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Research Article

OPTIMIZATION OF MEDIA FORMULATION FOR BACTERIAL ISOLATE MSB-6

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ABSTRACT

The bacterial isolate MSB-6 collected from Visakhapatnam coast of Bay of Bengal, Andhra Pradesh, India has shown considerable activity against Proteus *vulgaris* MTCC1771, Bacillus *subtilis* MTCC441 and Escherichia *coli* MTCC443. So the culture conditions for the production of bioactive compounds by MSB-6 are optimized by using these pathogens as standard. Galactose and Peptone are the best carbon and nitrogen sources respectively for the optimum growth of the bacterial isolate. The metabolite production is initiated after 24 h of incubation and reached its maximum after 48 h and decreased gradually thereafter. The fermentation yield is high at 35^0C and pH 7.0.

Keywords: Optimization, MSB-6, bioactive compound and pathogens.

INTRODUCTION

Bacteria from deep oceans are biologically active and taxonomically diverse[1]. These bacteria are the potent sources for antimicrobial peptides with various chemical structures[2,3]. Due to their ability of producing enormous novel antibiotics, they are important for pilot industrial production process. In antibiotic production process, the 2nd and 5th stages i.e. trophophase and idiophase are important[4]. It is evident that rather than idiophase, trophophase is very crucial in enhancing the quality and quantity of antibiotic yield[5]. As the trophophase is affected by the composition of culture media, we should optimize the cultural medium ingredients[6]. The medium composition should satisfy cell biomass and secondary metabolite production in all its elemental requirements. Medium composition includes nitrogen source, carbon source, inorganic salts, inhibitors, inducers etc[7]. The influence of nitrogen source on antibiotic production is very clearly evident in the production of antibiotics like erythromycin, leucomycin, candihexin, cephamycin etc. Carbon source will affect the antibiotic quality and quantity which was observed in penicillin production[8,9]. The quantity of carbon and nitrogen source will be decided based on C/N ratio. The presence of inducers, inhibitors and inorganic salts will show their significant impact on the quality of antibiotic. Even the competition for nutrients and space among marine micro organisms is a powerful differentiate factor to produce natural bioactive products which possess high medical and industrial values[10]. Other excellent technique for optimization of media formulation for high yield is experimental design[11].

In this study we have to find out an optimized medium composition for the selected microbial strain to get a desired product with high yield. In addition to the medium composition, fermentation parameters like temperature, pH and period of incubation are also should maintain at their optimum level to get a high yield with good quality. We have designed the optimization strategy to study the influence of physical and chemical conditions on the culture medium upon biosynthesis of bioactive molecules are reported.

MATERIALS AND METHODS

Upon isolation and screening of several bacteria from the marine sediment of Bay of Bengal at Visakhapatnam sea coast, the selected bacterial strain i.e. MSB-6 has to be submitted for

identification and 16S rRNA analysis. For obtaining pure and maximum growth, the optimization of production medium for MSB-6 is done. The bacterial strain is incubated at 37^{0}C in an orbital shaking incubator at 150 rpm and the samples are collected after every 24 hour interval. Cell free supernatant was tested against Proteus *vulgaris* MTCC1771, Bacillus *subtilis* MTCC441 and Escherichia *coli* MTCC443 using agar well diffusion technique aiming to obtain the highest productivity[12].

Effect of carbon and nitrogen sources on antimicrobial metabolites production:

Different carbon sources like arabinose, dextrose, fructose, galactose, glycerol, inosine, lactose, maltose, mannitol, mannose, sucrose and trehalose are added to the nutrient broth in 1% concentration at pH 7.0. The growth in the presence of sodium chloride is determined[13] and the growth of 12 carbon sources is determined on carbon utilization agar (ISP Medium 9; Difco) as described by Aassila[14]. Ammonium chloride, NaNO3, KNO3, L-aspargine, L-glutamine, tyrosine, casein, peptone, soybean meal yeast extract is studied by adding 0.2% to the nutrient broth at pH 7.0.

Effect of Incubation period

The fermentation is run in Shake-flasks containing nutrient broth and incubated at room temperature for optimum yields on rotator shaker operating at 150 rpm. At every 24 h interval, the flasks are harvested and centrifuged. The product is tested for the antimicrobial activity. The culture filtrate is extracted with ethyl acetate by using separating funnel and the extract is concentrated and tested for antimicrobial spectrum. The concentrated solvent extract (15µL) is tested for antimicrobial activity by employing agar diffusion method against the test organisms like *Proteus vulgaris* MTCC1771, *Bacillus subtilis* MTCC441 and *Escherichia coli* MTCC443.

Impact of pH and temperature on the production of bioactive metabolites

The effect of pH and temperature on the antimicrobial metabolite production by MSB-6 is studied by inoculating 24 hr old culture in nutrient broth. Affect of different ranges of pH (5-9) and temperature (150C-450C) on the production of antimicrobial metabolite is examined after 48 h of incubation.

Statistical analysis

The results analyzed in this chapter were the mean or SD (Standard Deviation) of three independent experiments.

RESULTS

Out of 12 carbohydrate molecules, galactose and sucrose are proved to be the best carbon source (Table-1) for the bacterial isolate MSB-6. In case of nitrogenous substances peptone and yeast extract are shown more positive results as nitrogen source (Table-2). The bacterial isolate MSB-6 utilizes the carbon and nitrogen source to reach maximum cell growth at 48th hour of incubation. The strain MSB-6 is started anti microbial metabolite production after 24 hours of incubation and reached to high levels after 48 hour of incubation and thereafter gradually declined its production (Table-3). As pH of the medium increases, the antibiotic production by MSB-6 is gradually increased up to the pH 7.0 (Table-4). Further increase in pH showed adverse effect on antibiotic production. The optimum temperature for antibiotic production by MSB-6 is 35^{0}C (Table-5). The utilization rate of components present in culture medium is unbalanced by the variation in temperature of fermentation medium[15].

Table 1: Role of different carbon sources on antibiotic production by *Bacteria (MSB-6):*

Carbon source (1%)	Diameter of growth inhibition zone (Mean± SD)(mm)		
	Proteus vulgaris (MTCC1771)	*Escherichia coli* (MTCC443)	*Bacillus subtilis* (MTCC441)
Arabinose	14±0.4^e	8±0.5^h	16±0.3^d
Dextrose	13±0.5^f	10±0.3^g	12±0.4^f
Fructose	15±0.3^d	19±0.2^b	16±0.2^d
Galactose	20±0.2^a	21±0.1^a	21±0.1^a
Glycerol	18±0.2^b	18±0.2^c	19±0.1^b
Inosine	6±0.4^g	5±0.4^i	3±0.4^g
Lactose	13±0.3^f	14±0.2^e	12±0.2^f
Maltose	17±0.2^c	19±0.2^b	18±0.3^c
Mannitol	11±0.^{4}	8±0.4^h	12±0.3^f
Mannose	15±0.4^d	12±0.3^f	16±0.4^d
Sucrose	20±0.2^a	19±0.1^b	19±0.2^b
Trehalose	18±0.2^b	17±0.1^d	15±0.3^e

Values expressed are a mean of the three replicates ± SD
Values indicated by different alphabets (^{a,b,c,d,e,f,g,h,i,j,k}) indicates significant difference at P ≤ 0.05 (DMRT)

Table 2: Influence of various nitrogen sources on antibiotic production by *Bacteria (MSB-6)*

Nitrogen source (1%)	Diameter of growth inhibition zone (Mean± SD)(mm)		
	***Proteus vulgaris* (MTCC1771)**	***Escherichia coli* (MTCC443)**	***Bacillus subtilis* (MTCC441)**
$(NH_4)_2SO_4$	6±0.4^{i}	8±0.3^{h}	10±0.2^{h}
NH_4Cl	12±0.3^{g}	11±0.3^{g}	11±0.1^{g}
$NaNO_3$	16±0.2^{e}	19±0.1^{c}	18±0.1^{c}
KNO_3	12±0.3^{g}	14±0.2^{e}	13±0.2^{e}
L-aspargine	18±0.2^{c}	16±0.2^{d}	16±0.2^{d}
L-glutamine	16±0.2^{e}	12±0.1^{f}	12±0.1^{f}
Tyrosine	17±0.1^{d}	14±0.1^{e}	8±0.2^{j}
Casein	11±0.3^{h}	12±0.2^{f}	9±0.2^{i}
Peptone	20±0.2^{a}	21±0.3^{a}	20±0.1^{a}
Soya bean Meal	14±0.3^{f}	16±0.3^{d}	13±0.3^{e}
Yeast Extract	19±0.2^{b}	20±0.2^{b}	19±0.1^{b}

Values expressed are a mean of the three replicates ± SD
Values indicated by different alphabets (^{a,b,c,d,e,f,g,h,I,j,k}) indicates significant difference at $P \leq 0.05$ (DMRT)

Table 3: Effect of incubation period on antibiotic production by *Bacteria (MSB-6)*

Incubation period (hours)	Diameter of growth inhibition zone (Mean± SD)(mm)		
	***Proteus vulgaris* (MTCC1771)**	***Escherichia coli* (MTCC443)**	***Bacillus subtilis* (MTCC441)**
0	0	0	0
24	11±0.6^{e}	14±0.2^{d}	8±0.5^{e}
48	24±0.2^{a}	22±0.4a	21±0.2^{a}
72	19±0.5^{b}	17±0.1^{b}	20±0.2^{b}
96	18±0.2^{c}	16±0.5^{c}	19±0.1^{c}
120	15±0.6^{d}	16±0.4^{c}	19±0.3^{c}
144	15±0.4^{d}	16±0.3^{c}	16±0.6^{d}

Values expressed are a mean of the three replicates ± SD
Values indicated by different alphabets (^{a,b,c,d,e,f,g,h,I,j,k}) indicates significant difference at $P \leq 0.05$ (DMRT)

Table 4: Impact of pH on antimicrobial metabolites production by *Bacteria (MSB-6)*

pH	Diameter of growth inhibition zone (Mean± SD)(mm)		
	***Proteus vulgaris* (MTCC1771)**	***Escherichia coli* (MTCC443)**	***Bacillus subtilis* (MTCC441)**
5	0	7±0	10±0
6	14±0.6^{d}	17±0	18±0
7	20±0.3a	21±0	20±0
8	19±0.2^{b}	18±0	16±0
9	17±0.2^{c}	18±0	16±0

Values expressed are a mean of the three replicates ± SD
Values indicated by different alphabets (^{a,b,c,d,e,f,g,h,I,j,k}) indicates significant difference at $P \leq 0.05$ (DMRT)

Table 5: Effect of temperature on antimicrobial metabolites production by *Bacteria (MSB-6)*

pH	Diameter of growth inhibition zone (Mean± SD)(mm)		
	Proteus vulgaris **(MTCC1771)**	***Escherichia coli*** **(MTCC443)**	***Bacillus subtilis*** **(MTCC441)**
15	0	0	0
20	10 ± 0.5^{e}	11 ± 0.6^{e}	10 ± 0.4^{e}
25	12 ± 0.2^{d}	11 ± 0.4^{e}	10 ± 0.6^{e}
30	18 ± 0.4^{b}	16 ± 0.3^{d}	17 ± 0.2^{d}
35	20 ± 0.2^{a}	21 ± 0.1^{a}	22 ± 0.4^{a}
40	18 ± 0.4^{b}	19 ± 0.5^{b}	20 ± 0.2^{b}
45	16 ± 0.3^{c}	18 ± 0.4^{c}	19 ± 0.3^{c}

Values expressed are a mean of the three replicates ± SD
Values indicated by different alphabets (^{a,b,c,d,e,f,g,h,I,j,k})
indicates significant difference at $P \leq 0.05$ (DMRT)

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