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Observations on the Growth of *Bacillus* (PSB) Under Modified Nitritional Conditions

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***Abstract*:** *Bacilluspolymyxa,* a Phosphate Solubilizing Bacteria (PSB)consideredto evaluate the influence of micro and macronutrients on its growth in controlled conditions. Influence of each factor in Pikovskaya'sgrowth media lead to prepare modified medium. The maximum growth of *Bacillus*was observed in modified Pikovskaya'smedium which contains Sucrose (10.0 gl^{-1}), $Ca_3(PO_4)_2$ (5.0 gl^{-1}), $(NH_4)_2$ SO_4 (0.7 gl^{-1}), NaCl (0.18 gl^{-1}), $MgSO_4$ $7H_2O$ (0.08 gl^{-1}), KCl (0.22 gl^{-1}) , Yeast extract (0.7 gl^{-1}), $MnSO_4$ (0.01 gl^{-1}), and $FeSO_4$ (0.012 gl^{-1}) at pH 7.1. The compound needed in higher doses are $(NH_4)_2$ SO_4, KCland Yeast extract. Whereas, NaCland $MgSO_4$ $7H_2O$ required in lower doses as compare to original Pikovskaya'smedia. Sucrose and $Ca_3(PO_4)_2$ are required in normal doses as prescribed in original media.The growth of *Bacillus* studied in the originally proposed and also in the modified medium with respect to its optical density.

Keywords: *Bacillus*, PSB, Pikovskaya's media, bacterial growth, micronutrients.

I. INTRODUCTION

Phosphorous is one of the important nutrients for the plants. Only 15 to 20% of applied phosphorous can recovered by crops and remaining gets fixed in the soil. Several reports have examined the ability of different bacterial species to solubilize insoluble inorganic phosphate compounds. A large number of bacteria including species of *Bacillus* have reported to enhance plant growth by their different plant growth promoting activities [1]. *Bacillus* is phosphate solubilizing, gram positive bacteria. They are aerobic and heterotrophic. Use of such natural products in crop cultivation helps in safeguarding the soil health and also the quality of produce [2].

Microorganisms are the most versatile and diversified in their nutritional requirements. The culture media consists of specific nutrient preparations, chemicals which supports the growth of microorganisms. Microbes can use the nutrients of culture media for cultivating them in vitro. PSBs can be cultured in different nutrient or growth media, viz., Pikovskaya's and Bunt and Roviraas modified by Louw and Webley[3]. By deleting or adding a constituent to defined media, we can assess necessity of that constituent for the growth of the microorganisms. However, for the routine laboratory cultivation and study of microorganism chemically defined media were used. After realizing this situation, microbiologists develop various techniques of defined culture media [4] and enrichment culture media [5].The maximum growth and other metabolic processesrequire optimum concentration of nutrients. Too low concentration may limit the growth and too high concentration may be inhibitory [6].

Microbiologists devised several media for the cultivation of microorganisms. Pikovskaya'smedia [7] is popular for the PSB. Reference [8] who designed and modified the media for phosphate solubilizing microorganisms based on the metabolic activity and phosphate dissolving capacity of bacteria isolated from wheat root soils. The strains of PSBs may respond and adapt differently over the time and may show maximum growth in improved concentration of constituents of nutrient media. Hence, it is aimed to study the influence of

different concentrations of elements of Pikovskaya'sculture media to achieve maximum possible bacterial population.

II. MATERIAL AND METHODS

The experiments were carried out during 2007-2008.The concentration (g/l) of the constituents of the original Pikovskaya's media is shown in the Table-I. The same constituents were used in modified media below and upper level of the original quantity with the difference of two units. It is shown as the experimental range in the Table-I. The study was made to observe the effect of different concentrations of nutrients with respect to growth and the duration at which the maximum growth is obtained from day one to twelve using optical density.Table-II.

The study of a particular compound was madeby omittingit from the originalmedium and rest of the compounds were taken as concentration as present in the same original media. The omitted compound was taken in the different concentrations to study the influence of such compounds on the growth of bacteria. The Pikovskaya'smedia was selected for the study, as it is commonly and commercially used.All chemicals used in the experiments were Sigma or BDH analar grade.

The media were prepared and taken into the 10 ml capacity test tubes, plugged and autoclaved at 15 atm. pressure for 30 minutes. The range of pH for *Bacillus* was studied from 6.6 to 7.4. The four days old inocula about 0.01 ml each of respective bacterial cultures were transfer aseptically into the test tubes containing respective medium in sterilized inoculation chamber. After the inoculation, the optical density of growth medium was measured at specific durations with UV-spectrophotometer and time and observations were noted.

The bioinoculantliquid culture of *Bacillus polymyxa*was confirmed from the Regional Centre of Organic Farming, Nagpur, Ministry of Agriculture, Govt. of India and Rajiv Gandhi Biotechnology Centre, RTM Nagpur University.

III. RESULTS AND DISCUSSION

Hydrogen ion concentration (pH): The growth and reproduction of the microorganism are influenced by the pH of the culture medium. Most bacteria show optimal growth in between pH 6.5 and 8.5. In the present investigations, *Bacillus*shows good growth at pH 7.1 which is just higher to that of Pikovskaya's basic medium. *Bacillus*is growing luxuriantly slightly in alkalinepH. Present results are in close agreement with [9].

TABLE-I: Composition of Pikovskaya's Media and Modified Pikovskaya's Medium for *Bacillus polymyxa*

Sr. No.	Nutrient	Pikovskaya's medium, g/l.	Pikovskaya's modified medium, g/l.	Experimental range (Min.to Max., g/l.)
1	Sucrose	10.0	10.0	02 to 20
2	$Ca_3(PO_4)_2$	5.0	5.0	1 to 19
3	$(NH_4)_2 SO_4$	0.5	0.7	0.1 to 1.9
4	NaCl	0.2	0.18	0.12 to 0.30
5	$MgSO_4 7H_2O$	0.1	0.08	0.02 to 0.20
6	KCl	0.2	0.22	0.12 to 0.30
7	Yeast extract	0.5	0.7	0.1 to 1.9
8	$MnSO_4$	Trace	0.01	0.006 to 0.08
9	$FeSO_4$	Trace	0.012	0.006 to 0.08
10	pH	7.0	7.1	6.6 to 7.4
11	Distilled Water	1000 ml	1000 ml	…

Magnesium sulphate [$MgSO_4$]: Magnesium is important in the activation of enzymes containing sulphydryl group (-SH) [10]. In present study 0.08 gl^{-1} magnesium sulphate in Pilovaskaya's medium needed for the growth of *Bacillus* and recorded optical density of 0.882. This concentration is lower than 0.1 gl^{-1} by Pikovskaya's basal medium. Reference [11] showed maximum growth of PSM at 0.05 gl^{-1} $MgSO_4$.

Sodium Chloride [NaCl]: *Bacillus* needs sodium chloride requirements in lesser amount. Present results showed 0.18 gl^{-1} of NaCl is sufficient for maximum growth and recorded 1.276 optical density. Similarly, number of workers described amount of requirement of NaCl in the medium. Reference [12] noted 0.1 gl^{-1}NaCl, and [13] 0.02 gl^{-1} for *Azospirilum*. Reference [14] showed *Bradyrhizobiumjaponicum*A1017 was very sensitive to salt and its growth was completely inhibited in a medium containing 0.15 M NaCl.

Ferrous Sulphate [$FeSO_4$]: Iron is required for nitrogenase and ferredoxin[15] for nitrogen fixation. The bacteria vary in their requirement and sensitivity for iron sulphate. In Pikovskaya's modified medium for *Bacillus* also required in trace amount 0.012 gl^{-1}. $FeSO_4$ was needed for maximum growth and shown 0.878 optical density. In *Azospirillum* 0.05 gl^{-1} $FeSO_4$ was sufficient for optimum growth of bacteria [13].

Calcium Phosphate [$Ca_3(PO_4)_2$]: Many microorganisms solubilize inorganic phosphates which are not available to plants. Microbial involvement in the solubilization of inorganic phosphate was first shown by [16], who observed the solubilization of tricalcium phosphate. The solubility of different phosphatic compounds in liquid medium studied by [17] tested all the microorganisms including *Psedomonas, Bacillus, Escherichia, Aspergillus* and *Penicillum* effectively solubilized tricalcium phosphate and hydroxiapatite. In the present study *Bacillus* required 5 gl^{-1} calcium phosphate similar to concentration as recorded by Pikovskaya's 5 gl^{-1} and recorded 1.142 optical density. Reference [18] found that 2 gl^{-1} calcium phosphate was necessary for maximum growth of *Rhizobium*. These values for *Bacillus* are in close agreement with the findings of [9].

TABLE-II: Growth of *Bacillus* in Pikovskaya's and Modified Pikovskaya's Medium

Day	**Optical Density of *Bacillus in* Pikovskaya's medium**	**Optical Density of *Bacillus* in Modified Pikovskaya's medium**
1	0.146	0.162
2	1.332	1.668
3	3.148	3.884
4	3.676	5.332
5	4.302	6.776
6	5.844	8.466
7	8.184	8.162
8	6.206	6.984
9	5.114	6.402
10	3.208	4.116
11	2.330	3.220
12	1.422	1.896

Ammonium Sulphate [$(NH_4)_2$ SO_4]:In Pikovskaya's basal medium *Bacillus* exhibit maximum growth at 0.5 gl^{-1} ammonium sulphate and in present experiment concentration 0.7 $gl^{-1}$was recorded, slightly higher for maximum growth of bacteria. Reference [19] reported the result of 0.66 gl^{-1} $(NH_4)_2$ SO_4 for growth of *Nitrosomonas*.

Yeast extract: Yeast extract consists of protein and other intercellular constituents. Yeast extract is an excellent stimulation for bacterial growth and is used frequently in culture media. It is rich source of B-Vitamins. In present study, *Bacillus* shown maximum growth at 0.7 gl^{-1} yeast and recorded 1.21 optical density. This recorded concentration of yeast is on high a side, which is 0.5 gl^{-1} in Pikovskaya's basal medium. In *Azospirillum*, the requirement of yeast quite less, 0.05 gl^{-1} [20].

Potassium Chloride [KCl]: Maximum growth was obtained at 0.22 gl^{-1}KCl for *Bacillus* in the present study against 0.20 gl^{-1} with that of Pikovskaya's basal medium. Reference [14] reported slightly higher concentration i.e. 0.3 M KCl for growth of *Bradyrhizobiumjaponicum* A 1017.

Manganese Sulphate [$MnSO_4$]: Manganese is a component of several host enzymes with an important role in Kreb's cycle but without specific role in the symbiosis. In this investigation $MnSO_4$ required 0.01 gl^{-1} for maximum growth of *Bacillus* in Pikovskaya's modified media and recorded 0.702 optical density. Similar to [21] reported the same requirements of Mn for *Azospirillium*.

Sucrose: Carbon source is the main source needed for the growth of the bacteria and it is important in elemental composition of microbial dry matter. Most strains of the different species use the substance as sole carbon and energy source as fructose, glucose and sucrose. In present study the growth was maximum in concentration at 10 gl^{-1} and recorded 0.876 optical density. The salts of organic acids such as succinate, lactate and pyruvate have been found satisfactory oxidisable carbon and energy sources in *Azospirillumlipoferum* and *A. brasilense*[12],[22].

Growth of bacteria: In Pikovskaya's medium the growth of bacterium was highest at 7^{th} day at optical density 8.184 while in modified medium it was at 6^{th} day at optical density 8.466. The cell density and efficiency of bacteria in modified media were better than the basal medium. *Bacillus* was grown in a defined medium with citrate as a sole carbon source showed maximum growth in the first phase of growth [23]. The growth of *PSB* studied in the originally proposed Pikovskaya's medium and also in the modified Pikovskaya's medium with respect to its optical density. It is observed that the compounds needed in higher doses areAmmonium sulphate, Potassium Chloride and Yeast extract. Whereas, Sodium Chloride and Magnesium suphate required in lower doses as compare to original Pikovskaya's media. Sucrose and Calcium Phosphate are required in normal doses as prescribed in original media. The results may be influence by accuracy of nutrient concentration and its purity. There may be a need to confirm the observations further with this.

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