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Research Article

Optimization of Cultural Parameters for Enhanced Biomass and Production of Bioactive Metabolite by *Alcaligenes faecalis* VuBc M20 isolated from Machilipatnam Sea Coast of Andhra Pradesh, India

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ABSTRACT
The present work was aimed to investigate the influence of appropriate culture medium by optimizing the cultural conditions for enhanced biomass and production of bioactive metabolite by *Alcaligenes faecalis* VuBc M20 which was selected based on its antibacterial potential observed during primary screening study. An attempt was made to examine the impact of optimized environmental parameters like incubation period, pH, temperature and salt concentration and effect of various carbon and nitrogen sources and minerals on bioactive metabolite production and biomass. From the obtained results, it was observed that the optimum pH and temperature for increased bioactive metabolite production were 7.0 and 37°C, respectively. The growth and metabolite production were found optimized with 3.0 % (w/v) glucose, 1.5 % (w/v) tryptone and 0.002 % L-aspartic acid as carbon, nitrogen and amino acid sources, respectively. At all these optimized conditions, *Alcaligenes faecalis* VuBc M20 showed relatively highest antibacterial activity against *Serratia marcescens* (MTCC 4822) when compared to the other tested bacteria namely *Bacillus cereus* (MTCC 430), *Bacillus subtilis* (MTCC 441) and *Escherichia coli* (MTCC 443).

KEYWORDS: Antibacterial activity; bioactive metabolites; biomass; cultural parameters; optimization

INTRODUCTION

The emergence of multi-drug resistance phenomenon in many pathogenic bacteria and the subsequent health consequences has caused a revival of interest in finding new reserves of bioactive compounds from natural sources [1]. Among the natural products, special interest is centered on the microbes that have been proved to be the natural repositories for an array of bioactive metabolites with diverse and promising applications in the field of pharmacology, biomedical and drug development research. Though, nearly 22,000 bioactive compounds have been reported from the marine biota, microbe-derived compounds of the marine ecosystem still remains as a relatively unexplored area of interest. Therefore, screening of microorganisms for the production of new and novel bioactive constituent continues to be an emerging and interesting approach in modern drug discovery programs [2, 3, 4]. In recent years, microbes especially bacteria dwelling in extreme marine habitats are the prime source for many important bioactive compounds with

an array of bioactivities such as cytotoxic, anticancer, antiproliferative, antitumor, antifouling and antibiotic properties [5]. In 2005, Elio *et al*. reported the prolific antibacterial activity of marine bacterium, *Marinomonasmediterranea* against nosocomial strains such as *Pseudomonas* sp. and *Staphylococcus aureus* [6]. Radjasa *et al*. reported the antagonistic activity of marine bacterium, *Pseudoalteromonasluteoviolacea* against both pathogenic and coral bacteria [7].

The nutritional sources like carbon, nitrogen and minerals; physico-chemical parameters like temperature, pH and incubation period play a pivotal role on growth and productivity of bioactive metabolites. Optimization of the culture conditions as well as these physico-chemical parameters is critically essential to have profound influence on increased production of cost effective bioactive metabolites and antimicrobial agents and thereby facilitate the drug discovery programs [4, 8, 9, 10, 11].

In the present study, an attempt was made to optimize the physical and nutritional parameters for the marine derived bacterial isolate *Alcaligenesfaecalis*VuBcM20 to get an effective and high yield of biomass production with improved antagonistic potential against some tested bacteria.

MATERIALS AND METHODS

Bacterial isolation and molecular characterization

Marine water samples were collected from random points of Machilipatnam sea coast of Andhra Pradesh, India and employed in isolation of marine bacteria following plating method. Different representative isolates obtained were subsequently sub-cultured and screened for antibacterial activity against some test bacteria. The isolate that showed better antibacterial activity was subjected to 16S rRNA sequencing (at Macrogen Inc., South Korea) for identification.

Test organisms

The test organisms used in the present study include *Bacillus cereus* (MTCC 430), *Bacillus subtilis* (MTCC 441), *Escherichia coli* (MTCC 41) and *Serratia marcescens* (MTCC 4822). All these cultureswere procured from Microbial Type Culture Collection (MTCC), IMTECH, and Chandigarh, India and were maintained on nutrient agar media slants.

Optimization studies

Effect of temperature on biomass and bioactive metabolite production

Effect of temperature on biomass and bioactivity of crude extract of bacterial isolate *Alcaligenes faecalis* VuBc M20 at different temperatures viz., 20, 25, 30, 35, 40 and 45 °C was studied by incubating the inoculated broth culture for 24 hrs. After incubation, biomass of the isolate in terms of optical density and bioactivity of the secondary metabolite of culture broth supernatant against tested bacteria in terms of zone of inhibition were recorded.

Effect of pH on biomass and bioactive metabolite production

Broth culture in test tubes inoculated with *Alcaligenes faecalis* VuBc M20 were incubated at different pH conditions namely 5.0, 6.0, 7.0, 8.0 and 9.0 to study the influence on biomass as well as bioactive metabolite production. After incubation, optical density for biomass and zone of inhibition for bioactivity of secondary metabolite were determined.

Effect of incubation time on biomass and bioactive metabolite production

The impact of different incubation periods viz., 24, 48, 72 and 96 hrs on the production of biomass and antibacterial activity of secondary metabolite of*Alcaligenes faecalis* VuBc M20 were determined according to the method described by [3] with slight modification.

Effect of agitation on biomass and bioactive metabolite production

The effect of agitation at 50, 100, 150 and 200 rpm on biomass as well as bioactive metabolite production by the isolate *Alcaligenes faecalis* VuBc M20 was investigated according to the method described by [12].

Effect of carbon sources on biomass and bioactive metabolite production

The influence of various carbon sources viz., glucose, fructose, lactose, sucrose and starch at 3% (w/v) concentration on biomass and bioactive metabolite production *Alcaligenes faecalis*VuBc

M20 was investigated and the results were recorded.

Effect of nitrogen sources on biomass and bioactive metabolite production

To study the impact of various nitrogen sources on biomass and bioactivity of *Alcaligenes faecalis* VuBc M20, different organic nitrogen sources such as soyabean meal, beef extract, tryptone, casein and glycine, and different inorganic nitrogen sources viz.,ammonium nitrate (NH_4NO_3), ammonium sulpahte ($(NH_4)_2SO_4$), ammonium chloride (NH_4Cl) and potassium nitrate (KNO_3) were added at the concentration of 1.5 % (w/v) to different boiling tubes containing broth medium inoculated with culture of *Alcaligenes faecalis*VuBc M20. After incubation, biomass and antibacterial activity were assessed [9].

Effect of NaCl concentration on biomass and bioactive metabolite production

The effect of salinity on growth and bioactive metabolite production by *Alcaligenes faecalis*VuBc M20 was studied by incubating the test tubes containing the medium supplemented with different concentrations of NaCl viz., 1%, 2%, 3%, 4% and 5% and inoculated with culture.The biomass and bioactive metabolite production for each NaCl concentration were estimated.

Effect of amino acids on biomass and bioactive metabolite production

The effect of various amino acids (0.002% w/v) such as L-aspartic acid, L-cysteine, L-lysine, L-histidine and L-glutamine on growth and bioactive metabolite production by *Alcaligenes faecalis* VuBc M20 was estimated by measuring optical density for biomass and zone of inhibition for antibacterial activity of secondary metabolite.

RESULTS

Molecular Characterization Of Bacterial Isolate

The extraction of DNA and its subsequent 16S rRNAsequence analysis confirmed the positive bacterial isolate M20 as *Alcaligenes faecalis* VuBc M20. The nucleotide sequences were submitted to the NCBI Gene Bank database as *Alcaligenes faecalis*VuBc M20with accession number KR921866.1.

Optimization Studies

Effect of temperature on biomass and bioactive metabolite production

From the optimization analysis for temperature, the production of bioactive metabolite by *Alcaligenes faecalis* VuBc M20 was observed to be high at 35 °C with a biomass of 3.79 g/L and it was found to exhibit maximum antagonistic activity against *S. marcescens* with a zone of inhibition of 16mm as compared to the other test organisms (Figure 1a).

Effect of pH on biomass and bioactive metabolite production

The effect of different pH conditions on biomass and bioactive metabolite production revealed that, *Alcaligenes faecalis* VuBc M20 exhibited highest biomass production and subsequent bioactive metabolite production at pH 7.0 (Figure. 2). At 7.0 pH, *Alcaligenes faecalis*VuBc M20 produced the biomass of 3.96 g/L showed highest bioactivity against *S. marcescens* (16mm diameter zone of inhibition) when compared to the other test organisms. Both the biomass and bioactivity of the organism increased gradually from pH 5.0 to pH 7.0 and then decreased (Figure 1b).

Effect of incubation on biomass and bioactive metabolite production

The effect of incubation on bioactivity of *Alcaligenes faecalis* VuBc M20showed that the production of antimicrobial metabolites was found to be maximum at 72 h of incubation with an enhanced biomass of 3.96 g/L. When the bioactivity of these metabolites was checked against a range of test organisms, it showed highest activity against *S. marcescens* with a substantial zone of inhibition of 16 mm (Figure 1 c).

Effect of agitation on biomass and bioactive metabolite production

When the effect of agitation on biomass and bioactive constituent production was evaluated, it was observed that at 150 rpm, the production of biomass and bioactive metabolites by the *Alcaligenes faecalis* VuBc M20 was found to be maximum. The production of biomass at 150 rpm was observed to be about 3.87 ± 0.11 g/L. The antagonistic activity of the isolate *Alcaligenes faecalis* VuBc M20 against test pathogens followed the similar trend and

showed highest activity against *S. marcescens* (Figure 1d).

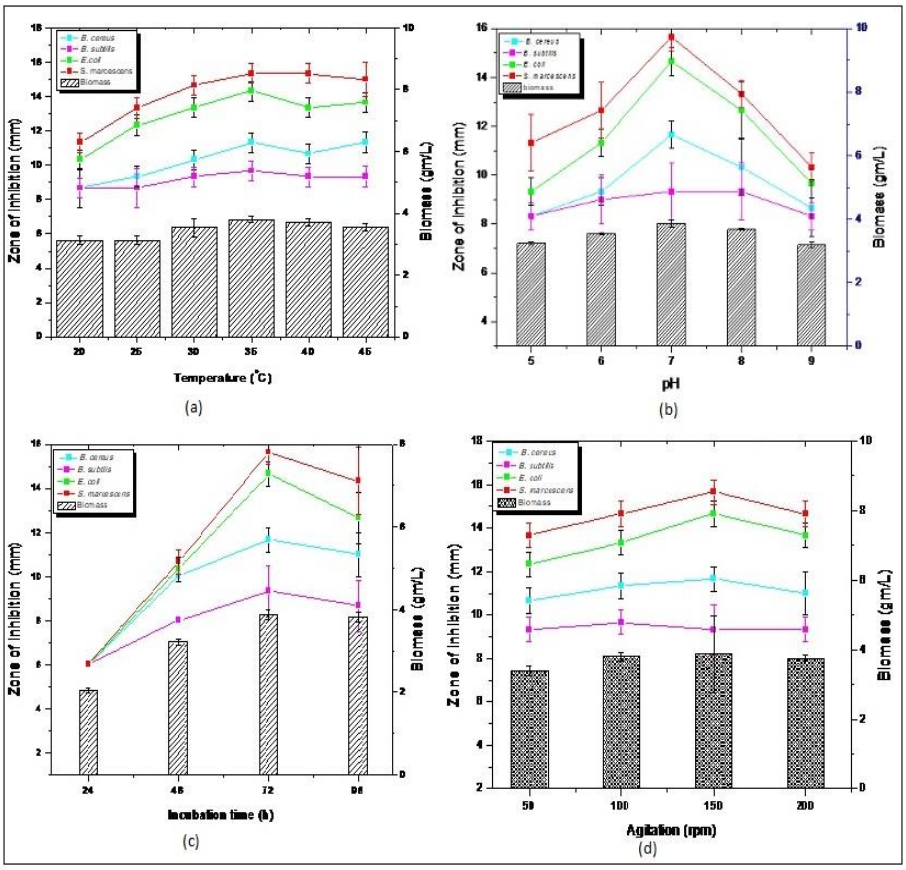

Fig. 1: Effect of (a) Temperature (b) PH (c) Incubation Time (d) Agitation on biomass and bioactive metabolite production by *Alcaligenes faecalis*VuBc M20

Effect of carbon sources on biomass and bioactive metabolite production

Among all the carbon sources utilized for optimization at concentration 0.5 and 1 gm/lt, *Alcaligenes faecalis* VuBc M20showed enhanced bioactive potential in glucose as carbon source with a biomass of 3.52 ± 0.12 g/L and 3.78 ± 0.07 g/L respectively. Meanwhile, highest bioactivity was shown against *S. marcescens* with a relative zone of inhibition of 12.67 mm and 14.67 mm respectively. The results are shown in Figure 2(a) and 2(b) .

Among all the carbon sources utilized for optimization at concentration 2 and 3 gm/lt, *Alcaligenes faecalis* VuBc M20showed enhanced bioactive potential in glucose as carbon source with a biomass of 3.68 ± 0.10 g/L and 3.76 ± 0.12 g/L respectively. Meanwhile, highest bioactivity was shown for glucose and lactose against *S. marcescens* with a relative zone of inhibition of 12.67 mm and 11.33 mm respectively. The results are shown in Figure 2(c) and 2(d).

Among all the carbon sources utilized for optimization at concentration 4 and 5 gm/lt, *Alcaligenes faecalis* VuBc M20showed enhanced bioactive potential in glucose as carbon source with a biomass of 3.67 ± 0.12 g/L and 3.87 ± 0.10 g/L respectively. Meanwhile, highest bioactivity was shown for fructose and lactose against *S. marcescens* with a relative zone of inhibition of 11.33 mm and 11.67 mm respectively. The results are shown in Figure 2(e) and 2(f).

Among all the carbon sources utilized for optimization, *Alcaligenes faecalis* VuBc M20showed enhanced bioactive potential in glucose as carbon source with a biomass of 3.78 ± 0.06 g/L. Meanwhile, highest bioactivity was shown against *S. marcescens* with a relative zone of inhibition of 15 mm. When a range of concentration of glucose was used to optimize the bioactivity of isolate *Alcaligenesfaecalis*VuBc M20; it was observed that at a concentration of 3.0 % (w/v) it showed highest activity with a biomass production of 4.17 g/L. It also showed an increase in antagonistic activity against the test microorganisms with highest being against *S. marcescens* with a zone of inhibition of 16 mm which is shown in Figure 5(a).

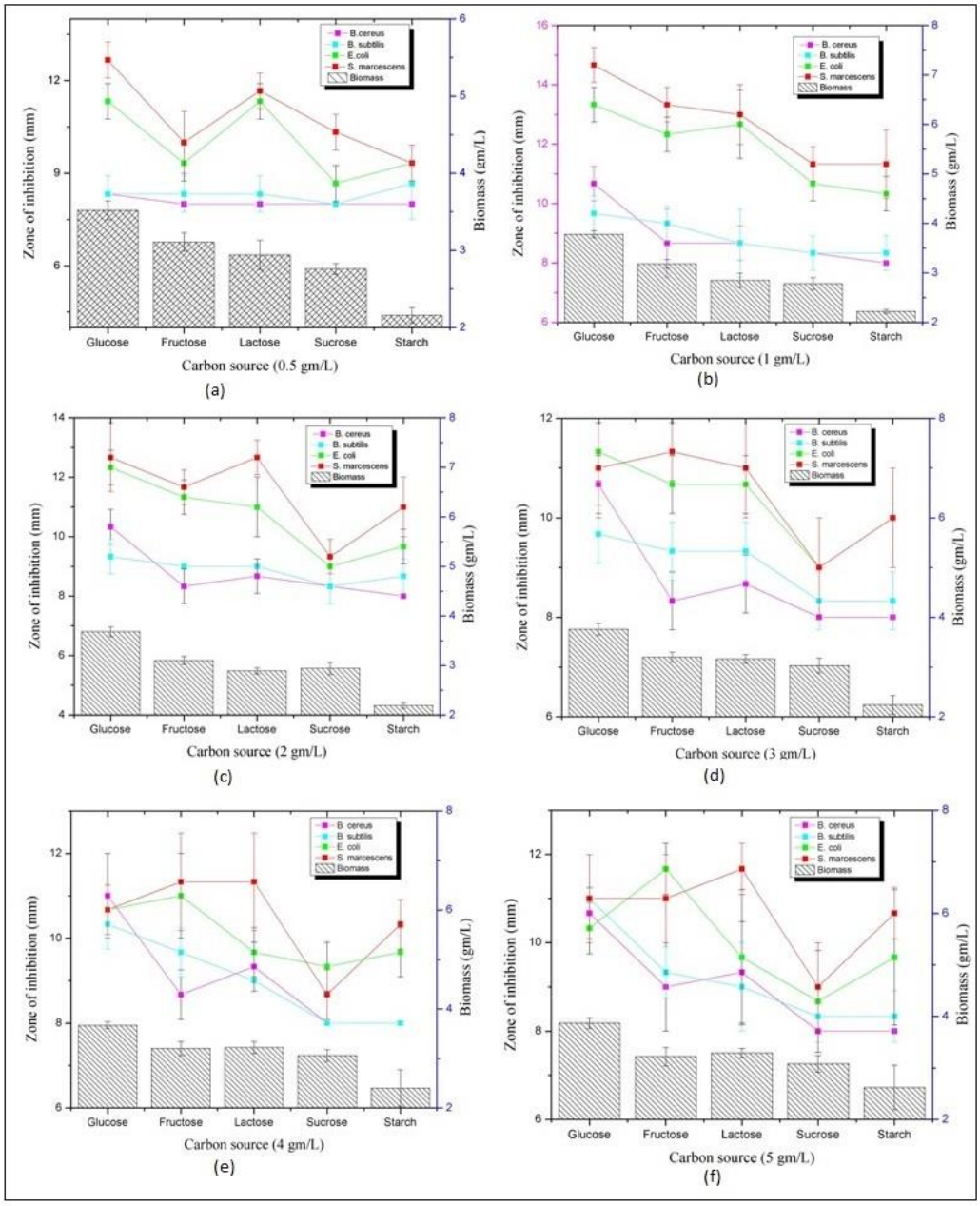

Fig. 2: Effect of Carbon Sources at different concentrations on production of biomass and bioactive metabolites by *Alcaligenes faecalis* VuBc M20

Effect of organic nitrogen sources on biomass and bioactive metabolite production

Among all the Organic Nitrogen sources utilized for optimization at concentration 0.5 gm/lt and 1.0 gm/lt, *Alcaligenes faecalis* VuBc M20showed enhanced bioactive potential in Beef Extract as Organic Nitrogen source with a biomass of 4.18 ± 0.09 g/L and 4.22 ± 0.12 g/L respectively. Meanwhile, highest bioactivity was shown against *S. marcescens* with a relative zone of inhibition of 15.33 mm for Beef Extract and 13.33 for Casein. The results are shown in Figure 3(a) and 3(b).

Among all the Organic Nitrogen sources utilized for optimization at concentration 1.5 gm/lt and 2.0 gm/lt, *Alcaligenes faecalis* VuBc M20showed enhanced bioactive potential in Beef Extract as Organic Nitrogen source with a biomass of 4.20 ± 0.13 g/L and 4.25 ± 0.08 g/L respectively. Meanwhile, highest bioactivity was shown against *S. marcescens* with a relative zone of inhibition of 14.00 mm for Casein and 13.33 for

Soya Bean Meal. The results are shown in Figure 3(c) and (d).

Alcaligenes faecalis VuBc M20 showed highest bioactivity when the supplied organic nitrogen source is tryptone with a biomass of 4.34 g/L and highest antagonistic activity was against *S. marcescens* with a zone of inhibition of 16 mm. When different concentrations of tryptone were used to optimize, at a concentration of 1.5 % (w/v); the biomass produced was observed to be about 4.75 g/L. In addition to that, *Alcaligenes faecalis* VuBc M20 showed potent antagonistic activity against both *E. coli* and *S. marcescens* with a zone of inhibition of 16 mm at 1.5 % (w/v) of tryptone which is shown in Figure 5(b).

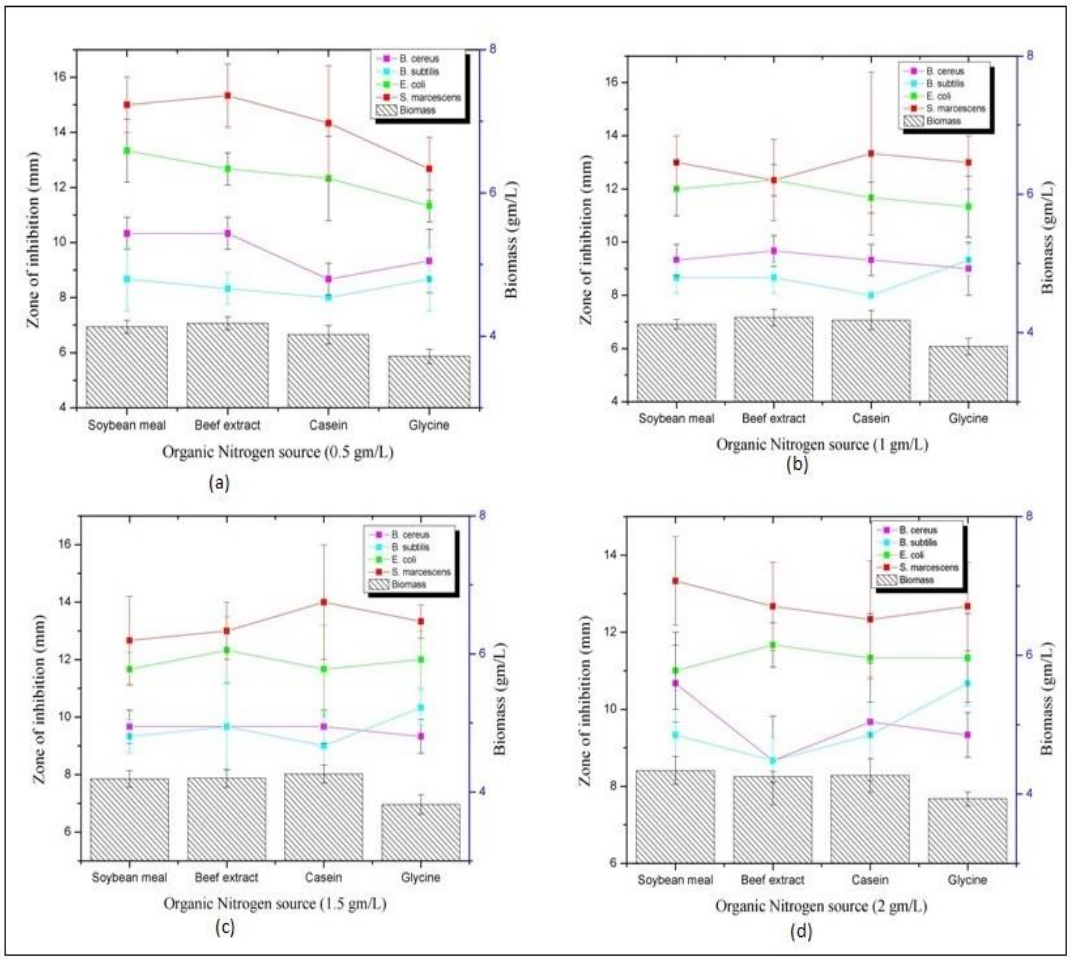

Figure 3: Effect of Organic Nitrogen Sources at Different Concentrations on Production of Biomass and Bioactive Metabolites by *Alcaligenes faecalis* VuBc M20

Effect of inorganic nitrogen sources on biomass and bioactive metabolite production

Among all the In-Organic Nitrogen sources utilized for optimization at concentration 0.5 gm/lt and 1.0 gm/lt, *Alcaligenes faecalis* VuBc M20showed enhanced potential in Ammonium Sulphate as In-Organic Nitrogen source with a biomass of 3.88 ± 0.08 g/L and 4.01 ± 0.11 g/L respectively. Meanwhile, highest bioactivity was shown against *S. marcescens* with a relative zone of inhibitions 14.33 mm and 14.67 for Ammonium Sulphate. The results are shown in Figure 4(a) and 4(b).

Among all the In-Organic Nitrogen sources utilized for optimization at concentration 1.5 gm/lt and 2.0 gm/lt, *Alcaligenes faecalis* VuBc M20showed enhanced potential in Ammonium Chloride as In-Organic Nitrogen source with a biomass of 4.19 ± 0.16 g/L and 4.13 ± 0.23 g/L respectively. Meanwhile, highest bioactivity was shown against *S. marcescens* with a relative zone of inhibitions 15.00 mm and 14.67 for Ammonium Chloride. The results are shown in Figure 4(c) and 4(d).

Among the various inorganic nitrogen sources, maximum bioactivity was observed in ammonium nitrate with a biomass produced was estimated to be about 4.19 g/L whereas the highest activity of *Alcaligenes faecalis* VuBc M20 was found against *S. marcescens*. When a range of concentrations of ammonium nitrate was used, at a sub minimal concentration of 1 % (w/v); highest bioactivity was achieved by *Alcaligenes faecalis* VuBc M20with an increased biomass production of 4.83 g/L and highest activity was observed against *S. marcescens* which is shown in Figure 5(c).

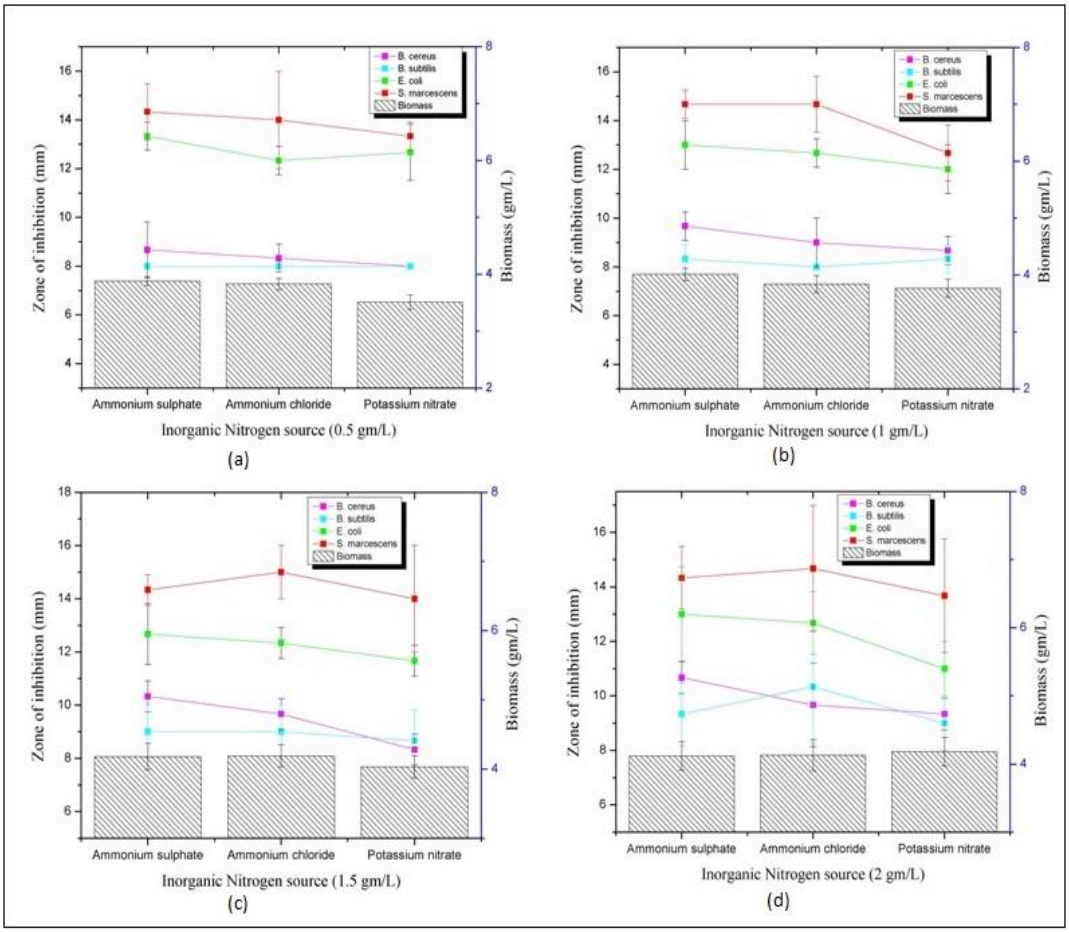

Fig. 4: Effect Of Inorganic Nitrogen Sources at Different Concentrations on Biomass and Bioactive Metabolite Production by *Alcaligenes faecalis*VuBc M20

Effect of NaCl concentration on biomass and bioactive metabolite production

Effect of salinity on bacterial growth and bioactivity of *Alcaligenes faecalis*VuBc M20 showed that at a substantial concentration of 3 % (w/v) of NaCl, *Alcaligenes faecalis* VuBc M20 showed highest biomass production and an increased antagonistic activity against test microorganisms. From the optimization analysis it was observed that the amount of biomass produced was about 4.74 g/L and the highest activity was observed against *S. marcescens* with a zone of inhibition of 15 mm which is shown in Figure 6(a).

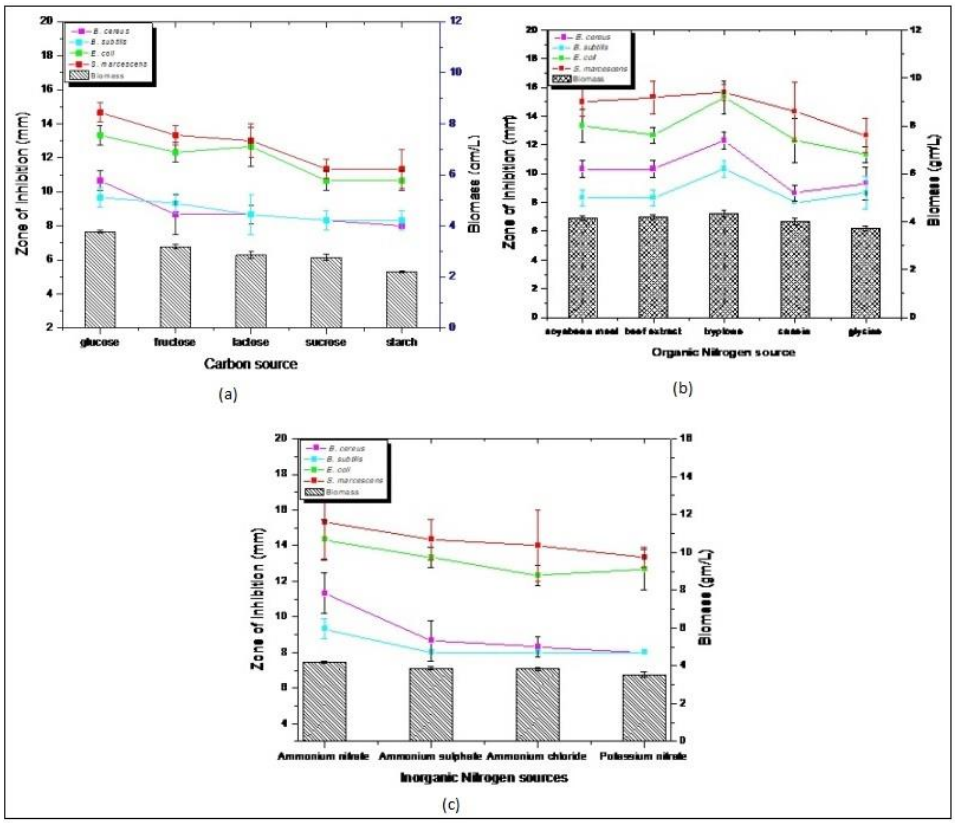

Figure 5: High Effect of Carbon, Organic Nitrogen and Inorganic Nitrogen Sources on Biomass and Bioactive Metabolite Production by *Alcaligenes faecalis*VuBc M20

Effect of amino acids on biomass and bioactive metabolite production

Optimization of different aminoacids on bioactivity of *Alcaligenes faecalis* VuBc M20showed that in presence of L-aspartic acid the production of biomass was maximum (4.36 g/L) and highest activity was against *S. marcescens* with a zone of inhibition of 15 mm.

When different concentration of L-aspartate was used for optimization, it was observed that *Alcaligenes faecalis* VuBc M20 showed maximum biomass of 4.86 g/L and a highest antagonistic activity against *S. marcescens* at a concentration of 0.002 % (w/v) which is shown Figure 6(b).

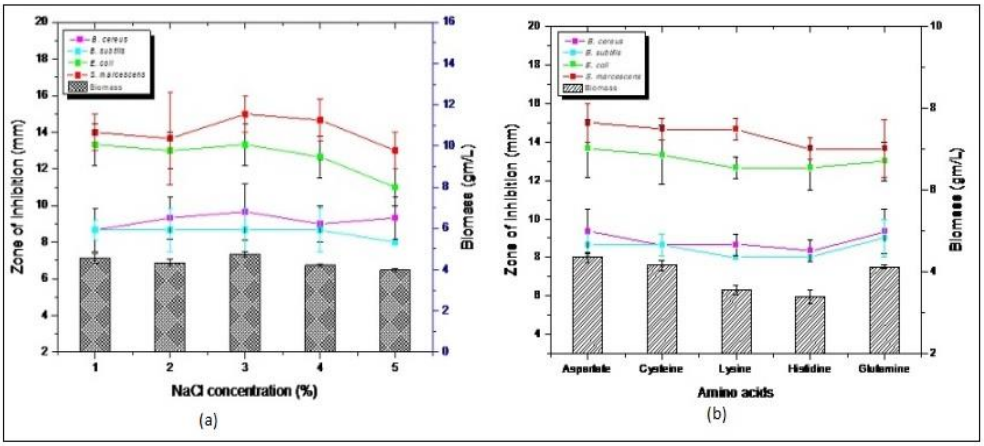

Fig. 6: Effect of NaCl Concentration and Different Types of Amino Acids on Production of Biomass and Bioactive Metabolites by *Alcaligenes faecalis* VuBc M20

DISCUSSION

The purpose of medium formulation and optimization of cultural parameters is to support efficient growth of microorganisms and thereby enhancing the chance of production of biomass and bioactive constituents by the microorganisms [3]. In the present study, the optimum temperature and pH required for *Alcaligenes faecalis* VuBc M20 for highest production of biomass and bioactive metabolite was observed to be 35°C and 7.0 respectively. This result was in accordance with earlier studies of [13] and [14] who reported that there is the requirement of an optimum temperature and pH of 40 °C and 6.8, respectively for the production of high levels of biomass and antibiotics. Incubation time also plays a pivotal role in bioactive metabolite and biomass production by microorganisms. *Alcaligenes faecalis* VuBc M20, in the present study, showed a highest level of biomass and bioactive metabolite production at incubation time of 72 h. The authors of [4] reported that *Bacillus subtilis* isolated from marine sponge produced highest level of biomass and antimicrobial metabolite after an incubation period of 72 h.

Optimization of carbon sources is one of the important criteria for enhanced biomass and for bioactive metabolite production by microorganisms. In the present study, among the different carbon sources used for optimization, glucose at a concentration of 3 % (w/v) served as the characteristic sole carbon source for both biomass and bioactive metabolite production. This result is in accordance with the results described by [15]. When the effect of different nitrogen sources for increased production of biomass and bioactive metabolites by *Alcaligenes faecalis* VuBc M20 were analyzed, it has been observed that tryptone at a concentration of 1.5 % (w/v) served as the best among the organic nitrogen sources used for optimization. The authors of [16] also reported about the enhanced production of biomass and bioactive metabolite by *Nocardia levis* MK-VL_113 with tryptone as the nitrogen source for optimization.

Salinity possesses a characteristic influence on bacterial metabolism and thereby has a profound implication in biomass and bioactive constituent's production by the concerned microorganisms. In the present study, 3 % NaCl with glucose and tryptone as carbon and nitrogen source found to be optimum for *Alcaligenes faecalis* VuBc M20for increased production of biomass and bioactive constituents. In paper [17],the authors reported that NaCl with 1.5 % concentration with starch and soy meal as carbon and nitrogen sources proved to be optimum for increased yield by *Nocardia cyriacigeorgia* KD-15.

The role of amino acids in production of biomass and bioactive metabolite by *Alcaligenes faecalis* VuBc M20 was assessed and it was observed that L-aspartic acid (0.002 %) served as the best amino acid source for the enhancement of biomass and bioactive metabolite production. This result is in complete accordance with the reports described in [9].

CONCLUSION

The present study furnishes an insight into the optimization of cultural and environmental parameters for enhanced biomass and antimicrobial metabolite production by the marine derived *Alcaligenes faecalis*VuBc M20. From the observation, it was concluded that the addition of 3 % (w/v) glucose as carbon source, 1.5 % tryptone as nitrogen source, 3 % NaCl, pH of 7.0 and temperature of 37 °C greatly favoured maximal production of biomass and bioactive metabolites by *Alcaligenes faecalis*VuBc M20. Thus, the present study could be further implemented in purification and characterization of potent bioactive metabolites by *Alcaligenes faecalis*VuBc M20 under the optimized parameters which will definitely provide a scope in the field of antibiotic research with novel and potent bioactive compounds.

CONFLICTS OF INTEREST

The authors declare that there is no conflict of interest regarding the publication of this research article.

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