The use of salicylaldehyde derivatives as a nitrogen source for antibiotic production by *Streptomyces hygroscopicus* CH-7

Sandra S. Konstantinović[1], Milica Z. Zlatković[1], Jovan T. Ćirić[1], Slavica B. Ilić[1], Gordana D. Gojgić Cvijović[2], Vlada B. Veljković[1]

[1]*Faculty of Technology, University of Niš, Leskovac, Serbia*
[2]*Institute for Chemistry, Technology and Metalurgy, Department of Chemistry, University of Belgrade, Belgrade, Serbia*

Abstract

In the present work, four derivatives of salicylaldehyde (salicylaldehyde-hydrazone, phenylhydrazone, semicarbazone and thiosemicarbazone) were synthesized using both conventional (95% ethanol) and green (crude glycerol from biodiesel production) solvents. The obtained compounds were identified by elemental microanalysis, as well as FTIR, UV/Vis and ^{1}H-NMR spectroscopic methods. Yields of 93–98% of the compounds in crude glycerol were achieved within 10–25 min. The derivatives of salicylaldehyde and crude glycerol were used as a nitrogen and carbon source, respectively, in the medium for antibiotic (hexaene H-85 and azalomycine B) production by *Streptomyces hygroscopicus* CH-7. The highest concentrations of hexaene H-85 and azalomycine B were achieved in the medium containing salicylaldehyde-thiosemicarbazone (198 and 69 μg/cm^3, respectively). Derivatives of salicylaldehyde also impacted the strain morphology. In the media with salicylaldehyde-phenylhydrazone and salicylaldehyde-thiosemicarbazone, *S. hygroscopicus* CH-7 grew like large dispersive pellets with long twisted filaments that produced the highest yield of the antibiotics.

Keywords: glycerol, salicylaldehyde, Streptomyces, antibiotic, morphology.

Available online at the Journal website: http://www.ache.org.rs/HI/

SCIENTIFIC PAPER

UDC 547.4/.5:66:615.33

Hem. Ind. **71** (6) 487–494 (2017)

Selection of an adequate solvent is a very important step in many organic reactions. The physicochemical and biological characteristics of the selected solvent significantly influence the reaction selectivity and yield. These properties are also very important for the product isolation, the reaction costs, the process safety and the environmental protection. Therefore, researchers have constantly been searching for environmentally-friendly solvents. During the last couple of years, different liquids, besides water, have been used as alternative solvents, such as ionic liquids and supercritical fluids [1].

Recent studies have shown that "pure" and crude glycerol from biodiesel production can successfully be used as a solvent in various reactions of organic synthesis, ensuring a high product yield and selectivity [2,3]. Because of its high boiling point and negligible vapor pressure, glycerol is an important alternative solvent for different catalytic and non-catalytic organic reactions. Being non-toxic, non-irritating, biodegradable, recyclable, environmentally-friendly, highly stable under typical storage conditions and compatible with many other chemicals, glycerol is considered as a "green" solvent [2,3].

The rapid growth of biodiesel industry has also expanded crude glycerol production as its main by-product [4]. The world production of glycerol is predicted to be 41.9 billion L by 2020 [3,5]. The increased crude glycerol production will make an additional pressure to already saturated glycerol market and generate environmental concerns associated with contaminated glycerol disposal [2]. Therefore, valorization of crude glycerol obtained during biodiesel production through new applications, despite the presence of impurities, has become an important issue for both glycerol market and commercial biodiesel production [2,3]. With such an approach, crude glycerol could be regarded as a desirable by-product and not as a waste with a disposal cost.

Crude glycerol originating from biodiesel production has already been used as a solvent in various processes such as Heck coupling and Suzuki cross coupling of halobenzenes [6], base-catalyzed aldol condensation, palladium catalyzed Heck carbon–carbon coupling [2], the Aza-Michael reaction of *p*-anisidine and the Michael reaction of indole [7]. Some Schiff bases can be also obtained in crude glycerol, such as vanillin-semicarbazone. The yield of vanillin-semicarbazone is increased by 17%, and the reaction time is shortened as compared with the use of conventional solvents, such as methanol and ethanol [8]. Besides the uses of Schiff

Correspondence: S.S. Konstantinović, Faculty of Technology, University of Niš, Leskovac, Serbia.
E-mail: sakisandra12@yahoo.com
Paper received: 24 January 2017
Paper accepted: 6 July 2017
https://doi.org/10.2298/HEMIND170124011K

bases in medicine and pharmaceutical industry due to their beneficial properties including antibacterial, antifungal and antitumor activities [9], they can be also used as a nitrogen source in antibiotic production by some microorganisms, such as *S. hygroscopicus*. Previous studies have shown that isatin Schiff base and its inclusion complexes can significantly improve the production of hexaene H-85 and azalomycine B by *S. hygroscopicus* CH-7 [10–12].

In the present work, several derivatives of salicylaldehyde (salicylaldehyde-hydrazone, salicylaldehyde--phenylhydrazone, salicylaldehyde-semicarbazone and salicylaldehyde-thiosemicarbazone) were synthesized in crude glycerol that originated from the alkali-catalyzed transesterification of edible sunflower oil as well as in ethanol (95 vol.%) as a conventional solvent The main goal was to increase the product yield and to shorten the reaction time, as well as to evaluate the obtained derivatives as a nitrogen source in the nutrition medium in antibiotic production by *S. hygroscopicus* CH-7. Finally, the work aims at increasing possibilities for utilization of crude glycerol in microbiological synthesis of value-added products.

EXPERIMENTAL

Chemistry

All chemicals used, except crude glycerol, were of reagent grade and used without further purification. They were purchased from Sigma Aldrich. The crude glycerol (impurities < 5%), a by-product in the laboratory production of biodiesel from sunflower oil, was obtained from the Laboratory for Chemical Engineering, the Faculty of Technology, Leskovac. For the purpose of synthesis, the potential excess of methanol was removed from the crude glycerol by distillation. The acidity of methanol-free crude glycerol was adjusted to pH 5 by addition of 85% phosphoric acid [13]. The inorganic salts formed in this stage were then removed by centrifugation (6080 RCF for 15 min).

Carbon, hydrogen and nitrogen were determined by microanalysis using a microanalyzer (model 1106, Carlo Erba, Devon, United Kingdom). Melting points were determined in capillary melting point apparatus (model Thomas-Hoover Uni-melt, USA) and were not corrected. The purity of the synthesized compounds was gained by thin layer chromatography (TLC) on silica gel using benzene:chloroform = 55:45, the compound being visualized by iodine vapors. The FTIR spectra were recorded with a spectrometer (Bomem MB-100, model Hartmann & Braun, Canada), using the KBr pellet (1 mg/100 mg) technique. The UV/Vis spectrum of 1×10^{-5} mol·dm^{-3} ethanol solution of synthesized compounds were recorded on a UV/Vis spectrophotometer (Lambda 15, Perkin-Elmer, USA).

The ^{1}H-NMR spectra were recorded in DMSO-d_6 solution on a spectrometer (500 MHz, model Gemini--200, Varian, Australia).

Synthesis of salicylaldehyde derivatives was conducted by the standard procedure [14]. Salicylaldehyde (0.1 mol) and hydrazine, phenylhydrazine, semicarbazide or thiosemicarbazide (0.1 mol) were dissolved in 95% ethanol as the conventional solvent. The mixture was refluxed at 50 °C and pH was adjusted to 4.5 by adding H_2SO_4. The products were collected by filtration and washed out with the used solvent. The experiments were repeated three times.

Salicylaldehyde derivatives were also synthesized in crude glycerol by using the same starting components and molar ratios as stated above. The mixtures were refluxed at 80 °C. The products, precipitated as a solid (salicylaldehyde-hydrazone, salicylaldehyde-phenylhydrazone and salicylaldehyde-semicarbazone) and as a crystal (salicylaldehyde-thiosemicarbazone), were filtered and washed with water. The experiments were repeated three times.

Antibiotic production by Streptomyces hygroscopicus CH-7

A strain *S. hygroscopicus* CH-7 (NCAIM (P) B--001336) was obtained from the Microbial Collection of the Faculty of Chemistry and the Institute of Chemistry, Technology and Metallurgy, Belgrade, Serbia [15,16]. *S. hygroscopicus* CH-7 was maintained as spore and mycelia suspensions in sterile glycerol (20%) which were prepared from speculated colonies grown at 30 °C on agar that contained the following (in g/dm^3): tryptone (Difco Laboratories, Detroit, United States), 5; yeast extract (Lab-M, United Kingdom), 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/dm^3): soluble starch, 15; yeast extract (Difco Laboratories, USA),1; $K_2HPO_4{\cdot}7H_20$, 1; NaCl, 3 (final pH adjusted to 7.2) prepared according to previously published data [10–12]. Flasks (250 cm^3) that contained 50 cm^3 of this broth were inoculated with 0.1 cm^3 of spore suspension and incubated at 28 °C with shaking at 220 rpm. The fermentation media were inoculated with 4 vol.% of a 48 h old preculture and shaken (220 rpm, rotary shaker, Heidolph, Model Unimax 2010, Schwabach, Germany) at 28 °C for 168 h. Media used for the fermentation were as follows: basal medium: (crude glycerol 15 g/dm^3; *L*-tryptophan 10 g/dm^3; $CaCO_3$, 3 g/dm^3; NaCl 3 g/dm^3; $MgSO_4{\cdot}7H_2O$ 0.5 g/dm^3; $(NH_4)_2HPO_4$ 0.5 g/dm^3; K_2HPO_4 1 g/dm^3) and modified media (crude glycerol 15 g/dm^3; salicyladehyde derivatives 10 g/dm^3; $CaCO_3$ 3 g/dm^3; NaCl 3 g/dm^3; $MgSO_4{\times}7H_2O$ 0.5 g/dm^3; $(NH_4)_2HPO_4$ 0.5 g/dm^3; K_2HPO_4 1 g/dm^3).

Determination of antibiotics

Antibiotics were measured spectrophotometrically (hexaene H-85 at λ_{max} = 364 nm and azalomycine B at λ_{max} = 252 nm) using a Perkin-Elmer Lambda 15 UV/Vis spectrophotometer (country). The mixture of 0.5 cm^3 fermentation broth and 2.0 cm^3 of 1-butanol for hexaene H-85 and ethyl acetate for szalomycine B was stirred and centrifuged.

Absorbance (*A*) of the extract was measured and used for calculating the concentration as follows:

γ (μg/cm^3) = 66.7A_{364}

γ (μg/cm^3) = 25.3A_{252}

where 66.7 and 25.3 are calculated extinction coefficients based on experimental measurements for hexaene H-85 and azalomycine B, respectively, [15,16].

The microbial growth was monitored by measuring dry biomass gravimetrically [16]. The broth (8 cm^3) was centrifuged at 4000 rpm for 15 min to separate the mycelial biomass. After that, the biomass was dried at 105 °C to the constant weight. During the fermentation, samples were periodically taken and microscopic photographs were obtained by Leica (Microsystem, Heerbrugg, Germany) using the Leica application Suite program (ver. 2.5.0, Leica microsystem, Switzerland). All experiments were performed in triplicate.

RESULTS AND DISCUSSION

Characterization of salicylaldehyde derivatives

The stoichiometric equation of synthesis is shown in Figure 1.

The synthesized compounds were characterized by physical and spectral studies (given below). Purity of the compounds was checked with TLC, and the melting points are in accordance with literature data [17,18].

Physicochemical characterization

Salicylaldehyde-hydrazone (SalH)

Colour: white; m.p: 98 °C; R_f(benzene:chloroform = 55:45) = 0.70; UV (EtOH): λ (nm)/ $\varepsilon \times 10^3$(dm^3 mol^{-1}·cm^{-1}): 218/0.8816, 294/1.0672, 344/0.9481; FTIR (cm^{-1}): 3443, 3292, 1580, 1341, 1201; ^{1}H-NMR (400 MHz, DMSO-d_6), δ (ppm): 11.13 (*s*, OH), 9.01 (*s*, –CH=N), 8.19 (*s*, NH), 6.93–7.15 (*m*, 4H); Combustion analysis for $C_8H_8N_2O$: Calcd.: C 61.75, H 5.92, N 20.57; found: C 61.71, H 5.96, N 20.59.

Salicylaldehyde-phenylhydrazone (SalP)

Colour: white; m.p: 142 °C; R_f(benzene:chloroform = 55:45) = 0.67; UV (EtOH), λ (nm)/ $\varepsilon \times 10^3$(dm^3 mol^{-1} cm^{-1}): 220/0.8568, 294/1.0124, 346/0.9036; FTIR (cm^{-1}): 3446, 3292, 1588, 1344, 1200; ^{1}H-NMR (400 MHz, DMSO-d_6), δ (ppm): 11.12 (*s*, OH), 9.11 (*s*, –CH=N), 8.21 (*s*, NH), 6.95–7.15 (*m*, 3H), 7.29–7.41 (*m*, 5H); Combustion analysis for $C_{13}H_{12}N_2O$: Calcd.: C 53.65, H 5.02, N 23.47; found: C 53.62, H 5.06, N 23.45.

Salicylaldehyde-semicarbazide (SalS)

Colour: bright yellow; m.p: 225 °C; R_f(benzene:chloroform = 55:45) = 0.64; UV (EtOH), λ (nm)/ $\varepsilon \times 10^3$(dm^3 mol^{-1}·cm^{-1}): 216/1.4625, 277/1.6623, 286/1.4581, 358/1.0758; FTIR (cm^{-1}): 3493, 3342, 3234, 3279, 1590, 1352, 1201; ^{1}H-NMR (400 MHz, DMSO-d_6), δ (ppm): 11.37 (*s*, OH), 9.18 (*s*, –CH=N), 8.16 (*s*, NH), 8.15 (*s*, NH_2), 6.82–6.88 (*m*, 3H); Combustion analysis for $C_8H_8N_3O_2$: Calculated. C 53.09, H 5.02, N 15.50; found: C 53.04, H 5.01, N 15.46.

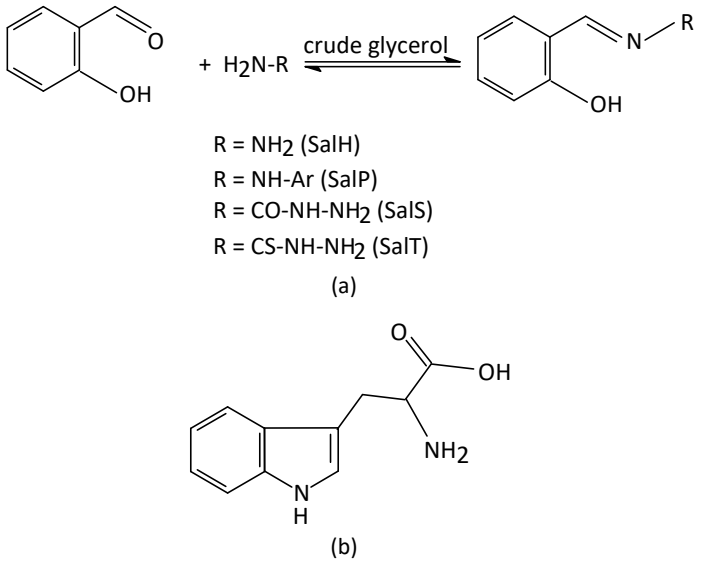

Figure 1. Reaction scheme of salicylaldehyde derivatives synthesis (a) and structure of tryptophan (b).

Salicylaldehyde-thiosemicarbazide (SalT)

Colour: bright yellow; m.p: 230 °C; R_f(benzene: :chloroform = 55:45) = 0.63; UV (EtOH), λ (nm)/ $\varepsilon\times10^3$(dm^3 mol^{-1}·cm^{-1}): 228/0.8285, 295/1.1471, 304/ /1.2771, 357/1.6001; FTIR (cm^{-1}): 3443, 3320, 3231, 3273, 1593, 1367, 1201; ^{1}H-NMR (400 MHz, DMSO-d_6), δ (ppm): 11.37 (*s*, OH), 9.68 (*s*, –CH=N), 8.37 (*s*, NH), 8.09 (*s*, NH_2), 6.77–6.68 (*m*, 3H).Combustion analysis for $C_8H_8N_3OS$: Calculated. C 49.25, H 4.62, N 21.55; found: C 49.21, H 4.65, N 21.52.

The ^{1}H-NMR spectra have a singlet at ~9.10 ppm, corresponding to azomethine HC=N group, as well as proton signals for –NH group in all compounds and $–NH_2$ group for semicarbazide and thiosemicarbazide. An ν(OH) stretching vibration appears at 3443-3493 cm^{-1} in the FTIR spectra of compounds. All spectra have exhibited ν(NH) stretching vibration in the region of 3273–3292 cm^{-1}. The band is strong, due its intramolecular hydrogen bond formation between azomethine and hydroxyl group. An appropriate in-plane deformation vibration at ~1350 cm^{-1} confirms the intramolecular hydrogen bonding of this group. The derivatives of salicylaldehyde also show ν(HC=N) stretching band at ~1585 cm^{-1} which confirms carbonyl-amine condensation reaction between salicylaldehyde and amine component. As it can be seen, the results obtained from elemental microanalysis and spectroscopic analysis is in accordance with structures suggested in Figure 1 [12,14].

Formation of salicylaldehyde derivatives in ethanol, as a conventional solvent, requires an acid-catalyzed reaction (sulfuric acid solution) because of water elimination and nucleophilic attack of the amine component on the salicylaldehyde carbonyl group (pH 4– –4.5). The reaction takes place at 50 °C within 30 min (SalH, SalP and SalT) or 40 min (SalS), Table 1.

In crude glycerol, the reaction occurred without the use of catalyst at 80 °C, and compared to the conventional solvent, the reaction times were shorter and the yields of salicyladehyde derivatives were higher (Δ = 2–8%), Table 1.

Among others, the advantages of glycerol, compared to the conventional solvent ethanol, are higher boiling point (290 *vs*. 78 °C), lower vapor pressure (<133.32 *vs*. 27997.69 Pa) and lower toxicity (lethal dose, 50% (LD_{50}) of 12.600 *vs*. LD_{50} of 5.628), which makes glycerol more suitable solvent for the synthesis.

Antibiotic production with salicylaldehyde derivatives in medium as a nitrogen source

The strain *S. hygroscopicus* CH-7 produces antibiotics such as hexaene H-85 and azalomycine B. Hexaene H-85 complex produced by *Streptomyces* CH-7 belongs to polyene antibiotic family. Although not fully characterized, it is structurally similar to mediomycins, linear polyenes consisting of a hexaene moiety and a conjugated oxo-triene group [19]. Azalomycine B (elaiophyline) is an antibacterial antibiotic with macrodiolide structure [20].

Polyene antibiotics are potent broad-spectrum antifungal compounds with low frequency of resistance among fungal pathogens. Well-known representatives of this group are nystatin A1 and amphotericin B used in clinical practice as antifungal and antiparasitic drugs. Even though new potential applications of polyene antibiotics in therapy of HIV infection and cancer are proposed, their use is limited due to serious side effects such as nephrotoxicity [21].

Both polyene and macrodiolide antibiotics are synthesized by polyketide syntheses (PKSs). Recently, characterization of modular PKSs responsible for the production of polyketides similar to mediomycins was reported [22]. Progress in genomic sequencing and understanding gene cluster evolution as well as chemical studies may significantly contribute to the improved production of known compounds and the discovery of new [23,24].

L-Tryptophan (Trp), as amino acid, has already been used as a nitrogen source, as well as some Schiff bases with isatin moiety, and the results were significant, considering antibiotic production by *S. hygroscopicus* CH-7 [10,11]. In this work, Trp was used as a nitrogen source in the basal medium, while in the modified media it was replaced with an equal amount of SalH, SalP, SalS or SalT. Table 2 shows the impact of basal and modified media on the concentrations of dry biomass and antibiotics, while the kinetics of fermentation is shown in Figure 2. Being very low, the error bars are shown but hardly visible due to low values (±2%).

Table 1.Reaction times and yields of salicylaldehyde derivatives synthesized in crude glycerol and ethanol; Δt – difference between reaction times in ethanol and glycerol; Δ – difference between reaction yields in ethanol and glycerol

Compound	Solvent						Δt / min	Δ / %
	Ethanol			Crude glycerol				
	Reaction time, *t* / min	Yield, %	Catalyst	Reaction time, *t* / min	Yield, %	Catalyst		
SalH	45	85	Yes	10	95	No	35	10
SalP	45	80	Yes	10	98	No	35	18
SalS	40	70	Yes	25	93	No	15	23
SalT	40	80	Yes	15	95	No	25	15

Table 2. Effects of tryptophan and derivatives of salicylaldehyde on the maximum dry biomass concentration (X_{max}) and the maximum concentration of antibiotics (C_{max}) achieved during the fermentation using S. hygroscopicus CH-7

Nitrogen source	X_{max} / g dm^{-3}	Hexaene H-85 c^{H}_{max} / μg cm^{-3}	Azalomycine B c^{E}_{max} / μg cm^{-3}
Trp	8.1	121	41
SalH	8.7	157	51
SalP	8.3	168	63
SalS	8.5	142	57
SalT	9.2	198	69

The concentration of dry biomass (Figure 2a) increases within 3 days of fermentation. The highest concentration of dry biomass was achieved in the medium with SalT as a nitrogen source after 2 days (9.2 g/dm^3).

The results show that used nitrogen sources have different effects on antibiotic production by *S. hygroscopicus* CH-7 (Table 2 and Figure 2b and c).

The concentration of hexaene H-85 (Figure 2b) increases within 3 days, while the concentration of azalomycine B (Figure 2c) increases within 5 days in all tested media. The highest concentration of antibiotics is achieved with SalT as a nitrogen source (198 μg/cm^3 for hexaene H-85 and 69 μg/cm^3 for azalomycine B). Concentrations of hexaene H-85 and azalomycine B are higher for 77 and 28%, respectively, than those in the basal medium. The obtained results indicate that medium containing L-tryptophan and Schiff base as a nitrogen source stimulates production of both antibiotics by *S. hygroscopicus* CH-7.

Effects of salicylaldehyde derivatives on the strain morphology

Composition of media has a great impact on the *Streptomyces* morphology. The strain used in the present study grows in the form of both single or branched filaments and single pellets. Formation of singles mall pellets, as it was reported, favors the production of secondary metabolites [25,26]. The *Streptomyces* morphology depends on the nitrogen source in the nutrition media. For instance, a strain of *Streptomyces fradiae* grows like pellets if the nutrition medium contains amino acids or inorganic ammonia salts as nitrogen sources [27]. If the nutrition medium contains soybean and inorganic ammonia salts as nitrogen sources, the strain *Streptomyces* VITSVK9 grows like long and twisted filaments [28]. The *S. hygroscopicus* CH-7 grows like filaments and pellets in the nutrition media containing different amino acids (phenylalanine, tryptophan, alalin, valine and arginine). During the fermentation with Schiff base as a nitrogen source, pellets and single filaments are dominant forms [29]. Figure 3 shows the morphology of *S.hygroscopicus* CH-7 after 7 days.

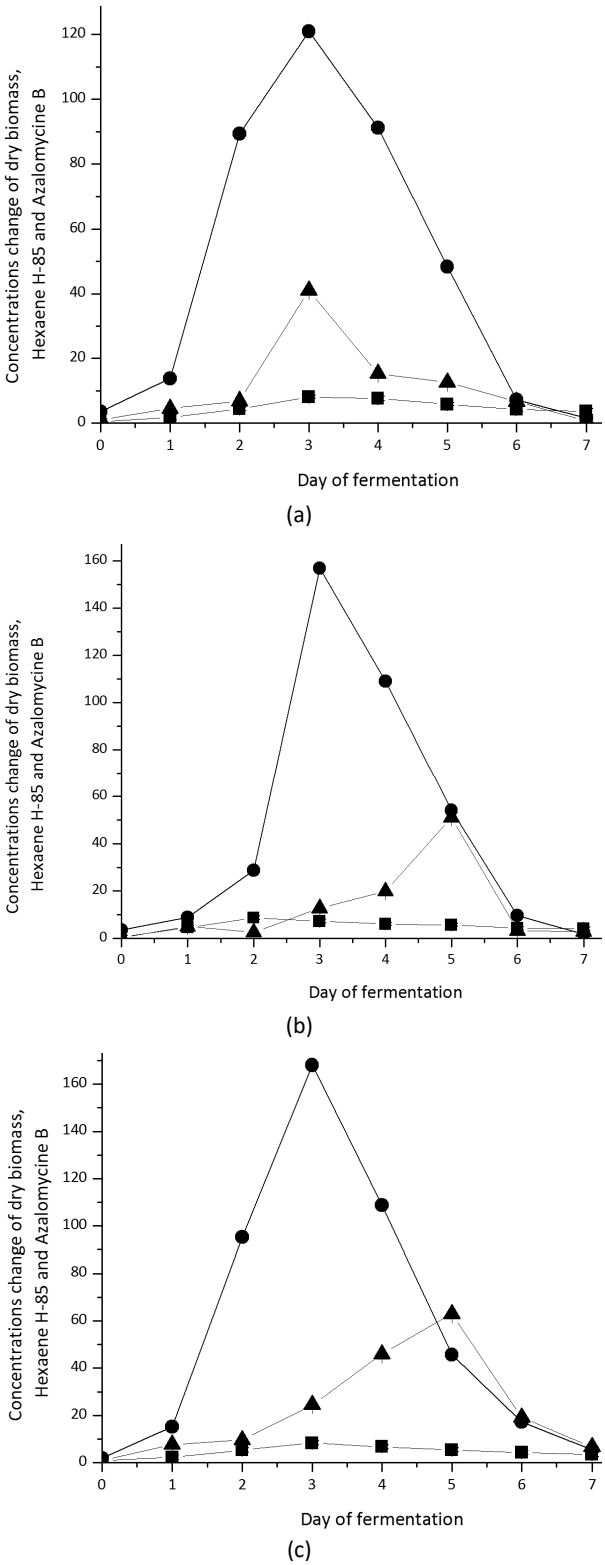

Figure 2. Concentrations of dry biomass (10^3 μg/cm^3) (■), hexaene H-85 (μg/cm^3) (●) and azalomycine B (μg/cm^3) (▲) during fermentation by S. hygroscopicus CH-7 in: a) basal medium, b) medium with SalH and c) medium with SalP (data are average of n = 3).

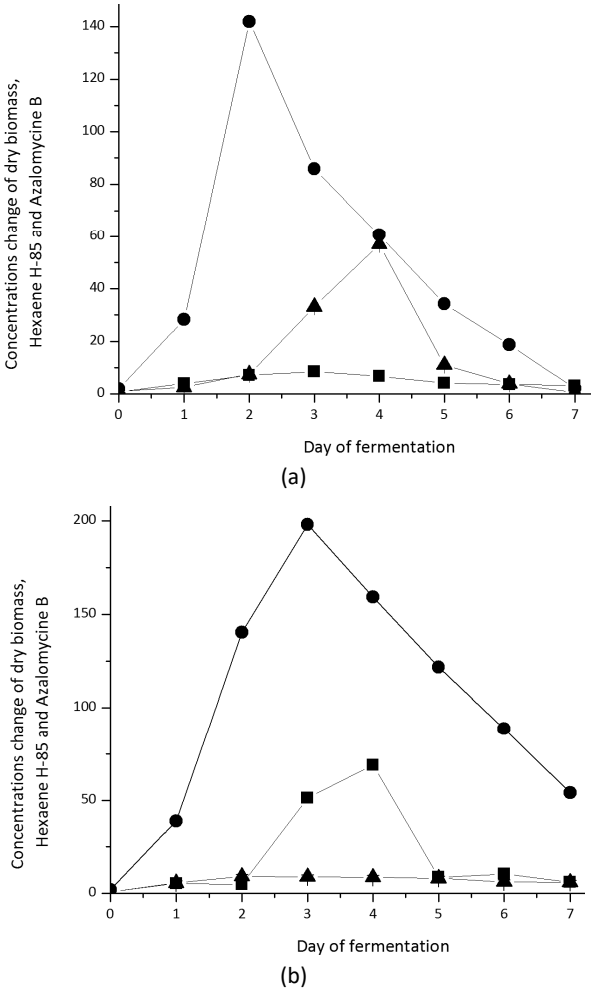

Figure 3. Concentrations of dry biomass (μg/cm^{3}x10^3) (■), hHexaene H-85 (μg/cm^3) (•) and azalomycine B (μg/cm^3) (▲) during fermentation by S. hygroscopicus CH-7 in: a) medium with SalS, and b) medium with SalT (data are average of n = 3).

In the basal medium, the strain grows like short and twisted filaments (Figure 4a) while in modified media it is in the form of short filaments (Figure 4b), branched and twisted filaments (Figure 4c) and large dispersive pellets with long twisted filaments. The highest yield of antibiotics was achieved in media with large dispersive pellets with long twisted filaments, with SalT and SalP as a nitrogen source. The results are in accordance with some previous work where isatin derivatives were used as a nitrogen source for antibiotic production. The highest production was also achieved in media where *S. hygroscopicus* CH-7 grows as pellets with branched filaments [11,22].

CONCLUSION

Synthesis of derivatives of salicylaldehyde were carried out using carbonyl-amine condensation reaction of salicylaldehyde and amines (hydrazine, phenylhydrazine, semicarbazide and thiosemicarbazide) in the stan-

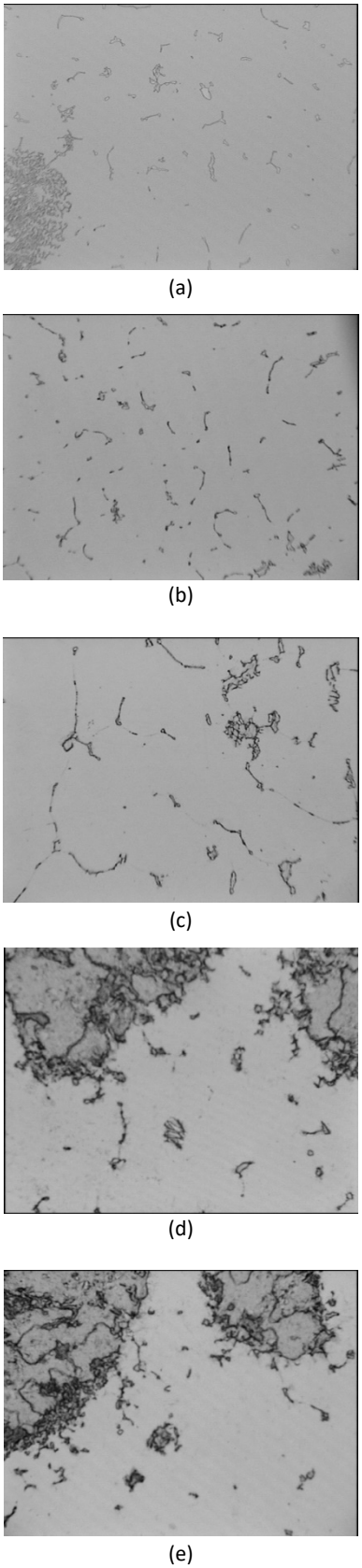

Figure 4. Photographs of S. hygroscopicus CH-7 in: a) basal medium and media with: b) SalS, c) SalH, d) SalP and e) SalT (magnification: 100×).

dard solvent 95% ethanol, and in crude glycerol, a green solvent obtained as a byproduct of the biodiesel production. The use of crude glycerol had significant advantages over the conventional solvent since the reaction time was reduced, the yield of the reaction was increased by 15–23% while the utilization of a catalyst was omitted. The synthesized compounds were shown to be good nitrogen sources in medium for production of antibiotics (hexaene H-85 and azalomycine B) by *S. hygroscopicus* CH-7. The highest concentrations of hexaene H-85 and azalomycine B were achieved in the medium with SalT (198 μg/cm^3 and 69 μg/cm^3, respectively). Derivatives of salicylaldehyde had also affected the strain morphology. The results have shown that *S. hygroscopicus* CH-7 grows like large dispersive pellets with long twisted filaments in media with SalT and SalP, in which also the highest yields of antibiotics were achieved.

The present study proves the utility of crude glycerol for application as a solvent in chemical syntheses of value-added products (Schiff bases) that can further improve the microbial synthesis of valuable antibiotics. In addition, the study has demonstrated that crude glycerol can be used as a carbon source in nutrition media for antibiotic production. In line with this, through the valorization of crude glycerol utilization, the present study might contribute to both economic and ecological issues like sustainability of biodiesel production and stabilization of glycerol market. In addition, by using crude glycerol, a problem of toxic solvents in chemical reactions, including syntheses of biological compounds, can be successfully resolved. High boiling point of glycerol makes it useful for higher reaction temperatures and also may lead to higher yields and reaction selectivity as well as shorter times.

Acknowledgement

This work has been funded by the Ministry of Education, Science and Technological Development of the Republic of Serbia (Project III 45001).

REFERENCES

[1]] Wolfson A, Snezhko A, Meyouhas T, Tavor D. Glycerol derivatives as green reaction mediums. Green Chem Lett Rev. 2012; 5: 7-12.

[2] Konstantinović SS, Danilović BR, Ilić SB, Savić DS, Vlada B. Veljković VB. Valorization of crude glycerol from biodiesel production. Chem Ind Chem Eng Q. 2016; 22: 461−489.

[3] Wolfson A, Litvak G, Dlugy C, Shotland Y, Tavor D. Employing crude glycerol from biodiesel production as an alternative green reaction medium. Ind Crops Prod.2009; 30: 78–81.

[4] Valerioa O, Horvath T, Ponda C, Misraa M, Mohantya A. Improved utilization of crude glycerol from biodiesel industries: Synthesis and characterization of sustainable biobased polyesters. Ind Crops Prod.2015; 78: 141–147.

[5] Nanda MR, Yuan Z, Qin W, Ghaziaskar HS, Poirier M, Xu C.Thermodynamic and kinetic studies of a catalytic process to convert glycerol into solketal as an oxygenated fuel additive. Fuel 2014; 117: 470-477.

[6] Wolfson A, Dlugy C. Palladium-catalyzed heck and suzuki coupling in glycerol. Chem Papers. 2001; 61: 228-232.

[7] Gu Y, Barrault J, Jerome F. Glycerol as an efficient promoting medium for organic reactions. Adv Synth Catal. 2008; 350: 2007-2012.

[8] Jovanović MB, Konstantinović SS, Ilić SB, Veljković VB. The synthesis of vanillin-semicarbazone in crude glycerol as a green solvent. Adv Technol. 2013; 2: 38-44.

[9] Arulmurugan S, Kavitha HP, Venkatraman BR. Biological activities of Schiff base and its complexes. Rasayan J Chem. 2010; 3: 385-340.

[10] Ilić SB, Konstantinović SS, Savić DS, Veljković VB, Gojgić-Cvijović G. The impact of Schiff bases on antibiotic production by Streptomyces hygroscopicus. Med Chem Res. 2010; 19: 690-697.

[11] Ilić SB, Konstantinović SS, Gojgić-Cvijović G, Savić DS, Veljković VB. The influence of Schiff base inclusion complexes with β-cyclodextrine on antibiotic production by Streptomyces hygroscopicus CH-7. Chem Ind. 2015; 69: 9-15.

[12] Ćirić JT, Konstantinović SS, Ilić SB, Gojgić-Cvijović G, Savić DS, Veljković VB. The impact of isatin derivatives on antibiotic production by Streptomyces hygroscopicus CH-7. Hem Ind. 2016; 70:123-128.

[13] Isahak WN, Ismail M, Yarmo MA, Jahim JM, Salimon J. Purification of crude glycerol from transesterification RBD palm oil over homogeneous and heterogeneous catalysts for the biolubricant preparation. J App Sci. 2010; 10: 2590-2595.

[14] Konstantinović SS, Radovanović BC, Cakić Z, Vasić V. Synthesis and characterization of Co(II), Ni(II), Cu(II) and Zn(II) complexes with 3-salycilidenehydrazono-2-indolinone. J Serb Chem Soc. 2003; 68: 641-647.

[15] Karadžić I, Gojgić-Cvijović G, Vučetić J. Hexaene H-85, A Hexaene H-85 macrolide complex. J Antibiot.1991; 12: 1452–1453.

[16] Vučetić I, Karadžić I, Gojgić-Cvijović G, Radovanović E. Improving HexaeneH-85 production by Streptomyces hygroscopicus. J Serb Chem Soc. 1994; 59: 973–980.

[17] Machado I, Fernández S, Beccoc L, Garatc B, Gancheffa JS, Reyb A, Gambino D. New fac-tricarbonyl rhenium(I) semicarbazone complexes: synthesis, characterization, and biological evaluation. J Coord Chem. 2014; 67: 1835-1850.

[18] Baruah D, Saikia UP, Pahari P, Dutta DP, Konwar D. Deprotection of oximes, imines, and azines to the corresponding carbonyls using Cu-nano particles on cellulose template as green reusable catalyst. RSC Adv. 2014; 4: 59338-59343.

[19] Cai P, Kong F, Fink P, Ruppen ME, Williamson RT, Keiko T. Polyene antibiotics from Streptomyces mediocidicus. J Nat Prod. 2007; 70:215–219.

[20] Kaiser H, Keller-Schierlein W, Metabolites of microorganisms. Part 202. Structure elucidation of elaiophylin: spectroscopic studies and degradation. Helv Chim Acta. 1981; 64:407-424.

[21] Zotchev SB. Polyene macrolide antibiotics and their applications in human therapy. Curr Med Chem. 2003; 10:211–23.

[22] Zhang L, Hashimoto T, Qin B, Hashimoto J, Kozone I, Kawahara T, Okada M, Awakawa T, Ito T, Asakawa Y, Ueki M, Takahashi S, Osada H, Wakimoto T, Ikeda H, Shin-ya K, Abe I. Characterization of Giant Modular PKSs Provides Insight into Genetic Mechanism for Structural Diversification of Aminopolyol Polyketides. Angew Chem Int Ed. 2017; 56:1740-45.

[23] Caffrey P, De Poire E, Sheehan J, Sweeney P. Polyene macrolide biosynthesis in streptomycetes and related bacteria: recent advances from genome sequencing and experimental studies. Appl Microbiol Biotechnol. 2016; 100: 3893-3908.

[24] Solovieva SE, Olsufyeva EN, Preobrazhenskaya MN. Chemical modification of antifungal polyene macrolide antibiotics. Russ Chem Rev. 2011; 80:103 – 126.

[25] Domingues FC, Queiroz JA, Cabral JMS, Fonseca LP. The influence of culture conditions on mycelial structure and cellulase production by Trichodermareesei Rut C-30.Enzyme Microb Technol. 2000; 26:394-401.

[26] Yang W, Hartwieg AE, Fang A, Demain A. Effects of carboxymethylcellulose and carboxypolymethylene on morphology of Aspergillus fumigatus NRRL 2346 and fumagillin production. Curr Microbiol.2003; 46: 24-27.

[27] Choi D, Park E, Okabe M. Dependence of apparent viscosity on mycelia morphology of Streptomyces fradiae culture in various nitrogen sources. Biotech Prog. 2000; 16:525-532.

[28] Saurav K, Kannabiran K. Diversity and optimization of process parameters for the growth of Streptomyces VITSVK9 spp. Isolated from Bay and Bengal, India. J Nat Env Sci. 2010; 1: 56-65.

[29] Ilić SB, The effect of composition and rheology of nutrition medium on kinetic of antibiotic production by bacteria Streptomyces hygroscopicus CH-7, Ph.D. Thesis, Faculty of Technology, University of Nis, Leskovac, 2010.

ИЗВОД

УТИЦАЈ ДЕРИВАТА САЛИЦИЛАЛДЕХИДА НА ПРОДУКЦИЈУ АНТИБИОТИКА ПОМОЋУ *Streptomyces hygroscopicus* CH-7

Сандра С. Константиновић[1], Милица З. Златковић[1], Славица Б. Илић[1], Гордана Д. Гојгић Цвијовић[2], Влада Б. Вељковић[1]

[1]*Универзитет у Нишу, Технолошки факултет, Лесковац*
[2]*Универзитет у Београду, Институт за хемију, технологију и металургију , Београд*

(Naučni rad)

Деривати салицилалдехида (салицилалдехид-хидразон, салицилалдехид-фенилхидразон, салицилалдехид-семикарбазон и салицилалдехид-тиосемикарбазон) су синтетисани како у 95% етанолу као конвенционалном растварачу, тако и у сировом глицеролу (добијеном при производњи биодизела) као зеленом растварачу. Једињења су окарактерисана елементарном микроанализом, FTIR, UV/Vis, и ^{1}H-NMR методама. Принос добијених једињења у сировом глицеролу (93–98%) је остварен у времену 10–25 min. Синтетисани деривати и сирови глицерол су коришћени као извори азота и угљеника, у хранљивој подлози при продукцији антибиотика (хексаена Х-85 и азаломицина Б) применом бактерије *Streptomyces hygroscopicus* CH-7. Највећа концентрација хексаена Х-85 и азаломицина Б је остварена у хранљивој подлози са салицилалдехид-тиосемикарбазоном (198 и 69 μg/cm^3). Деривати салицилалдехида утичу и на морфологију тестираног соја. *Streptomyces hygroscopicus* CH-7 расте у облику широких и дисперзних пелета, са дугим и увијеним филаментима у подлогама у којима је остварена највећа продукција антибиотика.

Ključne reči: Глицерол • Салицилалдехид • Streptomyces • Антибиотик • Морфологија