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Optimization of Antimicrobial Metabolites Production by *Streptomyces albidoflavus*

K.J.P. Narayana and M. Vijayalakshmi
Department of Microbiology, Acharya Nagarjuna University, Guntur-522 510, A.P, India

Abstract: Attempts were made to optimize the culture conditions for the production of antimicrobial metabolites by *Streptomyces albidoflavus*. Antimicrobial metabolites production was started after 72 h of incubation of culture broth and reached its maximum levels after 120 h and thereafter gradually declined. The culture medium adjusted to pH 7.0 supported the production of antimicrobial metabolites as compared to other pH levels and optimum temperature for antimicrobial metabolite production was found to be 35°C. Basal medium amended with maltose and soybean meal as carbon and nitrogen sources, respectively was proved to be the best for the production of bioactive metabolites. Among different minerals tested, only K_2HPO_4 showed positive influence on antibiotic production by the strain.

Key words: *Streptomyces albidoflavus*, antimicrobial metabolites, optimization

INTRODUCTION

Actinomycetes are gram-positive filamentous bacteria with high mol% G+C content. These are wide spread in nature and can be found with greater or less frequency in most ecological niche (Takahashi and Omura, 2003). Among actinomycete population from soils, *Streptomyces* species are reported to be the most abundant forms. They are the producers of most of the known bioactive metabolites. They include numerous potentially useful compounds providing the widest range and most promising array of pharmacologically and agriculturally active compounds. They are widely recognized as industrially important microorganisms because of their ability to produce many kinds of novel secondary metabolites including antibiotics (Bibb, 2005). The nutritional source like carbon, nitrogen and minerals, the environmental factors such as time, temperature and pH are found to have profound influence on antibiotic production by actinomycetes (Sanchez and Demain, 2002; Himabindu and Jetty, 2006). Optimization of the culture conditions is essential to get high yields of the metabolites. Hence, an attempt was made to optimize the nutrient levels as well as pH and temperature requirements of *Streptomyces albidoflavus* for the production of antimicrobial metabolites.

MATERIALS AND METHODS

A prevalent actinomycete strain was isolated from the laterite soil of Acharya Nagarjuna University campus and the culture was identified as *Streptomyces* sp. ANU 6277 that closely related to *Streptomyces albidoflavus* by 16S r RNA analysis and gene sequences are submitted to NCBI genbank with accession number EF 142856 (Narayana *et al*., 2007). Pure culture of the strain was maintained on Yeast extract-Malt extract-Dextrose (YMD) agar medium containing yeast extract, 0.4, malt extract, 1%, dextrose, 0.4% and agar, 1.5%, pH, 7.0.

Effect of incubation period: Shake-flask fermentations were run in 500 mL flasks containing 100 mL of YMD broth and were incubated at room temperature for optimum yields on a rotary shaker operating at 250 rpm. At every 24 h interval, the flasks were harvested and the biomass was separated from the culture filtrate. Biomass was determined in terms of total cell dry weight. Antimicrobial metabolites production determined in terms of their antimicrobial spectrum. The culture filtrates were extracted with ethyl acetate by using separating funnel. The solvent extracts were concentrated and tested for antimicrobial spectrum. The concentrated solvent extract (50 ppm) was tested for antimicrobial activity by employing agar diffusion method against the test organisms like *Bacillus subtilis*, *Pseudomonas aeruginosa* and *Fusarium udum* (Cappuccino and Sherman, 2004).

Impact of pH and temperature on the production of bioactive metabolites: The effect of pH and temperature on biomass and antimicrobial metabolites production by the strain was studied by inoculating 48 h old seed culture

Corresponding Author: M. Vijayalakshmi, Department of Microbiology, Acharya Nagarjuna University, Guntur-522 510, A.P, India

in YMD broth. Effect of different ranges of pH (5-9) and temperature (15-45°C) on the production of biomass and antimicrobial metabolites were examined after 120 h of incubation.

Effect of carbon and nitrogen sources on antimicrobial metabolites production: To determine the effect of carbon sources on biomass and antibiotic production, different carbon sources like arabinose, dextrose, fructose, galactose, glycerol, inosine, lactose, maltose, mannitol, mannose, sucrose and trehalose were added to the basal medium containing K_2HPO_4, 0.1%; $MgSO_4 7H_2O$, 0.01% and $CaCO_3$ 0.2%. Carbon compounds were added in 1% concentration to the basal medium supplemented with $NaNO_3$ (0.2%) as nitrogen source. Impact of various nitrogen sources such as ammonium sulphate, ammonium chloride, $NaNO_3$, KNO_3, L-asparagine, L-glutamine, tyrosine, casein, peptone, soybean meal and yeast extract was studied by adding nitrogen source (0.2%) to the basal medium containg an optimum amount of the superior carbon source (Majumdar and Majumdar, 1967). Final pH of the medium was adjusted to 7.0.

RESULTS AND DISCUSSION

The strain reached to maximum levels of cell growth after 96 h. Antimicrobial metabolite production by the strain was started after 72 h of incubation and reached to high levels after 120h of incubation and thereafter gradually declined its production (Table 1). Narayana *et al.* (2004) stated that the *Streptomyces* sp. isolated from virgin soil were elaborated maximum antimicrobial metabolites production after 120 h, which coincides with stationary phase of the culture.

The effect of pH and temperature on biomass and antimicrobial metabolite production by the strain is presented in Table 2 and 3. The optimum pH for biomass and antibiotic production was 7.0. The strain showed high levels of biomass and antibiotic production when culture medium incubated at 35°C. The strain was found to be strictly mesophilic for secondary metabolites production; extreme pH and temperature were unfavorable for antibiotic production. Bhattacharyya *et al.* (1998) showed that 30°C and 7.0 are the optimum temperature and pH for antibiotic production by *S. hygroscopicus* D1.5. Sujatha *et al.* (2005) reported that glucose and ammonium nitrate amended medium with pH 7.2 and incubated at temperature 30°C for 96 h are optimal for antibiotic production.

The impact of different carbon sources on biomass and antibiotic production by the strain is presented in Table 4. Among all the carbon sources, maltose amended basal medium proved to be the best for cell growth as well as antibiotic production by the strain fallowed by glycerol, trehalose, dextrose, rhamnose and galactose. Carbon sources like mannitol, inosine, mannose and arabinose were found to be moderately supported the biomass and antibiotic production by the strain.

Table 1: Effect of incubation period on biomass and antibiotic production by *Streptomyces albidoflavus*

Incubation period (h)	Biomass (mg mL^{-1})	Diameter of growth inhibition zone (mm) BS	PA	FU
0	3.1	0	0	0
24	9.4	0	0	0
48	15.2	0	4	4
72	24.7	4	5	7
96	41.5	13	15	18
120	39.3	20	22	24
144	38.6	18	20	22
168	35.9	12	16	18

BS- *Bacillus subtilis*; PA- *Pseudomonas aeruginosa*; FU- *Fusarium udum*

Table 2: Impact of pH on biomass and antimicrobial metabolites production by *Streptomyces albidoflavus*

pH	Biomass (mg mL^{-1})	Diameter of growth inhibition zone (mm) BS	PA	FU
5	13.8	0	3	4
6	41.8	10	14	16
7	42.5	20	23	24
8	29.2	5	10	11
9	6.1	0	0	0

BS- *Bacillus subtilis*; PA- *Pseudomonas aeruginosa*; FU- *Fusarium udum*

Table 3: Effect of temperature on biomass and antimicrobial metabolites production by *Streptomyces albidoflavus*

Temperature (°C)	Biomass (mg mL^{-1})	Diameter of growth inhibition zone (mm) BS	PA	FU
15	9.6	0	0	0
20	18.5	4	9	9
25	25.8	12	15	17
30	37.4	16	20	22
35	43.0	20	23	25
40	13.9	5	7	7
45	6.4	0	0	0

BS- *Bacillus subtilis*; PA- *Pseudomonas aeruginosa*; FU- *Fusarium udum*

Table 4: Role of different carbon sources on biomass and antibiotic production by *Streptomyces albidoflavus*

Carbon source (1%)	Biomass (mg mL^{-1})	Diameter of growth inhibition zone (mm) BS	PA	FU
Arabinose	13.2	3	4	7
Dextrose	31.4	10	14	16
Fructose	4.5	0	0	0
Galactose	19.0	4	9	11
Glycerol	29.7	15	16	18
Inosine	10.5	0	5	5
Lactose	3.6	0	0	0
Maltose	31.6	17	18	21
Mannitol	17.8	5	6	8
Mannose	20.8	8	7	11
Sucrose	3.2	0	0	0
Trehalose	30.5	15	15	18

BS- *Bacillus subtilis*; PA- *Pseudomonas aeruginosa*; FU- *Fusarium udum*

Table 5: Influence of different nitrogen sources on biomass and antibiotic production by *Streptomyces albidoflavus*

Nitrogen source (0.2%)	Biomass (mg mL^{-1})	Diameter of growth inhibition zone (mm)		
		BS	PA	FU
$(NH_4)_2SO_4$	14.2	6	10	12
NH_4Cl	7.8	3	5	7
NaNO3	31.6	14	18	19
KNO3	19.6	5	9	10
L-asparagine	34.5	15	20	22
L-glutamine	23.6	9	13	15
Tyrosine	17.5	3	7	9
Casein	22.9	8	9	11
Peptone	26.0	7	12	14
Soybean Meal	31.7	23	24	26
Yeast Extract	36.3	17	21	24

BS- *Bacillus subtilis*; PA- *Pseudomonas aeruginosa*; FU- *Fusarium udum*

Table 6: Role of minerals on biomass and antibiotic production by *Streptomyces albidoflavus*

Minerals	Biomass (mg mL^{-1})	Diameter of growth inhibition zone (mm)		
		BS	PA	FU
K_2HPO_4	42.5	22	26	27
KH_2PO_4	31.7	14	18	18
$MgSO_4$	40.1	21	24	26
$FeSO_4$	29.9	12	15	18
$CuSO_4$	20.9	7	11	14
$MnCl_4$	19.8	6	7	7
$ZnSO_4$	18.5	3	8	9
KCl	35.0	16	20	22
NaCl	39.6	17	22	25

BS- *Bacillus subtilis*; PA- *Pseudomonas aeruginosa*; FU- *Fusarium udum*

Antibiotic production was totally absent in the medium supplemented with fructose, lactose, raffinose and sucrose as sole carbon source. Carbohydrates such as glycerol, maltose, mannose, sucrose and xylose have been reported to interfere with the production of secondary metabolites (Demain and Fang, 1995). In the present study, the strain was found to produce high levels of biomass and antimicrobial metabolites in the medium supplemented with maltose as sole carbon source. With regard to carbon sources, species specific variation occurs within *Streptomyces* sp. for cell growth and production of secondary metabolites (Jonsbu *et al*., 2002).

Data on the effect of nitrogen sources on antimicrobial metabolites production by the strain is given in Table 5. Organic nitrogen sources were the best nitrogen sources for the antibiotic production by the strain than inorganic nitrogen source. Medium supplemented with soybean meal was found to be suitable for maximum antimicrobial metabolites production fallowed by yeast extract, tryptone, peptone and casein. High levels of biomass production was observed in medium contain yeast extract as sole nitrogen source. Himabindu and Jetty (2006) found that yeast extract is favorable for growth but not the antibiotic production.

Gesheva *et al*. (2005) reported that the growth and antibiotic production on synthetic media are unsatisfactory but a medium with a minimal amount of soymeal (0.5%) has been supported growth and antibiotic production in *Streptomyces hygroscopicus*.

Among different minerals tested, only K_2HPO_4 showed positive effect on antibiotic production fallowed by $MgSO_4.7\ H_2O$, NaCl, KH_2PO_4 and KCl. But $CuSO_4.5\ H_2O$, $FeSO_4.7\ H_2O$, $MnCl_2$ and $ZnSO_4.7H_2O$ exerted negative effect on cell growth and its secondary metabolites production (Table 6). Majumdar and Majumdar (1965) reported that K_2HPO_4 is required for maximum yield of neomycin by *S. fradiae*, whereas NaCl and the metals like Mn and Cu are without any effect, but $ZnSO_4.7H_2O$ caused decline of neomycin production. In the present study, optimum levels of culture conditions were determined for antibiotic production by *Streptomyces albidoflavus.*

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