Optimization of the Cultural parameters for Improved Production of Antimicrobial Metabolites by *Streptomyces gulbargensis* DAS 131^T

.

ABSTRACT

Aims: To investigate the influence of appropriate culture medium by optimizing the cultural conditions affecting the growth and bioactive metabolite production by *Streptomyces gulbargensis* DAS 131^T under submerged culture conditions in order to reduce the cost of fermentation process to improve the formation of antimicrobial compounds.

Place and Duration of Study: Department of Botany and Microbiology, January 2012 to May 2012.

Methodology: The impact of environmental parameters such as incubation period, pH, temperature and salt concentration and effect of various nutrients such as carbon and nitrogen sources and minerals on the antimicrobial metabolite production by *Streptomyces gulbargensis* DAS 131^T was evaluated by employing agar well diffusion assay. Growth was measured in the form of dry mycelial weight.

Results: The optimum pH and temperature for bioactive metabolite production were 7 and 35℃ respectively. Highest antimicrobial metabolite production was found when the strain was inoculated into the medium amended with glucose at the concentration of 2%, soya peptone at the rate of 1% and NaCl at the concentration of 5% and incubated for six days under shaking conditions. The metabolites showed good antimicrobial activity against Gram positive and Gram negative bacteria, as well as unicellular and multicellular fungi.

Conclusion:*S.gulbargensis* DAS 131^T isolated from the semi-arid soils of Gulbarga, Northern Karnataka province, India exhibited broad spectrum antimicrobial activity.It was found that the antimicrobial metabolite production by the strainwas positively influenced by carbohydrates, nitrogen sources and minerals.

Keywords: Optimization, Bioactive metabolites, Nutritional factors, Antimicrobial activity, Streptomyces gulbargensisDAS 131^T.

1. INTRODUCTION

The microbes are the source for many important drugs including antibiotics, antitumor compounds, Immunosuppressants, antiviral and antiparasitic agents. Over 10,000 of bioactive compounds have been produced by Actinomycetes which contribute to 45% of all

the bioactive secondary metabolites discovered [1]. Microbes dwelling in extreme habitats have been focused as an important source for novel compounds in recent years. The majority of studies with microbes from extreme environments were confined to bacteria and the actinomycetes from these habitats have been relatively less explored [2]. As highlighted in many reviews [3], natural products are the origin for most of the antibiotics in the market today. These products are an important source for both the existing and new drugs. Among these, actinomycetes are a biotechnologically priceless group of prokaryotes. Actinobacteria form a distinct line in the 16S rDNA tree and produce metabolites that have medical contribution from antibiotics to enzyme inhibitors. They are ubiquitously distributed in terrestrial, fresh water and extreme environments such as marine ecosystems and alkali soils [4]. They are considered to be the important group of microbes due to their ability to produce novel chemical compounds that are complex and commercially important (5). The solution to combat multidrug resistance of pathogens is to search for novel antimicrobial compounds so as to find a solution to overcome the global resistance to pathogenic bacteria.

It is widely accepted that alkaliphilic actinomycetes are a valuable source for medicinal and industrial products [6]. Extensive exploration of actinomycetes having unique therapeutic properties continues to be an important area of research. *Streptomyces* species belonging to actinomycetes have been known as prolific producers of useful bioactive metabolites. These species are also recognized as industrially important organisms for their ability to synthesize different kinds of novel secondary metabolites, accounting for 70- 80% of all natural compounds produced by actinomycetes. *Streptomyces* are well documented as source for novel drug metabolites [7]. Some of the important compounds obtained from the alkaliphilic*Streptomyces* species include Pyrocoll [8], Chinikomycin and Lajollamycin, Mediomycins A and B, Clethramycin [9], Bleomycin [10] and Caboxamycin [11] with anti-tumor, anti-parasitic and anti-microbial properties. Several studies were aimed at isolation of *Streptomyces* and screening them for new antibiotics. Novel actinomycetes documented and the products derived from poorly explored habitats stress the need to probe into new habitats [2].

Media supplemented with carbon, nitrogen sources [12], sodium chloride [13] and mineral salts [14] and physico-chemical parameters like temperature, pH and incubation period also play a major role on growth and production of anti-microbial metabolites. The type, addition, removal and concentration of carbon, nitrogen, and phosphate together with trace elements are reported to influence the antibiotic biosynthesis by *Streptomyces* [15]. In order to achieve the highest level of metabolite production, the optimization of process parameters is very critical [16, 17]. Hence an effort was made to understand the impact of different carbon and nitrogen sources, temperature, pH and incubation period on growth and bioactive metabolite production by *Streptomyces gulbargensis* DAS131^{T}

2. MATERIALS AND METHODS

2.1. Isolation

During the course of screening for industrially important microorganisms, an alkali-tolerant and thermo-tolerant actinomycete isolate identified as *Streptomyces gulbargensis* DAS 131^{T} was isolated from semi-arid soils of Gulbarga, Northern Karnataka province, India, by standard serial dilution technique using starch casein agar medium [18] and further maintained on Yeast extract malt extract dextrose (ISP-2) agar medium at 4°C [19]. The 16S rRNA gene sequence of thestrain has been deposited in the NCBI genbank with the accession number DQ317411 [20].

73 **2.2. Selection of culture conditions for the optimum production of bioactive**
74 **metabolites**

75 Antimicrobial metabolite production by the strain was optimized by using different
76 parameters such as incubation period, pH, temperature, NaCl, carbon, nitrogen sources and
77 minerals.
78

79 **2.3. Effect of Incubation period**

80 The growth pattern and bioactive metabolite production by the strain was studied at regular
81 intervals up to 10 days. One week old culture of *S. gulbargensis* DAS 131^{T}was cultivated in
82 ISP-2 broth (seed medium) comprising of yeast extract (0.4%), malt extract (1%), dextrose
83 (0.4%), $CaCO_3$-(0.2%) with pH7.2 at 37˚C for 48 h. Seed culture at a rate of 10% was
84 inoculated into the starch casein broth (production medium) consisting of soluble starch
85 (1%), sodium caseinate (0.2%), K_2HPO_4 (0.02%), $MgSO_4.7H_2O$ (0.02%) $FeSO_4$. $7H_2O$
86 (0.001%) with pH7.2.The fermentation process was carried out for 10 days under agitation at
87 150 rpm. At every 24 h interval, the flasks were harvested and the biomass was separated
88 from the culture filtrate. Biomass was determined in terms of dry weight and antimicrobial
89 metabolite production was determined in terms of their antimicrobial spectrum [21]. The
90 crude bioactive compound produced in the fermentation medium by the isolate was
91 extracted twice with equal volume of ethyl acetate (1:1) in a separating funnel at periodic
92 intervals. The solvent layer was collected and evaporated in a rotary evaporator under
93 vacuum. The crude residue thus obtained was dissolved in DMSO (dimethylsulfoxide) at a
94 concentration of 1000µg/ml and employed for antimicrobial activity against test
95 microorganisms like *Streptococcus mutans* (MTCC 497), *Staphylococcus aureus* (MTCC
96 3160), *Salmonella typhi* (ATCC 14028), *Pseudomonas aeruginosa* (ATCC 9027) and
97 *Candida albicans* (ATCC 10231) by agar well diffusion method [22].
98
99

100 **2.4. Effect of pH and temperature**

101 To determine the influence of initial pH on growth and bioactive metabolite production, the
102 strain was cultivated in the medium with different initial pH values ranging from 5 to 10 for six
103 days. The strain was inoculated into production medium and grown at temperatures ranging
104 from 20 to 50℃ at pH7 for six days to study the impact of temperature. The biomass and
105 bioactive metabolite production were estimated and optimal pH and temperature achieved in
106 this step was used for subsequent study.
107

108 **2.5. Effect of NaCl concentration**

109 The impact of salinity on growth and bioactive metabolite production by *S. gulbargensis* DAS
110 131^{T}was recorded by cultivating the strain in the fermentation medium amended with
111 different concentrations of NaCl (1-10%) at optimum pH and temperature for six days. The
112 salt concentration in which the strain exhibits optimum levels of bioactive metabolites was
113 fixed for further studies.
114

115 **2.6. Effect of carbon and nitrogen sources**

116 To determine the effect of carbon sources on biomass and bioactive metabolite production,
117 different carbon sources like galactose, lactose, fructose, sucrose, glucose, starch, mannitol,
118 arabinose, raffinose and rhamnose each at a concentration of 1% were added separately
119 into the production medium, maintaining all other conditions at optimum levels. The effect of
120 varying concentrations of the best carbon source (0.5 - 5%) on bioactive metabolite
121 production was examined. Similarly, the influence of various nitrogen sources on

122 antimicrobial metabolite production was evaluated by amending different nitrogen sources
123 like soya peptone, arginine, asparagine, meat extract, yeast extract, tryptone, soya flour,
124 casein, beef extract and glycine each at a concentration of 0.5% were individually
125 supplemented into the production medium containing an optimum amount of the superior
126 carbon source.The growth and production of bioactive metabolite was determined after six
127 days of incubation at optimum pH, temperature and salt concentration.Further, the impact of
128 varying concentrations of optimized nitrogen source (0.1-2%) was studied to standardize the
129 maximum antimicrobial metabolite production.
130

131 2.7. Impact of K_2HPO_4

132 To study the impact of K_2HPO_4 on growth and bioactive metabolite production, the strain
133 was grown in the fermentation medium amended with different concentrations of K_2HPO_4
134 (0.01 to 0.1%), maintaining all other conditions at optimum levels.
135

136 2.8. Statistical analysis

137 Results on cell growth and the production of bioactive metabolites by *S. gulbargensis* DAS
138 131^{T}exposed to different cultural conditions are statistically analyzed with two way analysis
139 of variance (ANOVA).
140

141 2.9. Bioassays

142 The metabolites produced by the strain under optimized conditions were tested against
143 bacteria and fungi by agar-well diffusion assay (22). The test microorganisms used to
144 evaluate the production of bioactive metabolites were *Staphylococcus aureus*(MTCC 3160),
145 *Streptococcus mutans* (MTCC 497), *Bacillus subtilis* (ATCC 6633), *Lactobacillus*
146 *casei*(MTCC 1423), *Lactobacillus acidophilus* (MTCC 495), *Xanthomonas campestris*
147 (MTCC 2286), *Bacillus megaterium* (NCIM 2187), *Escherichia coli* (ATCC 35218),
148 *Enterococcus faecalis* (MTCC 439), *Pseudomonas aeruginosa*(ATCC 9027), *Salmonella*
149 *typhi*(ATCC 14028), *Proteus vulgaris* (MTCC 7299), *Candida albicans* (ATCC 10231),
150 *Aspergillus niger*(ATCC 1015), *Aspergillus flavus*(ATCC 9643), *Fusariumoxysporum*(MTCC
151 3075) *and Penicilliumcitrinum*(MTCC 6489).
152

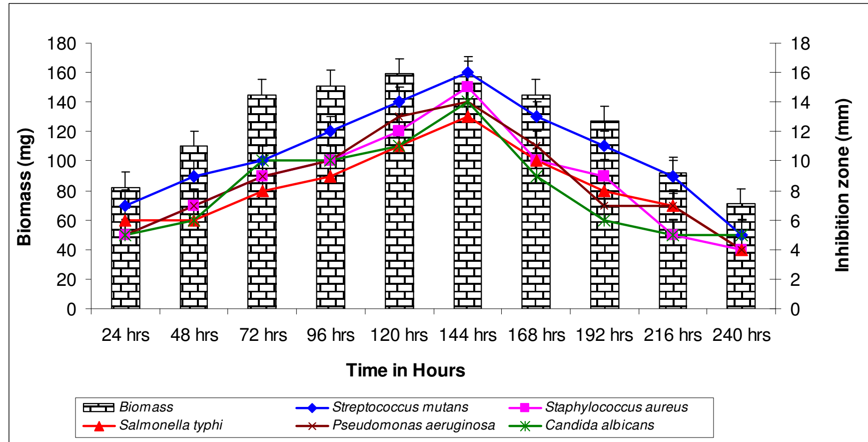
153 RESULTS AND DISCUSSION

154

155 3.1. Effect of incubation period

156

157 The growth pattern of *S. gulbargensis* DAS 131^{T}was studied on starch casein broth.
158 Exponential phase of the strain extended from lag phase after 24 h to 72 h. After that it
159 exhibited stationary phase from 96 h to 144 h of incubation, then declined (Fig.1).The results
160 revealed that the antimicrobial metabolite was early produced and reached maximum at the
161 stationary phase. The cessation of growth in the stationary phase is most commonly caused
162 by the exhaustion of the essential nutrients of the medium as well as accumulation of
163 undesirable metabolites.The secondary metabolites obtained from six day old culture
164 exhibited high antimicrobial activity against the test microorganisms. Thakur *et al*. [7] stated
165 that the maximum incubation period required for optimum growth and antibiotic yield by the
166 isolate *Streptomyces* sp. 201 was six days which was in complete accordance with the
167 earlier report [23]. The incubation period for the production of bioactive metabolites seems to
168 vary among *Streptomyces* strains. Metabolites elaborated from 5 day old culture of
169 *Streptomyces* sp. KGG32 [24] and *S.ramulosus*-AZ-SH-29[25] showed good antimicrobial
170 activity. Metabolites collected from 10-day old culture of *S.crystallinus* AZ-A151producing
171 Hygromycin-B exhibited good anti microbial activity[26].
172

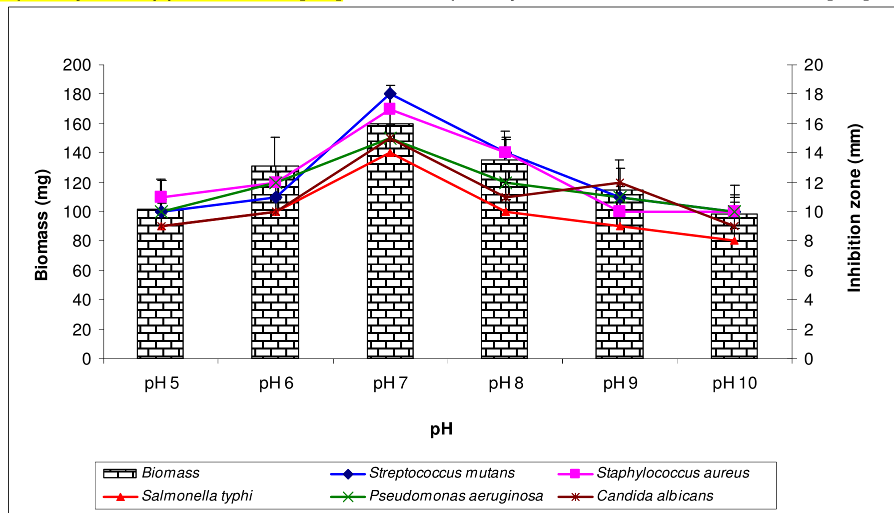


173
174
175 Fig.1. Growth pattern and anti-microbial activity of *S. gulbargensis* DAS 131^T.

176 **Data on cell growth and bioactive metabolite yield were statistically analyzed by Two-way ANOVA and*
177 *found to be significant at 1%.*
178

179 **3.2. Effect of initial pH and incubation temperature**

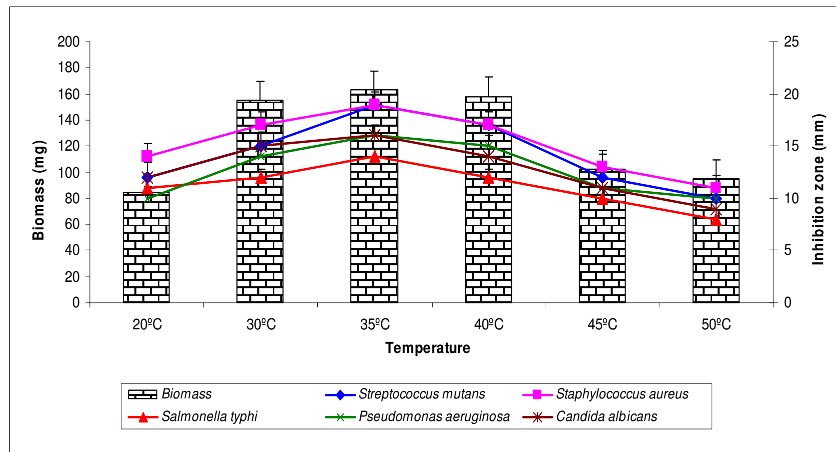
180 The environmental requirements and cultural conditions for growth and bioactive metabolite
181 production by *S. gulbargensis* DAS 131^{T}were studied. The antimicrobial metabolite
182 production was found to be influenced by pH of the medium. The maximum biomass and
183 bioactive metabolite production by the strain was obtained at pH 7 suggesting its inclusion in
184 the neutrophilicactinomycetes group (Fig. 2). Medium maintained at pH 7.0 was reported to
185 support enhanced anti-microbial metabolite production by *Streptomyces rochei* G 164[27],
186 *Streptomyces marinensis*[28], *Streptomycesalbidoflavus*[21], *Streptomycestorulosus* KH-4
187 [29], *Streptomyces* spp.VITSVK9[30] and *Streptomycescheonanensis* VUK-A [31].



188
189
190
191 Fig. 2. Effect of pH on growth and bioactive metabolite yield of *S. gulbargensis* DAS 131^T

192 **Data on cell growth and bioactive metabolite yield were statistically analyzed by Two-way ANOVA and*
193 *found to be significant at 1%.*
194

195 The effect of temperature on growth and bioactive metabolite production of the strain was
196 recorded (Fig.3). There was an increase in the growth as well as bioactive metabolite
197 production with the increase of incubation temperature from 20˚C to 35˚C. However further
198 increase in temperature (above 35˚C) resulted in the decline of growth and bioactive
199 metabolite production.In terms of its optimum temperature for growth, the organism
200 appeared to be mesophilic in nature. Atta *et al.* [26] reported that *Streptomycescrystallinus*,
201 AZ-A151 produced high levels of Hygromycin-B production at 35ºC. Ushakiranmayi *et al.*
202 [33] stated that the optimum temperature capable of promoting antimicrobial metabolite
203 produced by *Pseudonocardia* sp.VUK-10 isolated from Nizampatnam mangrove ecosystem
204 was 35ºC.
205
206

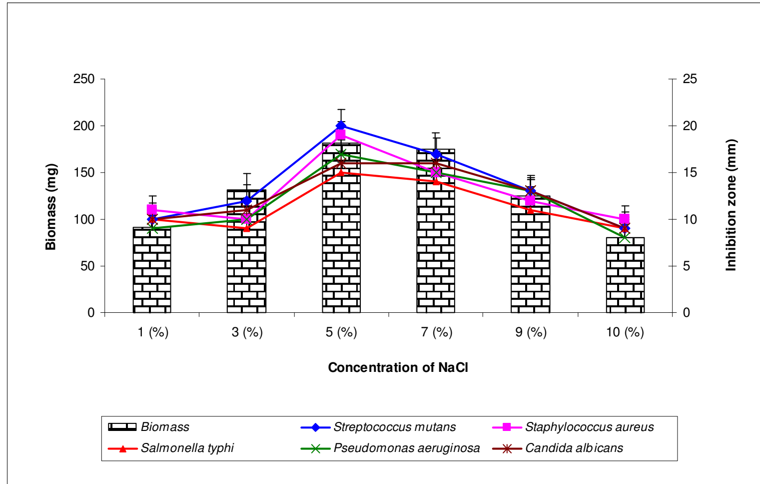


207
208
209
210 Fig.3. Effect of temperature on growth and bioactive metabolite yield of *S.gulbargensis* DAS
211 131^{T}.

212 **Data on cell growth and bioactive metabolite yield were statistically analyzed by Two-way ANOVA and*
213 *found to be significant at 1%.*
214

215 **3.3. Effect of NaCl**

216 Optimum salt requirement for bioactive metabolite production was examined in the
217 production medium supplemented with different salt concentrations ranging from 1-10%.
218 NaClat the concentration of 5% was found to be optimum for maximum growth as well as
219 antimicrobial compound production by*S.gulbargensis* DAS 131^{T}(Fig. 4). Further increase in
220 salt concentration reduced the antimicrobial agent biosynthesis. The requirement of NaCl for
221 the production of bioactive metabolites seems to be different among actinomycete strains.
222 Optimum NaCl concentration for maximum growth as well as antimicrobial metabolite
223 production was reported to be 2% for *Streptomyces tanashiensis* A2D [2], 1% for
224 *Streptomyces felleus* YJ1 [36] and 5% for *Streptomyces* VITSVK9 [30].
225
226

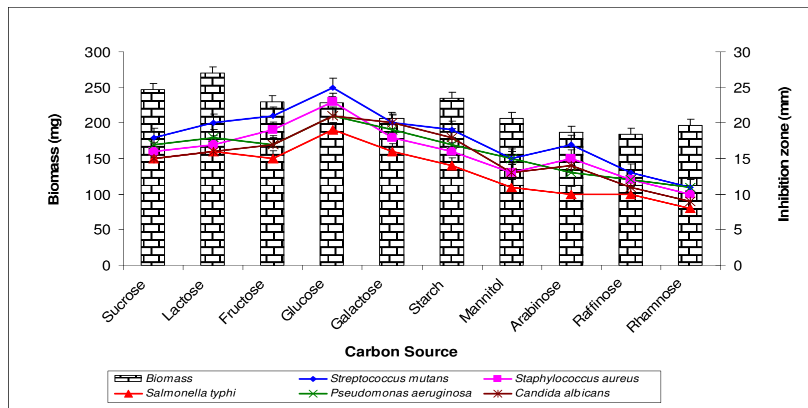


227
228
229 Fig. 4. Effect of NaCl on growth and bioactive metabolite yield of *S. gulbargensis* DAS 131^T.

230 **Data on cell growth and bioactive metabolite yield were statistically analyzed by Two-way ANOVA and*
231 *found to be significant at 1%.*
232

233 **3.4. Effect of carbon and nitrogen sources**

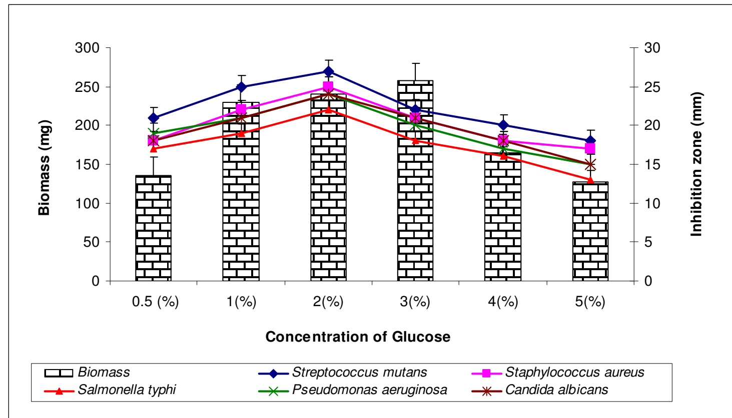
234
235 The effect of carbon sources on biomass and bioactive metabolite production by *S.*
236 *gulbargensis* DAS 131^{T}was evaluated. The production of biomass was high with lactose
237 followed by sucrose and starch, while significant bioactive metabolite production was
238 obtained by the strain in glucose amended media followed by galactose and fructose. El-
239 Enshasy *et al.* [39] reported that glucose and sucrose in pure or in polymer forms were the
240 best C-sources for erythromycin production. Antibiotic production from alkaliphilic
241 *S.tanashiensis* strain A2D was high in medium containing glucose as carbon source [2].
242 Similarly glucose was found to be the best carbon source for antibiotic production by
243 *Streptomyces torulosus*KH-4 [29], *S. griseocarneus* [40] and *S. kanamyceticus* M27 [41].
244



245
246
247 Fig. 5. Effect of different carbon sources on growth and bioactive metabolite yield of *S.*
248 *gulbargensis* DAS 131^T.

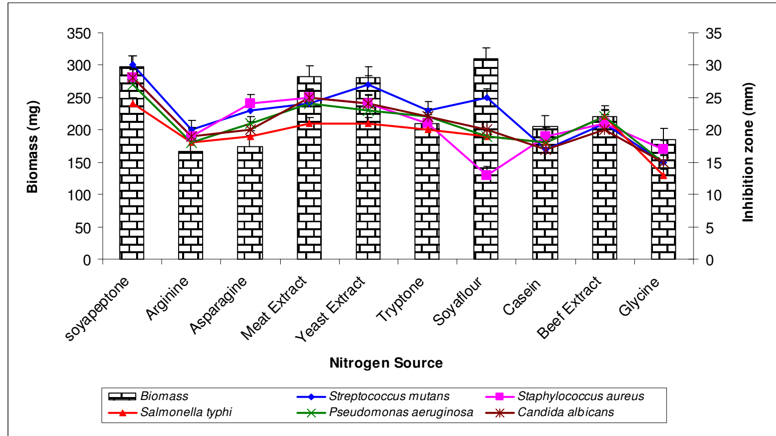
249 **Data on cell growth and bioactive metabolite yield were statistically analyzed by Two-way ANOVA and*
250 *found to be significant at 1%.*

251 As glucose emerged as the most preferred carbon source for bioactive metabolite production
252 by the strain, varying concentrations of glucose (0.5-5%) was tested to determine its optimal
253 concentration. It is noted that glucose at 3% and 2% concentrations showed optimal yields of
254 biomass and bioactive metabolites respectively (Fig.6).Medium containing 2% glucose
255 supported maximum levels of Natamycin production by *Streptomyces natalensis* and
256 *Thermomonospora* spp. [38, 42] while Atta *et al.* [25] reported that medium containing 2.5%
257 glucose supported antibiotic production by *Streptomyces ramulosus* AZ-SH-29.
258

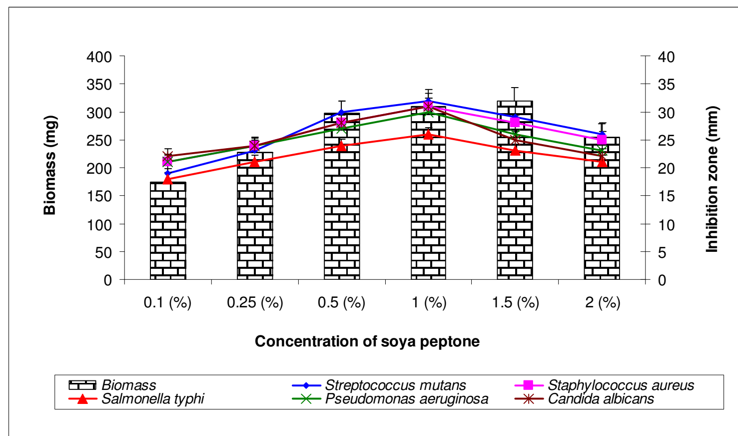


259
260
261 Fig.6. Effect of different concentrations of glucose on growth and production of bioactive
262 metabolite by *S.gulbargensis* DAS 131^T.

263 **Data on cell growth and bioactive metabolite yield were statistically analyzed by Two-way ANOVA and*
264 *found to be significant at 1%.*
265
266 Nitrogen sources are important for the production of bioactive metabolites by
267 microorganisms. Changes in the nature and concentration of nitrogen source seem to affect
268 antibiotic biosynthesis in different organisms. Different nitrogen sources were found to have
269 significant effect on growth and secondary metabolite production by *S. gulbargensis* DAS
270 131^T. Among the nitrogen sources tested amendment of soya peptone in the culture medium
271 enhanced the biomass and bioactive metabolite production by the strain (Fig. 7). Viana *et al.*
272 [43] recorded that soya bean flour increased the clavulanic acid production by *Streptomyces*
273 DAUFPE 3060. In contrast Thakur *et al.* [7] found that basal medium amended with
274 asparagine as nitrogen source was proved to be the best for 2-methylheptyl isonicotinate
275 production by *Streptomyces sp.*201.
276



277
278
279 Fig.7. Effect of different nitrogen sources on growth and bioactive metabolite production by
280 *S. gulbargensis* DAS 131^T.
281
282 **Data on cell growth and bioactive metabolite yield were statistically analyzed by Two-way ANOVA and*
283 *found to be significant at 1%.*
284
285 Influence of different concentrations of soya peptone on the production of bioactive
286 metabolites is represented in Fig.8. It is noted that soya peptone at a concentration of 1.5%
287 and 1% exhibited optimal production of biomass and bioactive metabolites respectively.
288 Himabindu and Jetty [44] reported that soya bean meal at a concentration of 1% and 0.5%
289 enhanced growth and gentamicin production by *Micromonospora echinospora.* Whereas Qin
290 Song *et al.* [36] stated that soya bean meal at a concentration of 2% increased the bioactive
291 metabolite production by *Streptomyces felleus* YJ1.
292

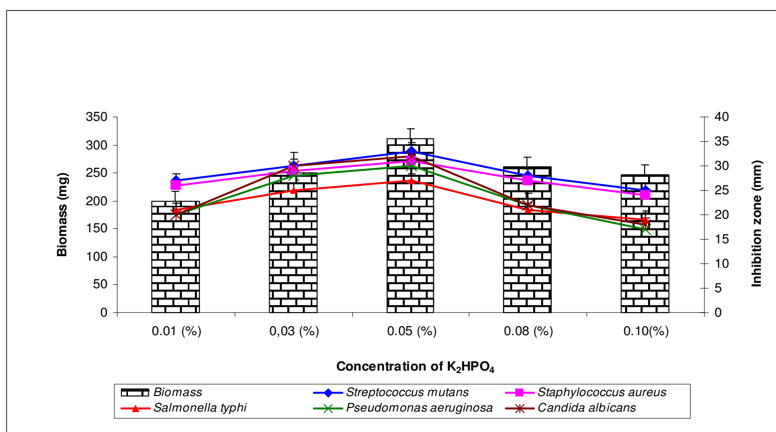


293
294
295 Fig.8. Effect of different concentrations of soya peptone on growth and production of
296 bioactive metabolite by *S. gulbargensis* DAS 131^T.
297 **Data on cell growth and bioactive metabolite yield were statistically analyzed by Two-way ANOVA and*
298 *found to be significant at 1%.*
299

300 **3.5. Effect of K_2HPO_4**

301 Effect of K_2HPO_4on biomass and bioactive metabolite production by the strain (Fig. 9) was
302 studied. A slight enhancement in growth and antimicrobial activity was obtained in medium
303 supplemented with 0.05% of K_2HPO_4. Ripa *et al.* [32] reported that among different minerals

304 tested, K_2HPO_4 showed positive influence on antibiotic production by *Streptomyces* RUPA-
305 08PR. Narayana and Vijayalakshmi [21] also recorded that K_2HPO_4 slightly enhanced the
306 production of biomass and bioactive metabolites of *Streptomyces albidoflavus.* Production of
307 gentamicin by *M.purpurea* and antibiotic tylosin by a *Streptomyces* sp. was inhibited by high
308 phosphate concentrations [45, 46].
309
310



311
312
313 Fig.9. Impact of K_2HPO_4 on growth and bioactive metabolite production of *S. gulbargensis*
314 DAS 131^T.
315 **Data on cell growth and bioactive metabolite yield were statistically analyzed by Two-way ANOVA and*
316 *found to be significant at 1%.*
317

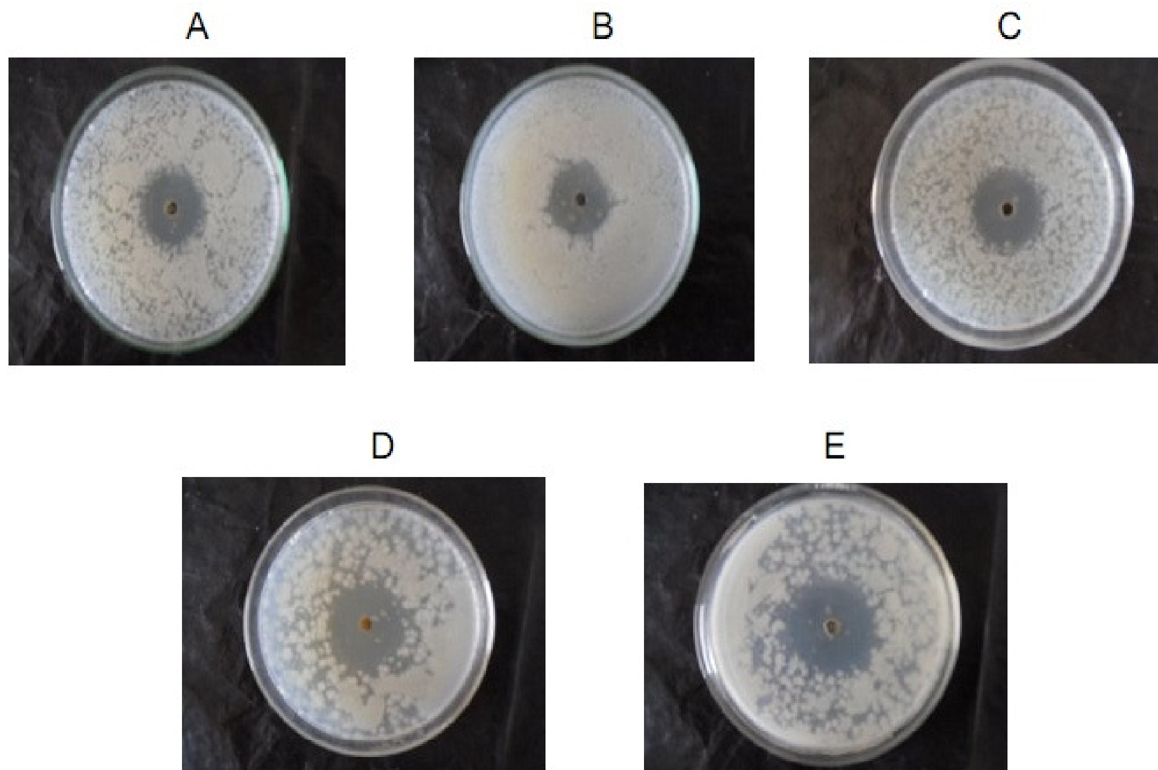
318 **3.6. Bioassays**

319 The antimicrobial metabolite produced by the strain under optimized conditions was tested
320 against various test bacteria and fungi (Table.1& Fig.10).Among the bacteria tested,
321 *Xanthomonas campestris*(MTCC 2286)and *Bacillus megaterium* (NCIM 2187)were highly
322 sensitive to the metabolites produced by *S.gulbargensis* DAS 131^Tfollowed by *Streptococcus*
323 *mutans*(MTCC 497)and *Enterococcus faecalis*(MTCC 439).Among the fungi tested, *Candida*
324 *albicans* (ATCC 10231)was highly sensitive to the metabolites produced by the strain
325 followed by *Aspergillus niger*(ATCC 1015) and *Aspergillus flavus* (ATCC 9643). A significant
326 antimicrobial activity was reported on the opportunistic and pathogenic bacteria and fungi
327 tested.
328
329 Table1. Antimicrobial activity of*S. gulbargensis* DAS 131^Tagainst opportunistic and
330 pathogenic bacteria and fungi under optimized conditions.
331

Antimicrobial Activity of Bioactive Metabolite Produced by S. gulbargensis DAS 131^T under Optimized Conditions	
Bacteria	
Test Microorganisms	***Zone of Inhibition****(mm)*
Staphylococcus aureus(MTCC 3160)	*31*
Streptococcus mutans(MTCC 497)	*33*

Bacillus subtilis(ATCC 6633)	*30*
Lactobacillus casei(MTCC 1423)	*32*
Lactobacillus acidophilus(MTCC 495)	*31*
Xanthomonas campestris(MTCC 2286)	*34*
Bacillus megaterium(NCIM 2187)	*33*
Escherichia coli (ATCC 35218)	*31*
Enterococcus faecalis(MTCC 439)	*33*
Pseudomonas aeruginosa(ATCC 9027)	*30*
Salmonella typhi(ATCC 14028)	*27*
Proteus vulgaris(MTCC 7299)	*28*
Fungi	
Candida albicans(ATCC 10231)	*32*
Aspergillus niger(ATCC 1015)	*27*
Aspergillus flavus(ATCC 9643).	*26*
Fusariumoxysporum(MTCC 3075)	*19*
Penicilliumcitrinum(MTCC 6489).	*20*

332
333



334
335

336 FIG. 10: Antimicrobial activity of *S.gulbargensis* DAS 131^T against
337 A.*Pseudomonasaeruginosa*(ATCC 9027)B. *Escherichia coli*(ATCC 35218)C.
338 *Salmonellatyphi*(ATCC 14028)D. *Bacillus subtilis*(ATCC 6633)E.*Candida albicans*(ATCC
339 10231).

340

341 **4. CONCLUSION**

342 In the present study *S.gulbargensis* DAS 131^T exhibited high antimicrobial activity when
343 cultured on production medium amended with glucose (2%),soya peptone (1%), NaCl (5%)
344 and K_2HPO_4 (0.05%)at pH 7 for six days of incubationat 35℃. Among the bacteria tested,
345 *Xanthomonas campestris* and *Bacillus megaterium* were highly sensitive to the metabolites
346 produced by the strain while *Candida albicans* exhibited high sensitivity followed by
347 *Aspergillus niger*among fungi.This is the first report on the optimization of bioactive
348 metabolites produced by *S.gulbargensis* DAS 131^T.
349

350 **ACKNOWLEDGEMENTS:**

351 Financial assistance from CSIR, New Delhi is gratefully acknowledged.

352 **COMPETING INTERESTS**

353 Authors have declared that no competing interests exist.
354

355 **AUTHORS' CONTRIBUTIONS**

356 UKM performed the experimental part, MVL& DA designed the study and SP performed
357 literature search and statistical analysis. All authors read and approved the final manuscript.
358

REFERENCES

[1]. Bérdy J. Bioactive microbial metabolites. J Antibiot. 2005; 58 (1): 1-26.

[2]. Singh LS, Mazumdar S, Bora TC. Optimization of process parameters for growth and bioactive metabolite production by a salt-tolerant and alkaliphilic actinomycete, *Streptomyces tanashiensis* strain A2D. Journal de Mycologie Medicale. 2009; 19 (4): 225-223.

[3]. Blunt JW, Copp BR, Munro MHJ, Northcote PT, Prinsep MR. Marine natural products. Natural Product Reports. 2010; 27 (2): 165-237.

[4]. Shuvankar B, Syed GD, Savita K. Biotechnological significance of actinobacterial research in India. Recent Research in Science and Technology.2012; 4(4): 31-39.

[5]. Hopwood DA. Therapeutic treasures from the deep. Nature Chemical Biology.2007; 3 (8): 457-458.

[6]. Mitsuiki S, Sakai M, Moriyama Y, Goto M, Furukawa K. Purification and some properties of keratinolytic enzyme from an alkaliphilic *Nocardiopsis* spp. TOA-1. Biosci Biotechnol Biochem. 2002; 66 (1):164-167.

[7]. Thakur D, Bora TC, Bordoloi GN, Mazumdar S. Influence of nutrition and culturing conditions for optimum growth and antimicrobial metabolite production by *Streptomyces* spp.201. Journal de Mycologie Medicale.2009; 19 (3): 161-167.

[8]. Dietera A, Hamm A, Fiedler HP, Good fellow M, Muller WA, Brun RP. An antibiotic, antiparasitic and antitumor compound produced by a novel alkaliphilic *Streptomyces* strain. J Antibiot. 2003; 56 (7):639-646.

[9]. Cai P, Kong F, Fink P, Ruppen ME, Williamson RT, Keiko T. Polyene Antibiotics from *Streptomyces mediocidicus*. J Nat Prod.2007; 70 (2):215-219.

[10]. Chen C, Si S, He Q, Xu H, Lu M, Xie Y. Isolation and characterization of antibiotic NC0604, a new analogue of bleomycin. J Antibiot.2008; 61(12):747-751.

[11]. Hohmann C, Schneider K, Bruntner C, Irran E, Nicholson G, Bull AT. Caboxamycin, a new antibiotic of the benzoxazole family produced by the deep-sea strain *Streptomyces* spp. NTK 937. J Antibiot. 2009; 62 (2):99-104.

[12]. Adinarayana K, Prabhakar T, Srinivasulu V, Rao AM, Jhansi LP. Optimization of process parameters for cephalosporin C production under solid state fermentation from *Acremonium chrysogenum*. Process Biochem. 2001; 39 (2): 171-177.

[13]. Noaman NH, Fattah A, Khaleafa M, Zaky SH. Factors affecting antimicrobial activity of *Synechococcus leopoliensis*. Microbiol Res. 2004; 159 (4): 395-402.

407
408 [14].Basak K, Majumdar SK. Mineral nutrition of *Streptomyceskanamyceticus* for Kanamycin
409 formation. Antimicrob Agents Chemother. 1975; 8 (4):391-395.
410

411 [15]. Loun'es A, Lebrihi A, Benslimance C, Lefebvre G, Germain P. Regulation of spiramycin
412 synthesis in *Streptomyces ambofaciens*: effect of glucose and inorganic
413 phosphate. J Microbiol Biotechnol. 1996; 45 (1-2): 204-211.
414

415 [16]. Moita C, Feio SS, Nunes L, Joa M, Curto M. Optimization of physical factors on the
416 production of active metabolites by *Bacillus subtilis* 355 against wood surface
417 contaminant fungi. Int Biodeterior Biodegrad. 2005; 55 (4): 261-269.
418

419 [17]. Krishnakumar S, Premkumar J, Alexis Rajan R, Ravikumar S. Optimization of potential
420 antibiotic production by salt-tolerant actinomycetes *Streptomyces* spp. MSU29
421 isolated from marine sponge. Int J Applied Bioengineering. 2011; 5(2): 12-17.
422

423 [18]. Kuster K, Williams ST. Selection of media for isolation of *Streptomycetes*. Nature. 1964;
424 202(4935): 928-929.
425

426 [19].Williams ST, Cross T. Isolation, Purification, Cultivation and Preservation of
427 Actinomycetes. In Booth C, editor. Methods Microbiology. 4: 295-334, 1971.
428

429 [20]. Dastager GS, Wen A, Dayanand BS, Mudgulkar KT, Shu PT, Xin, Xiao YZ.
430 *Streptomyces gulbargensis*sp.nov, isolated from soil in Karnataka, India.
431 Antonie van Leeuwenhoek. 2007; 91 (2): 99-104.
432

433 [21]. Narayana KJP, Vijayalakshmi M. Optimization of antimicrobial metabolites production
434 by *Streptomycesalbidoflavus*. Research journal of pharmacology. 2008; 2 (1): 4-
435 7.
436

437 [22]. Cappuccino JG, Sherman N. Microbiology, Laboratory manual. Person education, INC,
438 New Delhi; 2004.
439

440 [23]. Yarbrough GG. Screening microbial metabolites for new drugs, theoretical and practical
441 issues. J Antibiot. 1993; 46 (4):535-544.
442

443 [24].Oskay M. Effects of some Environmental Conditions on Biomass and Antimicrobial
444 Metabolite Production by *Streptomyces* spp., KGG32. Int J Agric Biol. 2011; 13
445 (3): 317-324.
446

447 [25].Atta HM, Bahobail AS, El-Sehrawi MH. Studies on Isolation, Classification and
448 Phylogenetic characterization of antifungal substance produced by
449 *Streptomyces albidoflavus*-143.New York Science Journal. 2011; 4 (3): 40-53.
450

451 [26].Atta HM, Afifi MM, Elshanawany AA, Abdoul-raouf AU, El-Adly AM. Production of
452 Hygromycin-B antibiotic from *Streptomycescrystallanius*. AZ-A151: II.
453 Parameters controlling of antibiotic production. Academia Arena. 2012; 4 (3):
454 37-52.
455

456 [27].Chattopadhyay D, Sen SK. Optimization of cultural conditions for antifungal antibiotic
457 accumulated by *Streptomycesrochei* G 164. Hindustan Antibiot Bull. 1997; 39
458 (1): 64-71.
459
460 [28] Adinarayana K, Ellaiah P, Srinivasulu B, Bhavani R, Adinarayana G. Response surface
461 methodological approach to optimize the nutritional parameters for neomycin
462 production by *Streptomyces marinensis* under solid state fermentation. Process
463 Biochemistry. 2003; 38 (11):1565-1572.
464
465 [29].Atta HM, Bayoumi R, El-Sehrawi M, Aboshady A, Al-Humiany A. Biotechnological
466 application for producing some anti-microbial agents by actinomycetes isolates
467 from Al-Khurmah Governorate. European Journal of Applied Sciences. 2010; 2
468 (3): 98-107.
469
470 [30]. Saurav K, Kannabiran, K. Diversity and Optimization of process parameters for the
471 growth of *Streptomyces* VITSVK 9 sp. Isolation from Bay of Bengal, India.
472 Journal of Natural and Environment sciences 2010; 1(2): 56-65.
473
474 [31].Ushakiranmayi M, Sudhakar P, Krishna N, Vijayalakshmi M. Influence of cultural
475 conditions for improved production of bioactive metabolites by *Streptomyces*
476 *cheonanensis* VUK-A isolated from Coringa Mangrove Ecosystem. Current
477 Trends in Biotechnology and Pharmacy. 2012; 6 (1): 99-111.
478
479 [32].Ripa FA, Nikkon F, Zaman S, Khondkar P. Optimal conditions for antimicrobial
480 metabolites production from a new *Streptomyces* spp.Rupa-08 isolated from
481 Bangladesh Soil. Mycobiology. 2009; 37 (3): 211-214.
482
483 [33].Mangamuri Usha Kiranmayi, Poda Sudhakar, Kamma Srinivasulu, Muvva
484 Vijayalakshmi. Optimization of culturing conditions for improved production of
485 bioactive metabolites by *Pseudonocardia* spp. VUK-10. Mycobiology. 2011; 39
486 (3): 174-181.
487
488 [34]. Kunnari T, Tuikkanen J, Hautala A, Hakala J, Ylihonko, K, Mantsala, P. Isolation and
489 characterization of 8- demethoxy steffimycins in *Streptomyces steffisburgensis*
490 by the nogalamycin biosynthesis genes. J Antibiot. 1997; 50 (6):496-501.
491
492 [35].Saha MR, Rifa FA, Islam MZ, Khondkar, P. Optimization of conditions and in vitro
493 antibacterial activity of secondary metabolite isolated from *Streptomyces* spp.
494 MNK 7. Journal of Applied Science Research. 2010; 6(5):453-459.
495
496 [36].Qin Song, Yun Huang, Hui Yang. Optimization of fermentation conditions for antibiotic
497 production by actinomycetes YJ1 strain against *Sclerotinia sclerotiorum*. J Agri
498 Sci. 2012; 4 (7): 95-102.
499
500 [37].Casida LE. Fermentation media, In: Casida, L.E. editor. Industrial Microbiology, John
501 Wiley and Sons, Inc, New York, USA; pp: 117-135, 1987.
502
503 [38].Farid MA, El-Enshasy HA, Ei-Diwany AI, El-sayed EA. Optimization of the cultivation
504 medium for Natamycin production by *Streptomyces netalensis*. J Basic
505 Microbiol. 2000; 40 (3):157-166.

506 [39].El-Enshasy HA, Mohamed NA, Farid MA, El-Diwany AI. Improvement of erythromycin
507 production by *Saccharopolyspora erythrea* in molasses based medium through
508 cultivation medium optimization. Bioresour Technol. 2008; 99(10): 4263-4268.
509

510 [40].Cruz R, Arias ME, Solveri J. Nutritional requirements for the production of phyla
511 zoloisoquinolinone antibiotic by *Streptomycesgriseocarneus* NCIMB 40447.
512 Appl Microbiol Biotechnol. 1999; 53(1):115-119.
513

514 [41].Pandey A, Shukla A, Majumdar SK. Utilization of carbon and nitrogen sources by
515 *Streptomyces kanamyceticus* M 27 for the production of an antibacterial
516 antibiotic. Afr J Biotechnol. 2005; 4(9): 909-910.
517

518 [42].Gupte M, Kulkarni, P. A study of anti-fungal antibiotic production by *Thermomonospora*
519 spp. MTCC 3340 using full factorial design. J Chem Tech Biotechnol.2003;
520 78(6): 605-610.
521

522 [43]. Viana DA, Carneiro-Cunha MN, Araujo JM, Barros-Neto B, Lima-Filho JL, Converti A *et*
523 *al.* Screening of variables influencing the Clavulanic Acid Production by
524 *Streptomyces* DAUFPE 3060 strain. Appl Biochem Biotechnol. 2010; 160(6):
525 1797-1807.
526

527 [44].Himabindu M, Annapurna J. Optimization of nutritional requirements for gentamicin
528 production by *Micromonospora echinospora.* Indian J Experimental Biol. 2006;
529 44: 842-848.
530

531 [45].Wu JY, Jenn-Wen H, Sin-Der SH, Wei-Chenand L, Yungehuan L. Optimization of
532 cultivation conditions for fungi chromin production from *Streptomyces padanus*
533 PMS-702. J Chin instChem Eng. 2008; 39 (6):67-73.
534

535 [46]. Majumdar MK, Majumdar SK. Effect of minerals on neomycin production by
536 *Streptomyces fradiae.* Appl Microbiol. 1965; 13 (2): 190-193.
537
538
539
540
541
542