Innovare Academic Sciences

International Journal of Pharmacy and Pharmaceutical Sciences

ISSN- 0975-1491 Vol 7, Supple 1, 2015

Original Article

OPTIMIZATION OF ANTIMICROBIAL METABOLITES PRODUCTION BY *STREPTOMYCES FRADIAE*

SUNANDA KUMARI KADIRI[1]*, NAGENDRA SASTRY YARLA

[1]Department of Microbiology, College of Science and Technology, Andhra University Visakhapatnam, India
Email: nandunagala@yahoo.co.in

Received: 24 Aug 2015 Revised and Accepted: 30 Dec 2015

ABSTRACT

Objectives: The aim of the present study to optimize the culture conditions for the production of secondary metabolites of a sponge-derived actinomycetes *Streptomyces fradiae*.

Methods: Effects of the incubation period, temperature and pH on biomass and antimicrobial metabolite production by *Streptomyces fradiae* was examined. Moreover, Effects of various carbon, nitrogen and minerals on biomass and antimicrobial metabolite production by *Streptomyces fradiae* were also assessed.

Results: Secondary metabolites production was started after 24 h of incubation of culture broth and reached its maximum levels after 96 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 secondary metabolite production was found to be 35 °C. Basal medium amended with arabinose 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 secondary metabolite production by the strain.

Conclusion: In the present study, optimum levels of culture conditions were determined for the production of antibiotic by *Streptomyces fradiae*.

Keywords: *Streptomyces fradiae*, Secondary metabolites, Cultural conditions, Optimization.

INTRODUCTION

Among the antibiotic-producing microbes, the class Actinobacteria represents a broad range of valuable and prominent sources of bioactive metabolites. Actinomycetes are responsible for the production of about most of the discovered secondary metabolites [1], antibiotics [2], anticancer agents [3], immunosuppressive agents [4], and enzymes [5]. Marine actinomycetes are one of the best sources of secondary metabolites and the vast majority of these compounds are derived from the single genus streptomyces. Streptomyces are distributed widely in terrestrial and marine habitats [6] and are of commercial interest due to their unique ability to produce novel metabolites. The nutritional source like carbon, nitrogen and minerals, the environmental factors such as time, temperature and pH are found to have a reflective influence on secondary metabolites production by actinomycetes [7, 8]. Optimization of the culture conditions is essentials 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 fradiae* for the production of antimicrobial metabolite(s).

MATERIALS AND METHODS

A prevalent actinomycete strain was isolated from an unknown sponge demospongiae and the culture was identified as *Streptomyces fradiae* that closely related to *Streptonyces fradiae* by 16 S rRNA analysis and gene sequences are submitted to NCBI genbank (Accession number under progress). Pure culture of the strain was maintained on yeast extract–malt extract dextrose (YMD) agar medium.

Effect of incubation period

Shake–flask fermentations were run in 500 ml flask 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 biomass was determined in terms of total cell dry weight. Antimicrobial metabolites production was determined in terms of their antimicrobial spectrum. The culture filtrates were extracted with ethyl acetate by using a 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.

Effect of pH and temperature on the bioactive production metabolites

The effects of pH and temperature on biomass and antimicrobial metabolites produced by the strain were studied by inoculating 48 h old seed culture in YMD broth. Effects of different ranges of pH (5-9) and temperature (15-45 °C) on the production of biomass and antimicrobial metabolites were also examined after 96 h of incubation and presented.

Table 1: Effect of incubation Period on biomass and antibiotic production by *Streptomyces fradiae*

Incubation period (h)	Biomass mg/ml	Diameter of growth		Inhibition zone (mm)	
		B. s	E. c	C. a	A. a
0	3.2			0	4
24	10.1	8	9	0	9
48	16.4	10	12	10	9
72	28.6	11	13	6	8
96	41.2	16	15	9	11
120	38.4	14	13	8	9
144	37.3	13	11	8	7
168	34.2	12	14	6	8

B. s–*Bacillus subtilis*, E. c–*E. coli*, C. a-*Candida albicans*, A. a-*Aspergillus awamori*

Table 2: Effect of pH on biomass and antimicrobial metabolite production by *Streptomyces fradiae*

pH	Biomass mg/ml	Diameter of growth		Inhibition zone (mm)	
		B. s	E. c	C. a	A. a
5	12.6	6	4	4	0
6	32.5	15	16	13	14
7	40.2	18	16	17	18
8	24.3	12	9	10	12
9	5.6	4	0	0	0

B. s–*Bacillus subtilis*, E. c–*E. coli,* C. a-*Candida albicans*, A. a-*Aspergillus awamori*

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

Temperature (°C)	Biomass mg/ml	Diameter of growth		Inhibition zone (mm)	
		B. s	E. c	C. a	A. a
15	8.2	0	0	0	0
20	15.7	6	4	4	3
25	23.2	11	16	15	16
30	34.6	15	12	13	13
35	41.1	18	17	19	20
40	14.3	11	11	13	15
45	6.2	0	0	0	0

B. s–*Bacillus subtilis*, E. c–*E. coli,* C. a-*Candida albicans*, A. a-*Aspergillus awamori*

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

Carbon source (%)	Biomass mg/ml	B. s	E. c	C. a	A. a
Fructose	9.2	7	6	6	4
Arabinose	29.6	14	16	15	12
Dextrose	28.5	12	13	14	14
Galactose	14.2	13	12	11	11
Lactose	3.6	3	2	2	5
Maltose	25.4	13	12	12	16
Sucrose	4.8	5	5	4	3
Mannitol	16.5	10	11	11	13

B. s–*Bacillus subtilis*, E. c–*E. coli,* C. a-*Candida albicans*, A. a-*Aspergillus awamori*

Table 5: Effect of different nitrogen sources on biomass and antibiotic production by streptomyces fradiae

Nitrogen Source (0.2%)	Biomass mg/ml	Diameter of growth		Inhibition zone (mm)	
		B. s	E. c	C. a	A. a
$NaNO_3$	20.5	15	16	12	11
KNO_3	16.8	14	13	12	12
$(NH_4)_2 SO_4$	9.6	6	6	7	5
NH_4Cl	2.8	5	4	3	3
Tyrosine	14.6	8	7	6	6
L–asparagines	27.2	16	17	18	17
L–glutamine	23.6	14	13	12	9
Casein	25.2	15	14	16	19
Peptone	20.1	16	12	12	11
Soybean meal	28.6	18	16	15	19
Yeast extract	20.4	14	14	16	15

Table 6: Effect of minerals on biomass and antibiotic production by *Streptomyces fradiae*

Minerals	Biomass mg/ml	Diameter of growth		Inhibition zone (mm)	
		B. s	E. c	C. a	A. a
K_2HPO_4	32.5	15	15	16	14
KH_2PO_4	16.3	13	12	12	13
$Mg SO_4$	26.8	14	16	12	11
$FeSO_4$	18.3	11	9	10	11
$CuSO_4$	14.5	12	10	11	12
$MnCl_4$	13.6	11	6	10	11
$ZnSO_4$	9.8	4	6	8	5

B. s–*Bacillus subtilis*, E. c–*E. coli,* C. a-*Candida albicans*, A. a-*Aspergillus awamori*

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, lactose, maltose, mannitol and sucrose were added to the basal medium containing K_2HPO_4, 0.1%, Mg SO_4. $7H_2O$, 0.2% and $CaCO_3$, 0.3%. Carbon compounds were added in 1% concentration to the basal medium supplemented with $NaNO_3$ (0.2%) as a nitrogen source. Effects of various nitrogen sources such as $NaNO_3$, KNO_3, ammonium sulphate, ammonium chloride, tyrosine, L-asparagines, L-glutamine, casein, peptone, soybean meal and yeast extract on antimicrobial metabolites production by the strain were studied by adding nitrogen source (0.2%) to the basal medium containing an optimum amount of the superior carbon source. Final pH of the medium was adjusted to 7.0.

RESULTS AND DISCUSSION

Antimicrobial compound and biomass production was monitored over a period of 14 d. Rate of antimicrobial metabolite production correlated with biomass. Antibiotic production was detected in culture broth after 24 h of incubation and reached maximum at stationary phase after 96 h of incubation (table 1). Mycelium growth gradually increased up to 96 h of incubation and entered into stationary phase. The condition of incubation influenced quantitatively the biosynthesis of antibiotics along with biomass as reported by A1 Zahrani [9].

The effects of pH and temperature on biomass and antimicrobial metabolite production by the strain are presented in table 2 and 3. The optimum pH for biomass and antibiotic production was found at 7.0. The strain showed high levels of biomass and antibiotic production when the culture medium was incubated at 35 °C. The strain was found to be strictly mesophilic for secondary metabolite production; extreme pH and temperature were unfavorable 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, Arabinose amended basal medium proved to be the best for cell growth as well as antibiotic production by the strain followed by dextrose and maltose. Carbon sources like mannitol, galactose and fructose were found to be moderately supporting biomass and antibiotic production by the strain. The antibiotic production was found to be less with carbon sources like fructose, sucrose and lactose. In the present study, the strain was found to produce high levels of biomass and antimicrobial metabolites in the medium supplemented with arabinose as sole carbon source.

The data on the effect of nitrogen sources on antimicrobial metabolites production by the strain is given in table 5. Organic sources were the best nitrogen sources for the antibiotic production by the strain than inorganic nitrogen source. Medium supplemented with casein meal was found to be suitable for maximum antimicrobial metabolites production followed by yeast extract and peptone. A high level of biomass production was observed in medium containing soybean meal as sole nitrogen source.

Among different minerals tested, K_2HPO_4 showed a high level of antibiotic production followed by $MgSO_4$, $FeSO_4$, KH_2PO_4, $CuSO_4$, $MnCl_4$ and $ZnSO_4$. In the present study, optimum levels of culture conditions were determined for antibiotic production by *Streptomyces fradiae*.

ACKNOWLEDGEMENT

Author (Sunanda Kumari Kadiri) wish to express immense gratitude to Department of Science and Technology [DST], Government of India for sponsoring a project under the Fast Track–Young Scientist Scheme with File no. SB/FT/lS-247/2012 Dated: 2.5.2013.

CONFLICT OF INTERESTS

Declared none

REFERENCES

1. Berdy J. Bioactive microbial metabolites. J Antibiot 2005;58:1-26.
2. Olano C, Mendez C, Salas JA. Antitumor compounds from marine actinomycetes. Mar Drug 2009;7:210-48.
3. Mann J. Natural products as immunosuppressive agents. Nat Prod Res 2001;18:417-30.
4. Peczynska-Czoch W, Mordarski M. Actinomycetes enzymes. In: M Good-fellow S.T; 1988. p. 219-84.
5. Williams M, Mordarski. Eds. Actinomycetes in biotechnology, Academic Press: London, UK; 1988. p. 219-84.
6. Pathom-aree WJEM, Stach AC Ward, K Horikoshi, AT Bull, M Goodfellow. Diversity of actinomycetes isolated from challenger deep sediment (10,898 m) from the Mariana Trench. Extrimophiles 2006;10:181-9.
7. Sanchez S, Demain AL. Metabolic regulations of fermentation processes. Enzyme Microb Technol 2002;31:895-906.
8. Himabindu M, A Jetty. Optimization of nutritional requirements for gentamicin production by *micromonospora echinospora*. Indian J Exp Biol 2006;44:842-8.
9. Al-Zahrani SHM. Studies on the antimicrobial activity of *streptomyces* sp. Isolated from Jazan. J King Abdulaziz Univ 2007;19:127-38.