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Veeramani S
P.G and Research Department of Botany and Microbiology A.V.V.M Sri Pushpam College, (Auto) Poondi. Thanjavur, Tamil Nadu, India

Babu S
P.G and Research Department of Botany and Microbiology A.V.V.M Sri Pushpam College, (Auto) Poondi. Thanjavur, Tamil Nadu, India

Tharanya M
P.G and Research Department of Botany, Kundhavai Naachiyaar Govt Arts College for women (Auto), Thanjavur, Tamil Nadu, India

Shijila Rani AS
P.G and Research Department of Botany and Microbiology A.V.V.M Sri Pushpam College, (Auto) Poondi. Thanjavur, Tamil Nadu, India

Ambikapathy V
P.G and Research Department of Botany and Microbiology A.V.V.M Sri Pushpam College, (Auto) Poondi. Thanjavur, Tamil Nadu, India

Correspondence
Veeramani S
P.G and Research Department of Botany and Microbiology A.V.V.M Sri Pushpam College, (Auto) Poondi. Thanjavur, Tamil Nadu, India

Studies on optimization of enhanced in a biomass production from marine Actinobacteria

Veeramani S, Babu S, Tharanya M, Shijila Rani AS and Ambikapathy V

Abstract
The study aimed at optimizing the biomass production of Actinobacteria by submerged fermentation. Conventional factor at a time was used as an initial screening process, i.e., one factor was varied, while keeping all the others constant. Different factors like pH, temperature, carbon nutrient source and incubation period were selected for the optimization process. The optimum pH, temperature, incubation periods and nutrient content were recorded are respectively. Different carbon and nitrogen sources were screened for optimum biomass production. Glucose at a concentration of 1.5% w/v and yeast extract at a concentration of 0.25% w/v was found to be the most effective for the mycelial biomass production. Further, inoculums were found to be the best for mycelial biomass production of *Actinomyces.* The maximum dry mycelial biomass obtained after combining these optimum conditions.

Keywords: Actinomycetes, biomass and optimization

Introduction
Actinomycetes, especially *Actinomycetes* species are a rich source of several useful bioactive natural products with potential applications Abdelfattah, (2009) [1]. They are prolific producers of secondary metabolites, many of which have commercial importance as antibiotics, antiparasitic and antifungal agents, anticancer and immunosuppressive agents. However, the ability of *Actinomycetes* cultures to form these bioactive products is not a fixed property but can be greatly increased or completely lost under different conditions of nutrition and cultivation. This is because antibiotic biosynthesis is a specific property of microorganisms which depends greatly on culture conditions. Improvement in the growth and antibiotic production can be carried out by manipulating the nutritional and physical parameters of the culturing conditions. Hence media composition plays a vital role in the efficiency and economics of the ultimate process. Therefore, designing an appropriate fermentation medium is of critical importance in the production of secondary metabolites. Changes in the nature and type of carbon and nitrogen sources have been reported to affect antibiotic biosynthesis in *Actinomycetes*. Also several cultivation parameters like pH, incubation period and temperature play a major role in the production of bioactive metabolites. The microscopic, morphological, biochemical and physiological characterization strongly suggests that the isolate belongs to the genus *Actinomycetes*. The present work describes the effect of different carbon and nitrogen sources and minerals on growth and bioactive compound production by the *Actinomycetes* strains. Further, the influence of pH, temperature and incubation time on growth and bioactive compound production was also studied (Usha Kiranmayi *et al*., 2016) [3].

Materials and Methods
Effect of pH (Ouardy Khay *et al*., 2013) [4]
To evaluate the effect of pH on growth and zone of inhibition was determined by changing the pH (5 to 11) by adjusting to required value by addition of 1N HCl or 1N NaOH of the optimized media containing best carbon and nitrogen source.

Effect of temperature
The optimized media containing best nitrogen, carbon sources at optimum pH was incubated at various temperatures ranging from 20 °C to 35 °C, to determine the optimum temperature required for maximum growth and production of Secondary metabolite.

Effect of incubation period (Papaspyridi *et al*., 2010) [5]
The optimum incubation period required for the growth and production of Zone of inhibition was determined by incubating the optimized media with best carbon, nitrogen and amino acid sources at optimum pH and temperature at different incubation periods (2-8 days).

Carbon source

To determine the optimum carbon source was selected, i.e. sucrose at a concentration of 1.5% (w/v). 250 ml Erlenmeyer flasks containing 100 mL of the following media: 0.2% yeast powder, 0.1% KH2PO4, 0.1% K2HPO4, 0.15% MgSO4.7H2O, 0.25% peptone and 1.5% of the respective carbon sources were added in individual flasks. Each flask was inoculated with 5% (v/v) of seed culture at 150 rpm, 25 °C for 10 days. The optimized carbon source was further studied for its optimum concentration from a range of 0.5-3.0% (w/v).

Nitrogen source

To determine the optimum nitrogen source six different nitrogen sources were selected i.e. yeast extract, peptone, ammonium sulphate, ammonium chloride, sodium nitrate, potassium nitrate at a concentration of 0.25% (w/v) each. Each 250 mL Erlenmeyer flask contained 100 mL of the following media: 1.5% glucose, 0.1% KH2PO4, 0.1% K2HPO4, 0.15% MgSO4.7H2O and 0.25% of the respective nitrogen sources in individual flasks. Each flask was inoculated with 5% (v/v) of the seed culture and maintained at 150 rpm, 25 °C for 10 days. The optimized nitrogen source was further studied for its optimum concentration from a range of 0.05-0.3% (w/v).

Results

Table 1: Invitro studies of optimization of biomass productions probiotics with different parameters (IU)

Control	**pH(5-11)**				**Temperature (˚C)**				**Incubation period (days)**			
	5	7	9	11	20	25	30	35	2	4	6	8
0.00	1.67	1.99	2.76	3.62	2.71	3.81	3.92	4.12	3.11	5.02	7.16	9.22
Control	**Nitrogen source (IU)**				**Potassium (IU)**				**Phosphorus (IU)**			
	0.00	1.25	1.84	2.33	4.12	1.18	1.67	2.81	3.33	1.41	2.03	2.62
0.00	1.25	1.84	2.33	4.12	1.18	1.67	2.81	3.33	1.41	2.03	2.62	3.81

Discussion

Development of an efficient fermentation process for the production of secondary metabolites by *Streptomyces* species requires examination of a diverse array of species-specific features, including physical and chemical factors. Carbohydrates and nitrogen play key roles as structural and energy compounds in cell. Also several cultivation parameters like pH, incubation period and temperature play a major role in the production of bioactive metabolites (Usha Kiranmayi *et al*., 2016) [3]. Thus, to determine the optimal medium and culture conditions for antibiotic production by the four isolates, various carbon and nitrogen sources were tested. We used a complex medium rather than a defined for optimization studies because antibiotic producing organisms usually produce limited quantities of antibiotics in defined media and growth is also lower (Dekleva *et al*., 2017) [6]. According to Guimaraes, the pH of the culture medium is one of the most important environmental factors, because it exerts a marked effect on the activity of several enzymes that catalyze metabolic reactions, as well as exerting significant influence on complex physiological phenomena such as membrane permeability and cell morphology. Changes in the initial external pH affect many cellular processes such as regulation and biosynthesis of secondary metabolites. Hence, an attempt was made to determine the optimum external initial pH for each isolate (Datta, and Kothary, 1993) [7]. The incubation temperature also was found to have an effect on growth as well as bioactive metabolite production. 30 ˚C was observed to be the optimum temperature for the growth of all the four *Streptomyces* species in this study (Sole *et al*., 1997) [8]. Except isolate, for which the optimum temperature for bioactive metabolite was 35 ˚C, all the other isolates showed maximum bioactive metabolite production at 30 ˚C. Generally, it has been observed that *Streptomyces* show progressive increase of biomass during the first 4-7 days of incubation. Antibiotic production usually starts on the second or third day but maximum antibiotic activity is recorded on ninth or tenth day, that is, in the stationary phase (Gogoi *et al*., 2018) [9]. It has been reported that two phases are observed during the propagation of antibiotic producers. In this study, it was clear from the results that the growth of the isolates was greatly influenced by the nature and type of nitrogen source supplemented in the medium. In comparison with the inorganic nitrogen sources, organic nitrogen sources induced relatively higher biomass yield as well as bioactive metabolite production (Singh *et al*., 2009) [10].

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