Statistical Optimization of Medium Components for Mass Production of Plant Growth-Promoting Microbial Inoculant *Pseudomonas trivialis* BIHB 745 (MTCC5336)

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Abstract Optimizing nutritional requirements for mass production of microbial inoculants in shortened time has relevance for their economical field application. Therefore, the present study aimed at selecting suitable growth medium, optimizing its components, and up-scaling inoculum production for plant growth-promoting *Pseudomonas trivialis* BIHB 745. Of the different media tested, the culture exhibited maximal viable colony count in trypticase soya broth with 17.6 % increased biomass on optimizing levels of carbon source, nitrogen source, and NaCl using response surface methodology. A twofold higher biomass with 9 h shorter incubation period was obtained in optimized medium in a bioreactor in comparison to shake flasks.

Keywords Medium optimization · Plant growth-promoting bacteria · *Pseudomonas trivialis*

Rhizobacteria exert beneficial effects on plants by enhancing nutrient status of soil and availability of nutrients, secreting growth-promoting hormones, and suppressing soil-borne pathogens. Utilization of microbial-resources in sustainable agriculture requires an assessment

of culture growth conditions for their bulk production. Designing medium for optimizing growth conditions is first step in fermentation processes. Statistical tools like response surface methodology (RSM) have been successfully employed to optimize growth conditions for enhanced activity and biomass production [1–3]. The information on optimization of growth conditions for plant growth-promoting *Pseudomonas* is limited to indole-acetic acid production by *Pseudomonas* sp. and *P. aeruginosa*, phenazine-1-carboxylic acid production by *Pseudomonas* sp., and biomass, phenazine and siderophore production by *P. fluorescens* [3–6].

Pseudomonas trivialis BIHB 745 (MTCC 5336, NCBI GenBank accession DQ536516) isolated from the Indian trans-Himalayas reported for high phosphate solubilization, stress tolerance, organic acid production, and plant growth-promotion was employed for present studies [7].

Nutrient broth, trypticase soya broth (TSB), King's B medium, *Pseudomonas* medium A, *Pseudomonas* medium No. 2, TSB with 2 % methanol and glucose yeast extract were tested for selecting medium supporting highest growth of the microbial inoculant. TSB components were optimized for biomass production statistically by RSM using Box-Behnken design. Casein enzyme hydrolysate, soya peptone, NaCl and dextrose/molasses were the independent variables (Online Resources 1, 2). Statistical software package MINITAB Inc., version 11.12 (PA, USA) was used to generate response surface graphs. Fisher's *F* test was used for statistical analysis of data.

The effect of agitation on scale-up inoculum production was studied at 200, 250 and 300 rpm in 20 l total volume and 14 l working volume bioreactor (Applikon Dependable Instruments, AE Schiedam). 8 l optimized medium with dextrose as carbon source was inoculated with 0.5 % of preculture prepared by growing the microbial inoculant in

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Fig. 1 Contour response surface plot showing effect of trypticase soya broth ingredients on *Pseudomonas trivialis* BIHB 745 biomass production. **a** dextrose and NaCl, **b** NaCl and CEH, **c** molasses and

CEH, **d** molasses and NaCl. Values in the box indicate biomass (g/l). SP soya peptone, CEH casein enzyme hydrolysate

optimized TSB medium at 28 ± 0.1 °C for 24 h at 180 rpm. The temperature was set at 28 °C and controlled within 0.1 °C of set point. The pH was maintained at 7.0 by controlled addition of 1 N NaOH. Antifoam (A6758, Sigma) was added automatically when required. Air flow was kept constant at 1 vvm. Biomass was determined from a 5 ml sub-sample of the culture at beginning of experiment and every hour until 24 h of growth.

The microbial inoculant exhibited maximum growth and highest specific growth rate in TSB (Online Resources 3, 4). The results corroborated relationship between medium

composition and biomass production [2]. Significant effect of dextrose/molasses, casein enzyme hydrolysate, soya peptone and NaCl as medium constituents indicated that minor variation in their concentration could affect biomass production (Online Resources 5–8). The optimized medium containing 3.0 g dextrose, 3.56 g NaCl, 29.0 g casein enzyme hydrolysate, 5.8 g soya peptone and 2.5 g KH_2PO_4 per litre generated 17.6 % higher biomass over the original TSB (Fig. 1a, b). The biomass obtained in medium with dextrose was 4 % higher than in medium with molasses as carbon source (Fig. 1c, d). Earlier, 19 % increased biomass

production of probiotic *Pseudomonas* strain has been reported in medium optimized for mannitol, glycerol, urea and NaCl [2]. Several reports are available on optimization of growth conditions for desired activity of *Pseudomonas* [1–3, 5, 8], limited information is available on biomass optimization of plant growth-promoting *Pseudomonas* strains [4, 6].

The higher biomass production in comparatively shorter fermentation time makes large-scale inoculum production more economical. The highest biomass of 5.38 g/l was observed at 300 rpm, followed by 5.23 g/l at 250 rpm and 4.92 g/l at 200 rpm (Online Resource 9). A twofold higher biomass production in bioreactor with 21 h run period as compared to 30 h incubation in shake flasks could be due to proper mixing of nutrients, maintenance of pH and dissolved oxygen in the bioreactor. The results are in accordance with a recent report where 2- to 3.3-fold higher phenazine-1-carboxylic acid production was obtained by double mutant of *Pseudomonas* sp. over single mutants [5].

The statistical design made possible to attain 17.6 % and twofold higher biomass production in shake flasks and bioreactor in shorter time, respectively, which implied reduced cost of production of the microbial inoculant.

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