Int.J.Curr.Microbiol.App.Sci (2020) *9*(6): 2594-2608

International Journal of Current Microbiology and Applied Sciences
***ISSN: 2319-7706* Volume 9 Number 6 (2020)**
Journal homepage: http://www.ijcmas.com

Original Research Article https://doi.org/10.20546/ijcmas.2020.906.316

Identification and Optimization of Fermentation Medium for Production of Antibacterial Compounds from Endophytic *Streptomyces* sp. GBTPR-167

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Keywords

Endophytic actinobacteria; *Streptomyces sp.*; Optimization; Fermentation medium;

Article Info

Accepted:
20 May 2020
Available Online:
10 June 2020

A B S T R A C T

Nutritional and environmental conditions are important parameters that influence growth and secondary metabolite production by actinobacteria. Endophytic actinobacteria (n=283) isolated from different tissues (root, needle and stem) of *Pinus roxburghii* of Pauri (Garhwal) region, India, were screened for their antibacterial potential. A potent endophytic actinobacterial strain, GBTPR-167, isolated from root tissue of *P. roxburghii* was identified on the basis of morphological, chemotaxonomic and 16S rDNA analysis as *Streptomyces* sp. The strain was found to produce bioactive compound with high antibacterial activity in FM-2 medium. OFAT based optimization of factors effect the production of bioactive compound and maximum production was observed in medium containing glucose as carbon source and casein as nitrogen source at pH 7.0, 180 rpm and 30ºC on 7th day of incubation. Further, *Streptomyces* sp. displayed salt tolerance up to 6% NaCl concentration however; maximum antibacterial activity was obtained at 2% NaCl concentration. The study holds importance as enhanced production of antibacterial compound can be achieved with these improved parameters. Also, optimization strategies may be undertaken by using statistical tools like design expert.

Introduction

Nature has always been an interesting source of bioactive products, particularly those derived from microorganisms. Even though microorganisms can be found literary throughout the world, efforts are still required to study the microbial biodiversity, given that there are still 99.99% of microbial taxa that waits to be discovered (Locey and Lennon, 2016). Endophytic microorganisms are an exceptional resource of therapeutic products (Pandey *et al.*, 2015). As the largest phylum under the bacterial kingdom, Actinobacteria has gained tremendous amount of attention from the scientific community as they produce secondary metabolites of medicinal and agricultural significance (Jakeline *et al.*, 2009; Barka *et al.*, 2016).

Among Actinobacteria, *Streptomyces* sp. is dominant, wide spread and produces two-third of the commercially and clinically important antimicrobial compounds (Berdy,

2012). The ability of *Streptomyces* to synthesize bioactive compounds can be increased or decreased under different conditions of nutrition and cultivation media. This is because antibiotic biosynthesis is a specific property of microorganisms which depends immensely on the culture conditions (Singh *et al.*, 2017). Many research workers have reported that altering the nutrient sources (carbon, nitrogen, phosphorus, minerals, trace elements) and environmental factors (pH, temperature, time, agitation) can greatly affect the production of secondary metabolites (Abbanat *et al.*, 1999; Himabindu and Jetty, 2006; Lin *et al.*, 2010; Sanchez *et al.*, 2010). Therefore, designing a suitable fermenting medium holds importance for mass production of secondary metabolites. Furthermore, the type of actinobacterial strain (*Streptomyces* sp.) used in the fermentation process has a significant role in the production of valuable secondary metabolites (Wang *et al.*, 2010; Mustafa, 2011). Keeping in view, the present study was undertaken to screen and optimize different fermentation medium for the production of antibacterial compounds from endophytic *Streptomyces* sp. GBTPR-167.

Materials and Methods

Microorganism used

The isolate GBTPR-167 was recovered from the root tissue of *Pinus roxburghii* in the previous study (Sharma and Baunthiyal, 2018). The isolate was maintained on the ISP-2 agar slants as well as 20% glycerol at -20°C.

Thirteen bacterial test organisms were used in the study. In which, six Gram positive bacteria [*Staphylococcus aureus* (MTCC-96), *Micrococcus luteus* (MTCC-106), *Mycobacterium smegmatis* (MTCC-06), *Streptococcus pneumoniae* (MTCC-1935), *Bacillus subtilis* (MTCC-441), *B. cereus* (MTCC-430)] and 7 Gram negative bacteria [including *Escherichia coli* (MTCC-739), *Pseudomonas aeruginosa* (MTCC-424), *Salmonella entrica* (MTCC-733), *Neisseria cinerea* (MTCC-3583), *Proteus vulgaris* (MTCC-426), *Klebsiella pneumoniae* (MTCC-4030), *Serratia mancesens* (MTCC-97)] were procured from MTCC, Institute of Microbial Technology, Chandigarh and maintained on their respective media.

Identification and characterization of isolated microorganism

The isolate was identified on the basis of morphology and biochemical characteristics according to the method defined in the Bergey's Manual of Systematic Bacteriology (Shirling and Gottlieb, 1966) and International *Streptomyces* Project (Locci, 1989). Molecular characterization and phylogenetic study of isolate GBTPR-167 was conducted according to the method defined previously by Semwal *et al.*, (2018). Spore shape and surface characteristics were described by Scanning Electron Microscopy (SEM) at Wadia Institute of Himalayan Geology, Dehradun, Uttarakhand, India (Kumar *et al.*, 2011).

Screening of fermentation medium for production of bioactive compounds with antibacterial activity

In order to select appropriate fermenting medium, 20 fermenting broth including ISP-4 broth, ISP-5 broth, production medium (PM), Fermenting medium-1 (FM-1), FM-2, liquid medium (LM), ISP-3 broth, starch casein broth (SCB), Luria Bertani (LB) broth, Arginine glycerol broth (AGB), FM-3, FM-4, FM-5, FM-6, FM-7, Latin square medium (LSM), ISP-2 broth, SS broth, Modified glucose soyabean meal broth (GSMB) and Soyabean mannitol liquid medium (SMLM)

were studied and screened for production of antibacterial compounds from isolate GBTPR-167 (Table 1).

The antibacterial activity was performed according to the method of Kumar *et al.*, (2012), in which, seed culture was grown in nutrient agar at 28°C for 72 hours at 180 rpm. 10% of seed culture was inoculated in different fermenting medium at 180 rpm with an incubation temperature of 28ºC for ten days for the production of antibacterial. Antimicrobial activity of the broth was monitored after every 24 hrs. The antagonistic activity was performed by agar well diffusion method on Mueller Hinton agar seeded with test organisms. The culture filtrate of fermenting medium displaying maximum antibacterial activity was selected for media optimization process through one factor at a time (OFAT) approach. Based on the results, FM-2 was found to be the best medium among the different tested medium for production of antibacterial compounds. Hence, further optimization experiments on the isolate GBTPR-167 were done using FM-2 medium.

Effect of cultural conditions on antibacterial compound production

Using OFAT approach, production of antibacterial compound from prominent isolate (*Streptomyces* sp. GBTPR-168) was optimized. In this study, different cultural conditions, including pH, temperature, agitation, inoculum size, carbon sources and nitrogen sources, were optimized for maximum production of antibacterial compound in FM-2 medium and their impact on growth of organism. The effect of pH was studied in the pH range of 4- 10 and incubated in a rotary shaker at 180 rpm for 7 days at 28ºC. The effect of temperature was studied at different temperatures viz. 15°C, 25°C, 30°C, 37°C, 45°C and 50°C in a rotary shaker at 180 rpm for 7 days at pH 7.0. To study the effect of agitation on growth and production of antibacterial compound, FM-2 medium was placed for fermentation at various rates of rpm viz. 100,120, 150, 180, 200 and 220 rpm for 7 days at 28ºC with pH 7.0 The influence of inoculum size was studied using various concentration of inoculum viz. 5%, 10%, 15%, 20% and 25% (v/v) on the growth and production of bioactive compounds at 37°C with pH 7.0 for 7 days of incubation. The effect of different carbon sources on the growth and bioactive metabolite production from isolate GBTPR-167 was studied by replacing glucose in the FM -2 medium with other carbon sources like xylose, sucrose, mannitol, lactose, fructose and maltose at 180 rpm for 7 days at 28ºC with pH 7.0. Further, concentration of carbon source showing maximum antibacterial activity from the isolate was furthered optimized at different levels viz. 0.0625%, 0.125%, 0.25%, 0.5% and 1% for enhancing the production of bioactive compounds. FM-2 already contains yeast extract, beef extract and peptone so their combinations (peptone+ beef extract (P+B); peptone+ yeast extract (P+Y) and beef extract+ yeast extract (B+Y)) were used as a nitrogen source along with casein during media optimization process for bioactive compound production at 180 rpm for 7 days at 28ºC. The mother culture was inoculated into FM-2 medium containing various concentration of NaCl in the range of 1-10% and incubated in a rotary shaker at 180 rpm for 7 days at 28ºC.The culture filtrate was then screened for antibacterial activity using agar diffusion method as described earlier.

Statistical analysis

All the experiments were performed in triplicates. The arithmetic mean, standard deviation (SD), standard error and significance of each experiment were calculated by one-way analysis of variances

(ANOVA) with Microsoft® Excel 2010. The level of significance $P < 0.05$ was considered statistically significant.

Results and Discussion

Identification and characterization of strain GBTPR-167

Morphological and physiological charcterization

In our previous study, 283 endophytic actinobacterial strains were isolated from various parts of the *P.roxburghii* (Sharma and Baunthiyal, 2018). Among them, strain GBTPR-167 displayed promising antibacterial activity and was thus chosen for optimization studies to enhance the production of bioactive compounds that contribute to its antimicrobial properties. Morphological studies revealed that the strain GBTPR-167 produced branched cream-colored mycelium on ISP-agar with yellow colored reverse colony pigmentation. The aerial mycelium of the isolate was observed to have a peculiar rectiflexible chain arrangement with smooth surface of spore (Figure 1). Chemotaxonomic analysis of the strain GBTPR-167 displayed that its whole cell hydrolysate was rich in LL-DAP (LL-diamino-pimelic acid) with no featured sugar thus representing chemotype I group.

The cultural and phenotypic traits are displayed in Table 2 and Table 3 respectively. No melanin production was observed on ISP-6 and ISP-7 agar as well as no diffusible pigment was secreted on different media. The isolate displayed moderate to better growth on different ISP agar media and optimum growth was obtained between 27°C to 37°C. It displayed growth at variable pH ranges from 5 to 10, which means it can tolerate lower pH but displayed good growth in alkaline conditions. Interestingly, the strain was found to tolerate upto 7% (w/v) of NaCl concentration. The isolate was capable to utilize major carbon sources and nitrogen sources except for DL-α-amino butyric acid and L-methionine, on which no sporulation occurred. The isolate displayed resistance against a number of antibiotics viz. amoxicillin, ampicillin, cefepime, trimethoprim, aztreonam and also combination of ampicillin/sulbactum and amoxicillin/clavulanate. On the basis of morphological, chemotaxonomic and phenotypic traits, the strain GBTPR-167 was identified as *Streptomyces* sp.

Molecular identification

The analysis of amplified 16s rDNA gene sequence of the isolate GBTPR-167 using BLAST tool identified it as *Streptomyces* at genus level with 99% homology to the sequence of *S. pratensis* ch24, *S. griseus* NBRC 15744, *S. erumpens* NBRC 15403. The strain was given an accession number MF138869. A phylogenetic tree was constructed to display the comparative relationship with various strains of *Streptomyces* as shown in Figure 2.

Screening of fermentation media for maximum antimicrobial activity

During preliminary screening, *Streptomyces* sp GBTPR-167 was tested for its antimicrobial effect against six Gram positive bacteria and seven Gram negative bacterial pathogens (Sharma and Baunthiyal, 2018). It was observed that the strain possess broad spectrum antagonistic activity against all bacterial pathogens except *Serratia marcesens*. Many researchers have represented the bioactive potential of endophytic actinobacteria against various bacterial pathogens (Kaur, 2016; Salam *et al.*, 2017). The production of secondary metabolite by actinobacteria was influenced

by nutrients present in the production medium. As a consequence, *Streptomyces* sp. GBTPR-167 was screened in twenty different fermenting medium for growth as well as antibacterial activity and found that FM-2 was suitable for production of antibacterial compound (Figure 3). A significant zone of inhibition (mm) was observed in culture filtrate in FM-2 medium against *M. luteus* (32.3±0.5 mm) followed by *B. subtilis* (25.7±0.5 mm), *S. pneumoniae* (17.7±0.5 mm), *P. vulgaris* (16.7±0.5 mm), *S. aureus* (16.7±0.9 mm), *S. entrica* (16.3±0.5 mm), *N. cinerea* (15.3±0.5 mm), *E .coli* (14.7±0.5 mm), *P. aeruginosa* (12.3±0.5 mm), *M. smegmatis* (10.3±0.5 mm), *K. pneumoniae* (09.7±0.5 mm) and *B. cereus* (09.3±0.5 mm) respectively. The organism produced antibacterial compound from the first day with gradual increase in activity reaching highest on 7th day of fermentation in FM-2 medium (Figure 4). Some studies have shown maximum production of bioactive compounds observed on 4th (Oskay, 2009; Osman *et al.*, 2011), 7th (Wadetwar and Patil, 2013) and 10th (Ripa *et al.*, 2009) day of incubation by various species of *Streptomyces*. From the results, maximum sensitivity of *Streptomyces* sp. GBTPR-167 was observed against *M. luteus*. Therefore, *M. luteus* was selected to optimize cultural conditions for the production of antibacterial compounds from *Streptomyces* sp. GBTPR-167.

Effect of cultural conditions on antibacterial compound production

Culture condition optimization of an isolate has always been a key factor for increased production of bioactive compounds which is prominently influenced by different nutrients and physical parameters.

pH

As shown in Figure 5 (a), cell biomass of the isolate GBTPR-167 was highest at neutral pH and slightly declined towards alkaline pH of the medium while, under acidic conditions, the growth decreased. Maximum inhibitory activity was achieved at pH 7.0 that gradually decreased while moving either toward acidic or basic pH. In earlier studies, it was reported that optimum pH for production of secondary metabolite varies from pH range (6.0 to 8.0) (Song *et al.*, 2012). In a similar study by Hassan *et al.*, (2001), it was found that maximum secondary metabolite production was achieved at pH 7.0.

Temperature

Temperature plays a critical role in microbial secondary metabolite production. The isolate produced bioactive compounds at variable temperatures (Figure 5 (b)) but no production was observed at very low (15°C) or high temperatures (50°C). The optimum temperature at which highest activity occurred and maximum cell biomass production observed was at 30°C. On increasing the temperature, growth of the isolate as well as production of the bioactive compound decreased. In a similar study by Bundale *et al.*, (2015), it was shown that the incubation temperature for production of secondary metabolite for *Streptomyces* sp. usually ranges between 25°C to 37°C.

Agitation

Aeration required for the production of bioactive compounds is facilitated by agitation for proper supply of oxygen to the bacterial cells. Maximum antibacterial activity by *Streptomyces* sp. GBTPR-157 was observed at agitation of 180 to 200 rpm (Figure 5(c)). Similar studies by researchers also stated maximum antibacterial activity for *Streptomyces* at 150 rpm (Wadetwar and Patil, 2013) and 180 rpm (Mandava *et al.*, 2012).

Table.1 Composition of various fermenting medium screened for production of antibacterial compound

Sr. No.	Name of Medium	Composition	Reference
1.	**ISP-4 broth**	Starch-10.0; K_2HPO_4-1.0; $MgSO_4.7H_2O$-1.0; NaCl-1.0; $(NH_4)_2SO_4$-2.0; $CaCO_3$-2.0; $FeSO_4.7H_2O$-0.001; $MnCl_2.7H_2O$.0.001; $ZnSO_4.7H_2O$-0.001; pH-7.2 ± 0.2	**Rao *et al.*, 2016**
2.	**ISP-5 broth**	L-asparagine-1.0; K_2HPO_4-1.0; Trace salt (g/L)-1ml $FeSO_4.7H_2O$-0.01; $MnCl_2.4H_2O$-0.01; $ZnSO_4.7H_2O$-0.01; pH-7.0 ± 0.2	**For current study**
3.	**PM**	Tryptone-5.0; Dextrose-10.0; Yeast extract -2.0; K_2HPO_4-2.0; $MgSO_4.7H_2O$-0.5; NaCl-2.0; pH-7.0 ± 0.2	**Roy and Banerjee, 2015**
4.	**FM-1**	Glucose-5.0; Yeast extract-5.0; Peptone-5.0; Beef extract-5.0; Corn steep liquor-4.0; Starch-20.0; Soyabean meal-10.0; $CaCo_3$-4.0; $CoCl_2$-0.02; pH-7.2 ± 0.2	**Yan *et al.*, 2010**
5.	**FM-2**	Starch-24.0; Glucose-1.0; Yeast extract-5.0; Peptone-3.0; Beef extract -3.0; $CaCo_3$-3.0; pH-7.0 ± 0.2	**Inahashi *et al.*, 2011**
6.	**Liquid medium**	Soyabean meal-15.0; Starch -20.0; Yeast extract -5.0; Peptone-2.0; $CaCo_3$-4.0; pH-7.2 ± 0.2	**Huang *et al.*, 2012**
7.	**SCB**	Starch-10.0; Casein-0.3; KNO_3-2.0; NaCl-2.0; K_2HPO_4-2.0; $MgSO_4.7H_2O$-0.05; $CaCO_3$ -0.02; $FeSO_4.7H_2O$-0.01; pH -7.0 ± 0.2	**Kandpal *et al.*, 2012**
8.	**ISP-3**	Oat meal-20.0; Trace salt (g/L)-1ml $FeSO_4.7H_2O$-0.001; $MnCl_2.4H_2O$-0.001; $ZnSO_4.7H_2O$-0.001; pH-7.0 ± 0.2	**For current study**
9.	**LB broth**	Casein enzymatic hydrolysate-10.0; Yeast extract -5.0; NaCl-10.0; pH-7.0 ± 0.2	**Kandpal *et al.*, 2012**
10.	**AGB**	L-arginine-1.0; Glycerol-12.5ml; $K_2HPO_4$1.0; NaCl-1.0; $MgSO_4.7H_2O$ -0.5; $CuSO_4.7H_2O$- 0.001; $FeSO_4.7H2O$-0.01; $ZnSO_4.7H2O$-0.001;pH-7.0 ± 0.2	**Gandotra *et al.*, 2012**
11.	**FM-3**	Glucose-10.0; Starch-10.0; Glycerol -10.0 ml; Corn steep liquor-2.5 ml; Peptone-5.0; Yeast extract-2.0; NaCl-1.0; $CaCO_3$ -3.0; pH-7.0 ± 0.2	**Bisht *et al.*, 2012**
12.	**FM-4**	Glucose-4.0; Peptone -15.0; Yeast extract-3.0; Fe(III) citrate hydrate-0.3; pH-7.6 ± 0.2	**Sunaryanto and Mahsunah *et al.*,**

			2013
13.	**FM-5**	Malt extract-20.0; Corn steep liquor -20.0 ml; NaCl-5.0; K_2HPO_4-0.2; $CaCO_3$-0.2; pH-7.0 ± 0.2	**Ohkuma *et al.*, 1999**
14.	**FM-6**	Starch-25.0; Soyabean meal -15.0; Yeast extract -2.0; $CaCO_3$-4.0; pH-7.4 ± 0.2	**Takatsu *et al.*, 1985**
15.	**FM-7**	Glucose-10.0; Na_2HPO_4-0.69; K_2HPO_4-0.26; $MgSO_4.7H_2O$-0.2; Na(Fe)EDTA- 0.01; NaCl-0.3; Trace salt (g/L)-1 ml H_3BO_4 -1.5X10^{-3}; $MnSO_4$-8X10^{-4}; $ZnSO_4.7H_2O$-6X10^{-4}; $CuSO_4.7H2O$-1X10^{-4}; $NaMoO_4.2H_2O$- 2.5X10^{-5}; $CoSO_4.7H_2O$-1X10^{-6}; pH-6.8 ± 0.2	**Bieber *et al.*, 1997**
16.	**LSM**	Glucose-10.0; Starch-10.0; Glycerol -10.0 ml; Soyabean oil-1.6ml; Corn steep liquor-2.5 ml; Peptone-5.0; Yeast extract-5.0; NaCl-1.0; $CaCO_3$ -3.0; Histidine -1.0; pH-7.0 ± 0.2	**Bisht *et al.*, 2012**
17.	**ISP-2 Broth**	Yeast extract-4.0; Malt extract-10.0; Dextrose-4.0; pH-6.8 ± 0.2	**For current study**
18.	**SS broth**	Soluble starch -25.0; Glucose -10.0; Yeast extract -2.0; $CaCO_3$-3.0 Trace salt (g/L)-1ml $CuSO_4.5H_2O$-5.0; $FeSO_4.7H_2O$-5.0; $ZnSO_4.H_2O$-5.0; $MnSO_4.4H_2O$-5.0; pH-7.0 ± 0.2	**Umasankar *et al.*, 2010**
19.	**GSMB**	Glucose-10.0; Soyabean meal-10.0; NaCl-5.0; Tryptophan-2.5; pH-7.0± 0.2	**For current study**
20.	**SMLM**	Soyabean-12.0; Mannitol-20.0; pH-7.2 ± 0.2	**Li *et al.*, 2008**

Table.2 Cultural traits of *Streptomyces* sp. GBTPR-167

Media	Substrate mycelium	Aerial mycelium	Growth
ISP-2 (Yeast extract-malt extract) agar	Light yellow	Cream	+++
ISP-3 (Oat meal) agar	Yellow	Cream	++
ISP-4 (Inorganic salt starch) agar	Yellow	Cream	+++
ISP-5 (Glycerol asparagines) agar	Cream	White	+++
ISP-6 (Peptone yeast extract iron) agar	Light yellow	White	+++
ISP-7 (Tyrosine) agar	Yellow	White	+++

+++, Good; ++, Moderate; +, Poor

Table.3 Phenotypic traits of *Streptomyces* sp. GBTPR-167

Traits	GBTPR-167	Traits	GBTPR-167
Sporulation	Good	**Growth at temperature**	
Spore chain	Rectiflexible	4 ºC & 10 ºC	-
Gram reaction	+	20 ºC	+
Melanin pigment	-	27 ºC	+++
Diffusible pigment	-	37 ºC	+++
Starch hydrolysis	++	45 ºC & 50 ºC	-
Casein hydrolysis	+	**Growth at NaCl (w/v)**	
Gelatin hydrolysis	-	0-3 %	+++
Catalase	+	4-5%	++
Oxidase	+	6-7 %	+
H_2S production	-	8-12%	-
Citrate utilization	-	**Growth at pH**	
Nitrate reduction	+	4	-
MR test	-	5	+
VP test	+	6	++
Indole test	-	7-10	+++
Carbon utilization		**Growth in presence (w/v)**	
Xylose	++	Crystal violet (0.001)	+
L-Sucrose	+++	Phenol (0.1%)	+
D-Maltose	+++	Sodium azide (0.01%)	++
Mannitol	++	(0.02%)	-
Inositol	++	**Antibiotic sensitivity test**	
Lactose	+++	Ampicillin (25 mcg)	R
Fructose	+++	Trimethoprim (25 mcg)	R
Sodium acetate	++	Chloramphenicol (25 mcg)	S
Sodium citrate	+++	Neomycin (30 mcg)	S
Nitrogen Utilization		Streptomycin (25 mcg)	S
L-Cysteine	+++	Ofloxacin (2U)	S
L- Valine	+++	Cefepime (50 mcg)	R
L- Histidine	+++	Nitrofurantoin (100 mcg)	S
L-Glutamic acid	+++	Vancomycin (10 mcg)	S
L-Serine	++	Gentamycin (30 mcg)	S
L-Arginine	+++	Tobramycin (30 mcg)	S
L-Proline	+++	Amikacin (30 mcg)	S
DL-α-amino butyric acid	+	Amoxicillin / sulbactum (30/15 mcg)	R
L-Hydroxyproline	+++	Rifampicin (30 mcg)	S
L- Methionine	+	Amoxicillin / clavulanate (50/10 mcg)	R
L- Threonine	+++	Amoxicillin (30 mcg)	R
Pottasiun nitrate	+++	Aztreonam (50 mcg)	R

+++, Good; ++, Moderate; +, Poor; +w, weakly positive; ±, doubtful; -, Negative; R, Resistant; S, Sensitive

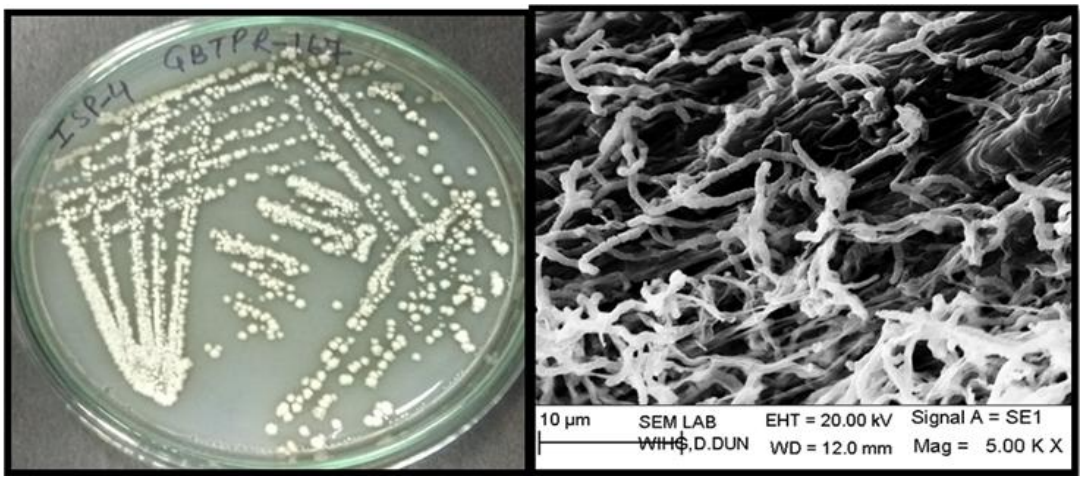

Figure.1 Culture morphology on ISP-4 medium and scanning electron micrograph of isolate *Streptomyces* sp. GBTPR-167 showing spore chain morphology

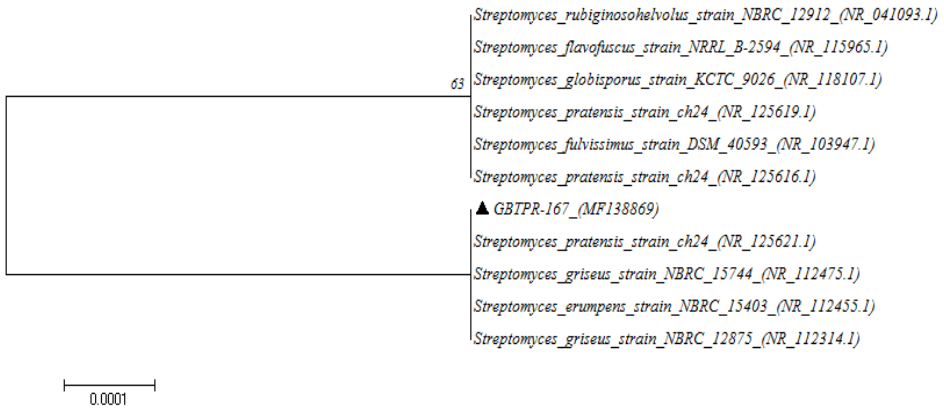

Figure.2 A phylogenetic tree was constructed using neighbor-joining method representing relationships between the representative strains and nearest strains of *Streptomyces*, based on 16S rDNA gene sequences. A number of nodes indicate bootstrap values based on 1000 replicates. Bar 0.0001 substitutions per nucleotide position

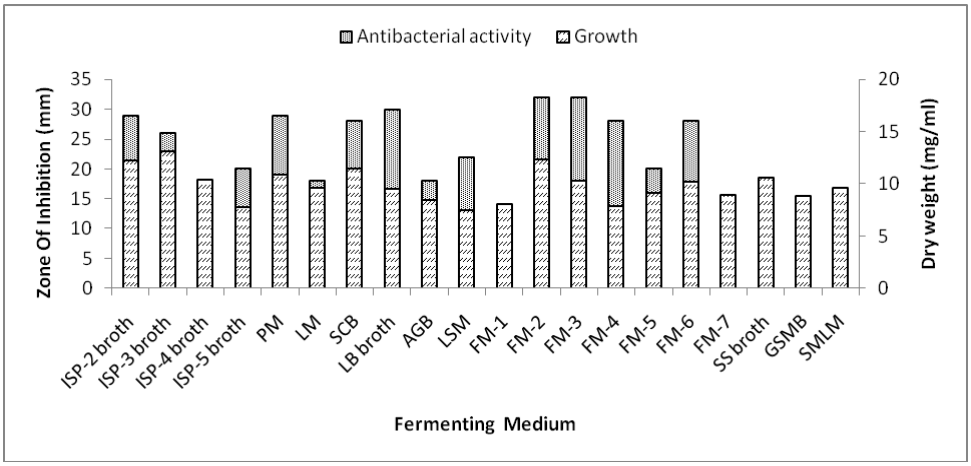

Figure.3 Screening of various fermenting medium for production of secondary metabolite against *M. luteus*

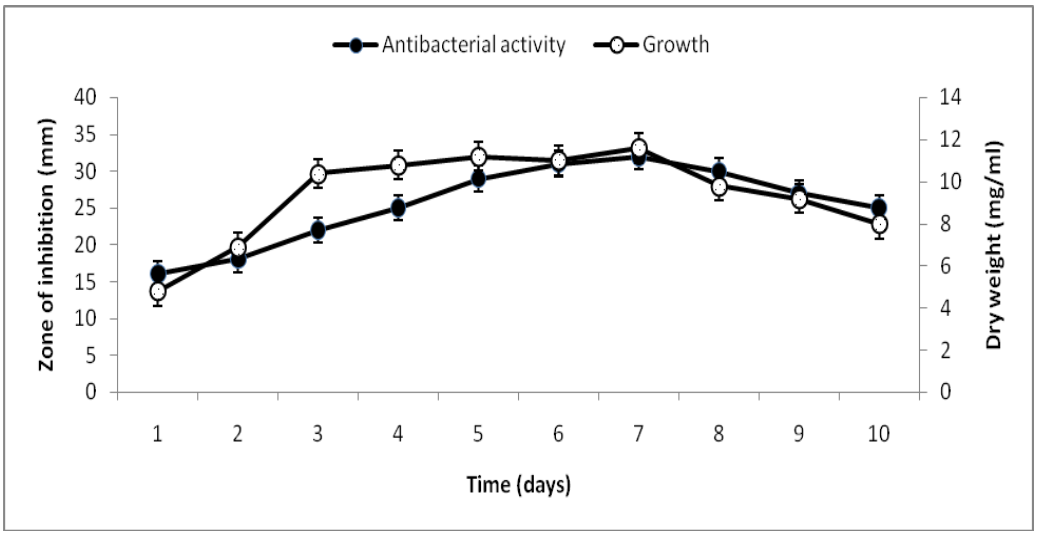

Figure.4 Effect of incubation period on production of bioactive compound

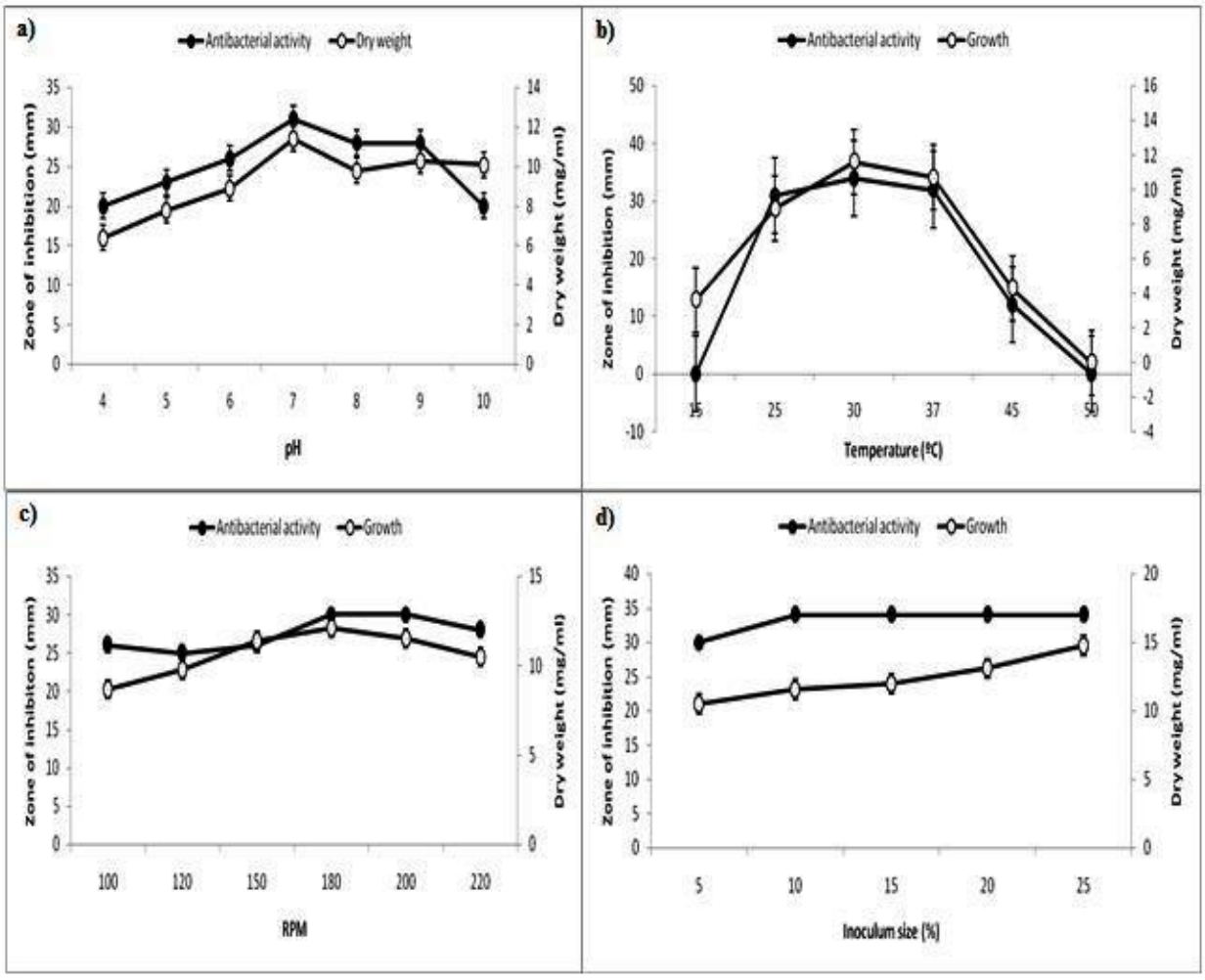

Figure.5 Effects of (a) pH (b) Temperature (c) Agitation and (d) Inoculum size on growth and antibacterial compound production by *Streptomyces* sp. GBTPR-168

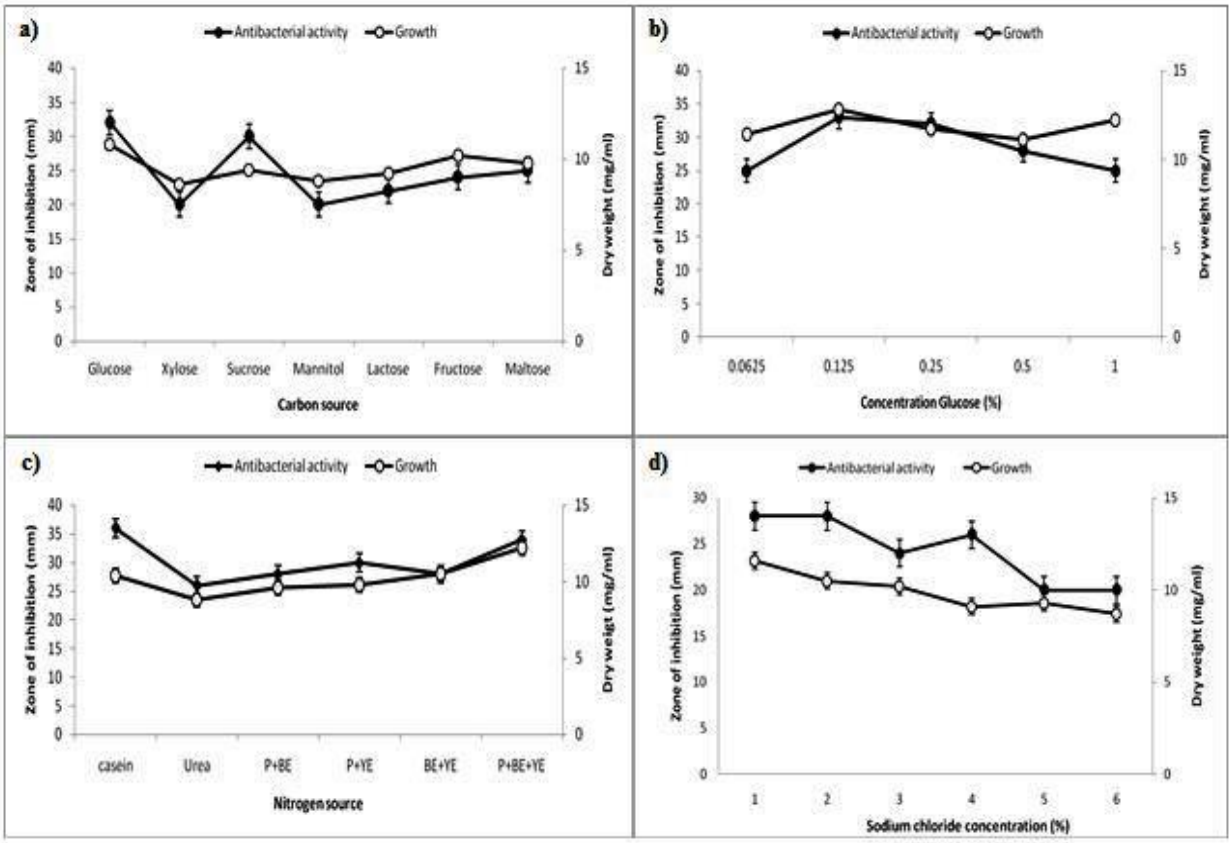

Figure.6 Effects of (a) Carbon source (b) Concentration of glucose (c) Nitrogen source and (d) sodium chloride concentration on growth and antibacterial compound production by *Streptomyces* sp. GBTPR-168

Inoculum size

It was observed that maximum bioactive compound production from *Streptomyces* sp. GBTPR-157 was observed at 10% (v/v) of inoculum size and the inhibitory activity remains same with rise in the inoculum size (Figure 5(d)).The results were consistent with Wadetwar and Patil (2013), wherein maximum inhibitory activity was achieved with *Streptomyces* at 10% of inoculum size.

Carbon source

Utilization of various carbon sources produced various levels of bioactive metabolites (Figure 6 (a)). In all carbon sources, the isolate displayed growth but maximum antibacterial activity was obtained when glucose was used as carbon source, followed by sucrose. Further, maximum antimicrobial potential of the strain was observed at 0.125 and 0.25 (%, w/v) concentration of glucose with an inhibition zone of 32.3±0.5 mm (Figure 6 (b)) though no significant change in the growth of isolate was observed.

In similar reports, *Streptomyces* sp. JGR-04 (Ganesan *et al.*, 2017), *S. sannanensis* RJT-1 (Vasavada *et al.*, 2006) and *S. psammoticus* BT-408 (Sujatha *et al.*, 2005) utilised glucose as best carbon source and as a good inducer for the production of bioactive compound as well as growth. In some cases, sucrose, a disaccharide, was also found to be good source of carbon for production of secondary metabolite (Mangamuri *et al.*, 2014).

Nitrogen source

Both inorganic and organic nitrogen sources have a key role in production of secondary metabolites. Sometimes, utilization of intricate nitrogen sources accelerates the production of secondary metabolites in *Streptomyces* (Mangamuri *et al.,* 2014). The effect of different nitrogen sources along with various combinations were screened the result of which are displayed in Figure 6 (c). The results showed that significant antibacterial activity was observed when the isolate was grown in medium containing casein, while no significant increment was observed in the growth as compared to the control (P+BE+YE). In similar studies, combination of tryptone, yeast extract and L-asparagine was found to be best sources of nitrogen for antibiotic production from *Streptomyces* sp. (Ripa *et al.,* 2009; Saha *et al.,* 2010; Ababutain *et al.,* 2013).

Sodium chloride

Figure 6 (d) reveals that *Streptomyces* sp. GBTPR-167 was able to produce maximum antimicrobial activity in fermenting medium containing 2% NaCl concentration, while tolerating upto 6% salt concentration. The result was in accordance to study by Arasu *et al.,* where *Streptomyces* strains ERI-1, ERI-3 and ERI-26, utilized 2% sodium chloride for production of antimicrobial compound with higher antagonistic activity (Arasu *et al.,* 2014). Similarly, PrashithKekuda and Onkarappa (2017), also found that *S. variabilis* strain PO-178 produced maximum inhibitory metabolite at 2% NaCl concentration in their study.

The promising endophytic strain, *Streptomyces* sp. GBTPR-167, produces broad spectrum antibacterial compound in FM-2 medium. The results indicated that the isolate produced antibacterial compound under specified cultural conditions. Overall, increase in antibacterial activity of *Streptomyces* sp. GBTPR-167 was observed after the optimization process. Future studies can be focused on the purification of compound and may lead to novel bioactive compound.

Acknowledgements

The authors acknowledge TEQIP-II, G B Pant Institute of Engineering and Technology, Ghurdauri for providing financial assistance and necessary facilities for the present study.

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How to cite this article:

Pushpendra Sharma, Shweta Ranghar and Mamta Baunthiyal. 2020. Identification and Optimization of Fermentation Medium for Production of Antibacterial Compounds from Endophytic *Streptomyces* sp. GBTPR-167. *Int.J.Curr.Microbiol.App.Sci.* 9(06): 2594-2608.
doi: https://doi.org/10.20546/ijcmas.2020.906.316