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CHARACTERIZATION OF MEDIA FOR IMPROVED GROWTH OF *PSEUDOMONAS*

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ABSTRACT

Pseudomonas culture was provided by Department of Biotechnology, Career College, Bhopal. Different medium like Nutrient Broth, Minimal and Luria Broth were used to characterize the growth of *Pseudomonas*. Growth was measured by turbidity measurements at 600nm after 48 hrs it was observed that maximum growth was appeared in Minimal Media then in Nutrient Media and then less growth was measured in Luria but after 120 hrs turbidity was increased in Minimal Media and Luria Media but decreased in Nutrient Media. After 48 hrs, the dry weight was calculated as 15.0, 9.1 and 7.2 mg/ml in Minimal Media, Luria Media and Nutrient Media respectively. Hence enhanced growth was observed in Minimal Media thus this media can be used for large scale production of *Pseudomonas*.

KEYWORDS: *Pseudomonas*, Minimal Media, Turbidity.

INTRODUCTION

Pseudomonas is a motile, nonsporulating, rod shape, gram negative obligate aerobic bacteria that inhabits many environments, including plants, soil, and water surfaces. *Pseudomonas* is considered to be the most potential group of plant growth promoting rhizobacteria. (Gardner et al., 1984; Moeinzadeh et al., 2010). Fluorescent Pseudomonads are effective biocontrol agents against plant diseases (Howell and Stipanovic 1980; Kloepper et al. 1980; Scher and Baker 1982; Weller and Cook 1983).

Fluorescent pseudomonads are ubiquitous bacteria that are common inhabitants of the rhizosphere, and are the most studied group within the genus *Pseudomonas*. They comprise of *P. aeruginosa*, the type species of the genus; *P. aureofaciens*, *P. chlororaphis*, *P.*

fluorescens (four biotypes), *P. putida* (two biotypes), and the plant pathogenic species *P. cichorii* and *P. syringae* (Dwivedi and Johri, 2003). Pseudomonads are ideal biocontrol antagonists because of their adaptive metabolism and ability to produce an array of inhibitory compounds. When introduced as seed or seedling inoculants, they can grow with the advancing root and successfully colonize and compete for nutrients, to the exclusion of the pathogen (Schroth and Hancock, 1982, Thomashow and Weller, 1990). Many potential biocontrol microbes are found on the plant roots at the site of pathogen infection. They inhibit the growth of pathogens by producing secondary metabolites such as iron-scavenging compounds, antibiotics and volatile substrates, which act in combination to inhibit pathogen establishment (Burr and Caesar, 1984, Davidson, 1988, Schippers 1988.

Plant growth-promoting ability of these bacteria is mainly because of the production of indole-3-acetic acid (IAA; Patten and Glick 2002), siderophores (Leong 1986; Neilands and Leong 1986; Schippers et al. 1987) and antibiotics (Colyer and Mount 1984; Gutterson et al. 1986). Production of antibiotics such as phenazine-1-carboxylic acid (PCA) (Gurusiddaiah et al. 1986; Mavrodi et al. 1998), pyocyanin (Watson et al. 1986), 2-acetamidophenol (Slininger et al. 2000), pyrrolnitrin (Arima et al. 1964), pyoluteorin (Howell and Stipanovic 1980), phenazine-1-carboxamide (PCN) (Chin-A-Woeng et al. 1998), 2,4-diacetylphloroglucinol (Shanahan et al. 1992), viscosinamide (Nielsen et al. 1999) and tensin (Nielsen et al. 2000) in different species of pseudomonads has been reported. By keeping above described views, the present study aimed at Characterization of Media for enhanced growth of *Pseudomonas.*

MATERIALS AND METHODS

Bacterial culture

Strain of *Pseudomonas* was provided by the Department of Biotechnology and Biochemistry, Career College, Bhopal. The bacterial strain was purified in Minimal medium and incubated at 28 ± 2^0 C for 48 hrs.

Inoculation of *Pseudomonas* in different broths

Loopfull culture of *Pseudomonas* obtained from the Minimal media plate was inoculated in each flask containing Nutrient Broth, Luria Broth and Minimal Broth and then incubated at 28 ± 2^0 C.

Measurement of growth of *Pseudomonas* by Spectrophotometer

Optical density at 600nm was measured in each flask containing *Pseudomonas* Nutrient Broth, *Pseudomonas* Luria Broth and *Pseudomonas* Minimal Broth keeping uninoculated respective broths as blank at different time interval ie. 24 hrs, 48 hrs, 72 hrs, 96 hrs and120 hrs.

Calculation of Dry Weight of *Pseudomonas*

Dry weight was calculated for each broths containing the cultures at different time interval. Firstly, 1 ml of cultures from each broths were transferred to centrifuge tubes of 1.5ml, then centrifuged at 10000 rpm for 10 mins. Supernatant was discarded and the tubes containing the pellet were kept for air drying for overnight then the weight of cells was measured.

RESULTS AND DISCUSSION

Measurement of optical density of *Pseudomonas* in different medium shown in Table 1 below:

Medium	O.D. (after 24hrs)	O.D. (after 48hrs	O.D. (after 72hrs)	O.D. (after 96hrs)	O.D. (after 120hrs)
Minimal	0.363	1.090	1.549	1.609	1.610
LB	0.182	0.428	1.080	1.424	1.501
Nutrient Broth	0.136	0.653	1.143	0.719	0.468

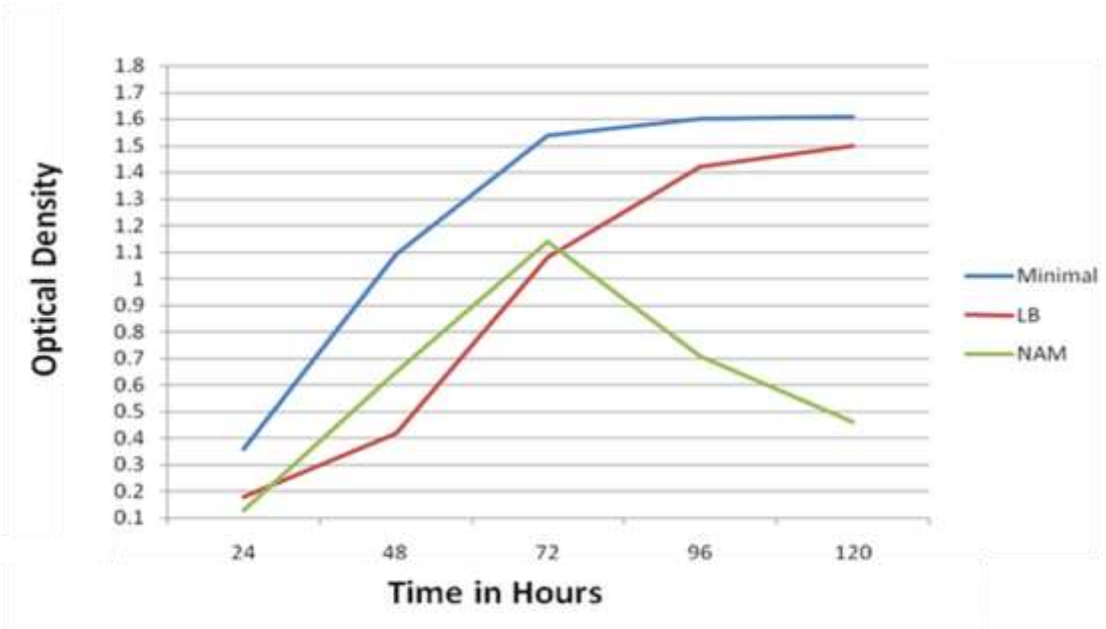

Fig 1: **Growth measurements (Optical Density) of *Pseudomonas* in different medium at diffetent Time interval.**

Dry weights of *Pseudomonas* in different medium shown in table 2.

Medium	Dry weight in mg/ml				
	24 hrs	48 hrs	72 hrs	96 hrs	120 hrs
Minimal	6.1	15.0	81.6	99.5	154.0
LB	5.4	9.1	17.4	79.5	99.8
Nutrient Broth	4.0	7.2	17.4	34.2	44.2

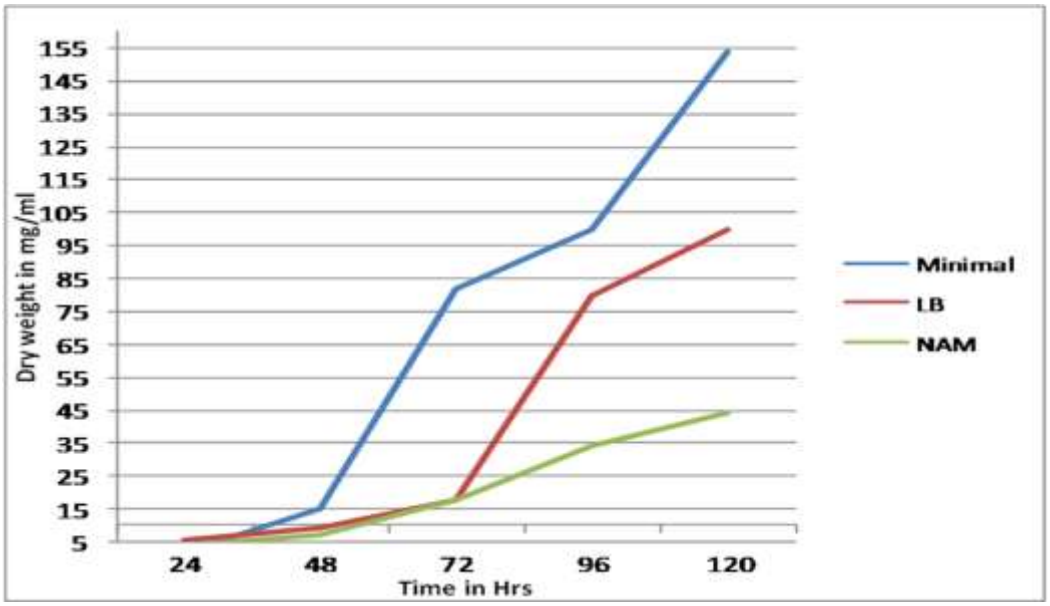

Fig 2: Dry weight of *Pseudomonas* in different medium at diffetent Time interval.

CONCLUSION

Characterization of the media is one of the key factors to maximize the yield of the product under study. When Optical Density of *Pseudomonas* was taken at 600nm the Media in which the production of *Pseudomonas* was maximum in Minimal broth. In Minimal Broth the optical density and dry weight were higher as compare to other broth it means that the Media component was accurate for production of *Pseudomonas*.

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