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 $\lambda_{max} = 364$ nm (Hexaene H-85) and $\lambda_{max} = 252$ 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.

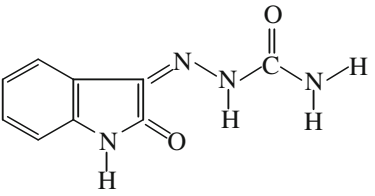
General methods of preparation of Schiff bases

Equimolar amounts of isatin and thiosemicarbazide, semicarbazide, and phenylhydrazine 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 $CaCl_2$ (Konstantinović *et al.*, 2007). The structures of Schiff bases are given in Fig. 1.

Fig. 1 Structures of Schiff bases



isatin-3-thiosemicarbazone



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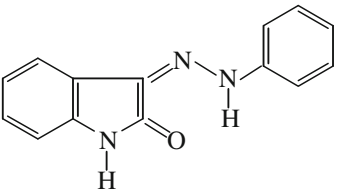

isatin-3-phenylhydrazone

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. ^{1}H 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 $\nu(NH_2)$, 3239, 3132 ν(NH), 1710 ν(C=O), 1585 ν(C=N), 1250 ν(C=S). UV/VIS (DMF, λ (nm/$\varepsilon \cdot 10^3$(mol^{-1} dm^3 cm): 349/0.946 $\pi \rightarrow \pi^*$, 366/1.325 $\pi \rightarrow \pi^*$ ^{1}H NMR (DMSO, δ, ppm) 6.9–7.7 (m, 4H, Ar), 8.69, 9.05 (s, 2H, NH_2), 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 $\nu(NH_2)$, 3237, 3126 ν(NH), 1704, 1686 ν(C=O), 1595 ν(C=N). UV/VIS (DMF, $\nu(cm^{-1}/\varepsilon \cdot 10^3(mol^{-1}\ dm^3\ cm)$: 321.8/3.121 $\pi \rightarrow \pi^*$, 271.8/2.662 $\pi \rightarrow \pi^*$. ^{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, $\nu(cm^{-1}/\varepsilon \cdot 10^3(mol^{-1}\ dm^3\ cm)$: 398.5/2.260 $\pi \rightarrow \pi^*$, 258.5/1.625 $\pi \rightarrow \pi^*$, 207.5/2.914 $\pi \rightarrow \pi^*$. ^{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 bean (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

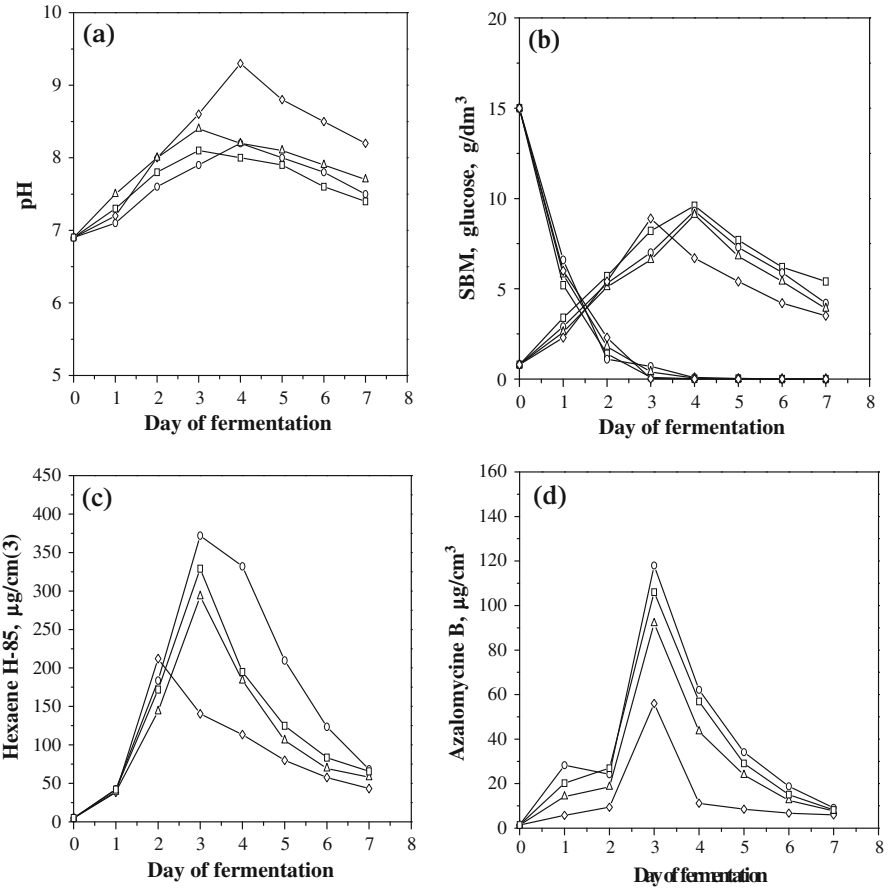

Fig. 2 Change of pH (**a**), concentration of glucose and dry biomass (**b**), concentration of Hexaene H-85 (**c**), and Azalomycine B (**d**) in basal medium (-◇-) and media with Schiff bases: ITC (-○-), ISC (-Δ-), and IPH (-□-)

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

Table 1 Impact of Schiff bases on maximum specific rate of glucose utilization (k_{max}), maximum concentration of dry biomass (X_{max}), and maximum production (C_{max}) and yield of antibiotics (Y_{max}) during the fermentation of *S. hygroscopicusa*

Nitrogen source	k_{max}	X_{max}	Hexaene H-85		Azalomycine B	
	d^{-1}	g dm^{-3}	C^{H}_{max} μg cm^{-3}	Y^{H}_{max} μg $g_{s.b}$	C^{A}_{max} μg cm^{-3}	Y^{A}_{max} μg $g_{s.b}$
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}). 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.

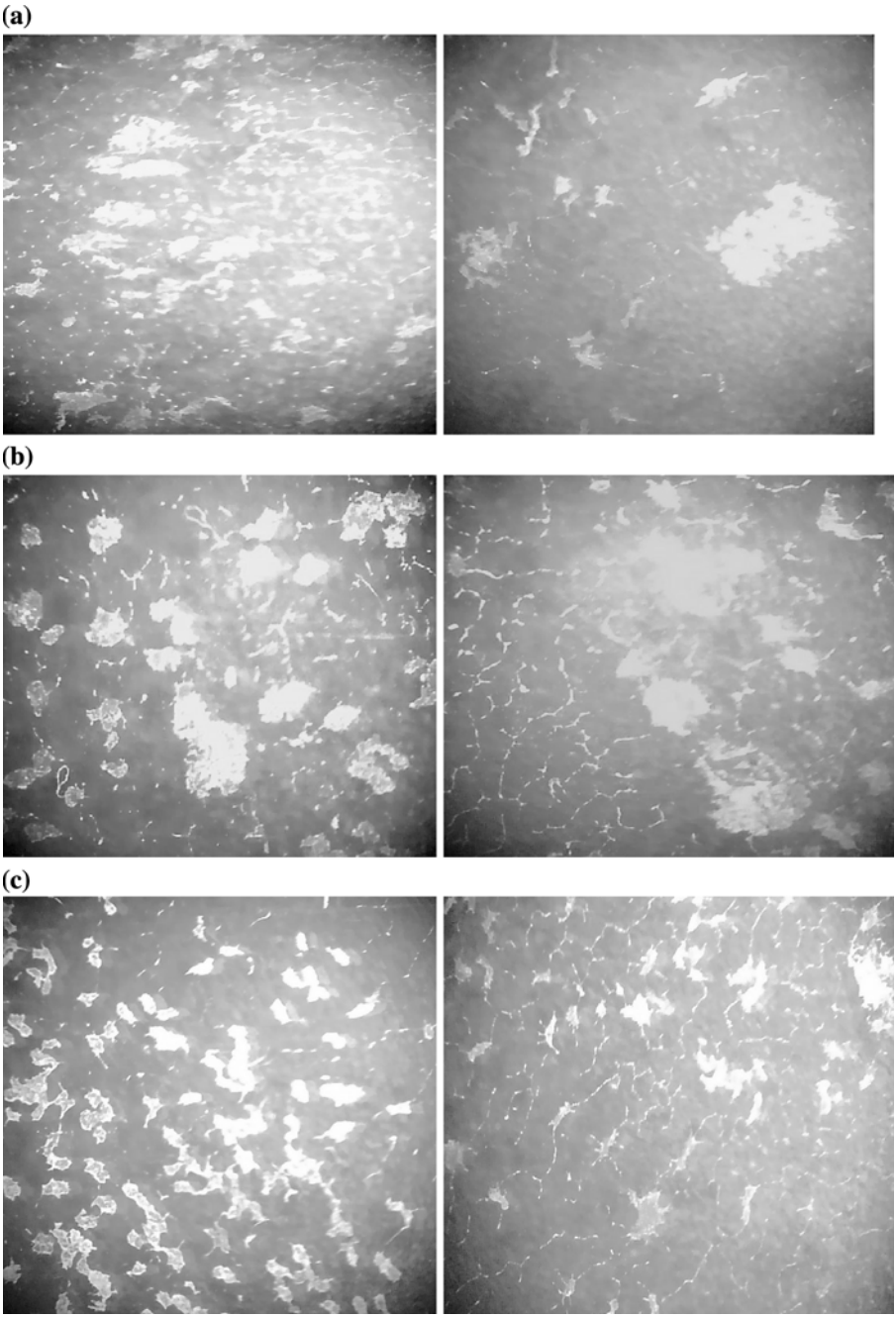

Fig. 3 Morphology of *S. hygroscopicus* in basal medium and media with Schiff bases: **a** ITC, **b** ISC, and **c** IPH

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 fillaments	38.75	12.29
ISC	Pellets, single, weakly branched fillaments	31.50	9.89
IPH	Pellet, a little of sinlge fillaments	36.15	11.64

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