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Research Article

Development of Liquid Formulation for Pink Pigmented Facultative Methylotrophs (PPFMs)

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ABSTRACT

The present investigation was carried out at Department of Plant Pathology and Agricultural Microbiology, PGI MPKV, Rahuri.The efficient PPFMs isolates MT_{10}, MT_{23} *and* MT_4 *which were superior in producing IAA, GA, nitrogen fixation and sidrophore production were selected for development of liquid formulation. These three isolates were collectively grown by using media with a different composition.M3 media found a higher growth of PPFMs as compared to other media. The M3 media was further standardized by using different concentration of cell protectants. The luxuriant cell count (cfu) of all Methylobacterium was observed on the LM5 medium and devised as consortium or liquid formulation. The shelf life studies of liquid consortium was done by taking periodical count at monthly interval up to the 12 months. The PPFMs population in liquid formulation maintain its standard up to 210 days (seven months) which clearly shows that the selected liquid formulation having a good potentiality as a biofertilizer.*

Key word: *PPFMs bacteria, Standardized Media, Cell protectants, Shelf life, Bio fertilizer.*

INTRODUCTION

The Pink-pigmented facultative methylotrophs (PPFMs) are ubiquitous inhabitants of *phyllosphere* and *rhizosphere* of plants. PPFMs produces plant growth promoting substance such as Indole acetic acid (IAA), Gibberlic acid (GA) etc. which are known to stimulate plant growth (Koenig et al., 2002); fix the atmospheric nitrogen (Sy et al., 2001); solubilise mineral phosphate (Jones et al., 2007); and chelation of inorganic compound such as iron. Due to these importance, the PPFMs have received a great deal of attention as bio inoculants or bio fertilizer for use in agriculture. In the present study, the attempt has been made to prepare a liquid formulation of three selected best isolates of PPFMs and also to study their shelf life so as to use as liquid bio fertilizer in agriculture.

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MATERIALS AND METHODS

1. PPFMs isolates:-The three selected best isolates MT_4, $MT_{10}$and MT_{23} having higher potential of producing IAA production (22.43µg/ml, 27.65 µg/ml and 25.43µg/ml GA production 70.54µg/ml 67.25 µg/ml and 64.58 µg/ml sidrophore production 0.57 µmoles, 0.62 µmoles and 0.52 µmoles and also having good ability of nitrogen fixation 1.17 mg/g of malate 1.03 mg/g of malate and 1.28 mg/g of malate respectively have been selected to prepare a liquid formulation.
2. Media with different chemical composition.
3. Selected media with different cell protectants.

RESULT AND DISCUSSION

Liquid bioinoculants are special liquid formulations containing not the desired microorganisams and their nutrients, but also, special cell protectant or substances that encourage the longer shelf life and tolerance to adverse conditions (Beaker et al., 1984). Also, a liquid inoculants formulation made from local low cost material may be useful to the small producer especially in overcoming some of the problems associated with processing of the carrier (Chandra et al., 1995).

Standardization of medium

The three selected PPFMs isolates (MT_4, MT_{10} and MT_{23}) were formulated for their mass production as a liquid consortium by comparing the respective selective media of these isolates and the appropriate medium was designed by preparing various combinations, considering the common ingredients, concentration of ingredients pH level etc. (Table 1).

The medium no. M3 was found to be the most suitable medium.

Table 1: Standardization of media for Pink Pigmented facultative Methylotrophs (PPFMs)

Sr No	Chemicals gm/lit	Composition of five test media				
		M1	M2	**M3**	M4	M5
1	NH_4Cl	0.1	0.2	**0.5**	0.3	0.2
2	K_2HPO_4	1.0	0.5	**0.7**	0.5	0.3
3	KH_2PO_4	0.1	0.2	**0.5**	0.4	0.3
4	$Mgso_4.7H_2O$	0.1	0.5	**1.0**	0.7	0.3
5	$Cacl_2$	0.2	0.1	**0.2**	0.2	0.1
6	Glucose	8.0	8.0	**10**	10	5.0
7	Methanol(ml)	5	5	**5**	5	5

2. Standardization of Liquid formulation.

The M3 medium was further formulated by using different concentrations of cell protectants like arabinose, trehalose, glycerol, PVP (polyvinylpyrolidone) and Fe-EDTA in different concentrations at pH 7.0 and were devised as liquid medium LM1, LM2, LM3, LM4 and LM5 (Table 2)..All the media were tested and compared for growth, by transferring 1% inoculum .The flasks were kept in a temperature controlled shaker rotary shaker at 150 rpm at 30^0c for five days. After incubation, the growth was observed on sterilized Ammonium mineral salt agar plates. The plates were incubated at 30ºC for 72hrs and observed for growth by comparing *cfu* count on each devised medium, where the maximum *cfu* of all *Methylobacterium* observed, was selected as standard appropriate *Methylobacterium* consortium medium. The luxauriant cell count (*cfu*) of all *Methylobacterium* was observed on the LM5 medium and devised as consortium.

Similarly Deaker et al. (2004) reported the accumulation of the desiccant protectant trehalose in strains of *Rhizobia*, might result in better survival under desiccation stress. Similarly, the accumulation of

exopolysaccharide (EPS) might act as a barrier reducing excessive water loss. Polymeric adhesives such as gum arabic, methyl cellulose and polyvinyl pyrollidone (PVP) have improved survival.

Chandra et al. (2012) tested several compounds *viz.* polyvinylpyrrolidone, Fe EDTA, glycerol, trehalose, glucose, mannitol etc. for their ability to promote survival of bacteria after inoculation.

Similar results were also reported by many workers viz., the liquid *Azospirillum* formulated by amendments of trehalose (10 mM), PVP (2 %) and glycerol (10 mM) in N2 free malic acid broth and also standardized doses of liquid formulation (10 ml/kg seed, 150 ml/ha seedlings and 300 ml/ha) for seed treatment, seedling root dipping and soil application respectively (Vendan & Thangaraju, 2006).

Table 2: Standardization of Liquid formulation

Sr no	Ingradients	LM1	LM2	LM3	LM4	LM5
1	Standard medium	M3	M3	M3	M3	**M3**
2	Fe.EDTA (g)	0.25	0.75	1.0	0.5	**1.0**
3	Arabinose (g)	0.25	0.5	0.5	0.25	**0.75**
4	Glycerol(ml)	10.0	6.0	4.0	8.0	**4.0**
5	PVP(g)	24.0	18.0	20.0	22.0	**20.0**
6	Trehalose	0.25	0.5	0.5	0.25	**0.75**
7	Distilled water(lit)	1L	1L	1L	1L	**1L**
8	PH	7.0	7.0	7.0	7.0	**7.0**

3. Shelf life of consortium

The shelf life studies of liquid consortium was done by taking periodical count at monthly interval and the optimum count (*cfu*) were observed up to the 12 months(Table 3). The *cfu* count of *Methylobacterium* after one month of inoculation was in the range of 10.45 x 10^9cfu/ml of formulation. The viable count of three *Methylobacterium* isolates decreases but maintain its population standard up to the 210 days and then stop its growth suddenly from 240 days onwards. This shows that the population maintains its standard up to 210 days. So the test medium LM5 was selected as the best liquid consortium.

The present observations correlates the findings of Khavazi et al. (2007) who maintained 10^6 - 10^7 and 10^9 Rhizobia per gram till 7 and 12 months in coal based carrier.Aseri et al 2008 reported that Liquid formulation supporting growth of three biofertilizer organisms viz., *Pseudomnas Bacillus* and *Thiobacillus* using different concentrations of cell protectant like arabinose, trehalose, glycerol, polyvinylpyrolidone (PVP) were formulated with liquid medium maintaining good population even after 210 days.

Table 3: Shelf life studies/longvity of *Methylobacterium* isolates in liquid formulation

Sr No	Number of days	Liquid Formulation *cfu*/ml of formulation
1	30	10.45 x 10^9
2	60	10.03 x 10^8
3	90	9.72 x 10^8
4	120	7.12 x 10^8
5	150	5.08 x 10^8
6	180	3.10x 10^8
7	210	1.06 x 10^8
8	240	--
9	270	--
10	300	--
11	330	--
12	360	--

CONCLUSION

From the above data it is confirmed that the liquid formulation is prepared by using a three best suitable isolates of PPFMs and their shelf life is maintain up to 210 days (seven months) which is prerequisite period for any bio fertilizer.

REFERENCES

Aseri, G. K., Jain, N., Panwar, J., Rao, A. V., & Meghwal, P. R. (2008). Biofertilzers improve plant growth, fruit yield, nutrition, metabolism and rhizosphere enzyme activities of pomogranet (Punica ganatum L.) in Indian Thar Desert. *Scientia Horticulture*. *117*(2), 130-135.

Beaker, M. E., Blumbergs, J. E., Ventina, E. J., & Rapoport, A. I. (1984). Characteristics of Cellular Membranes at Rehydration of Dehydrated Yeast *Sacchromyces cerevisiae*. *Appl. Microbiol*., *19*, 347-352.

Chandra, K. (1995). Lime pellating an useful approach for effective Rhizobium inoculatin programme in acid soil of Manipur. *J. North Eastern Council*. *15*, 13-15.

Chandra, K., Greep, S., Ravindranath, P., & Srivathsa, R. S. H. (2012). Liquid Biofertlizers: Minisrty of Agriculture, Department of Agriculture and Co-operation, Governament of India.

Deaker, R., Roughley, R. J., & Kennedy, I. R. (2004). Legume seed inoculation technology-a review. *Soil Biol Bioch 36*, 1275-1288.

Jones nirmalnath, P., Krishnaraj, P. U., Kulkarni, J. H., Pranav, C., Alagwadi, A. R., & Vasudev, A. R. (2007). Pink Pigmented Facultative Methylotrophs Solubilize Mineral Phosphates. Paper presented at 48th Annual Conf., AMI, held at IIT, Chennai, between 18-21.

Koenig, R. L., Morris, R. O., & Polacco, J. C. (2002). tRNA is the source of low level trans-zeatin production in *Methylobacterium* spp. *J. Bacteriol., 184*, 1832-1842.

Khavazi, K., Rejali, F., Seguin, P., & Miransari, M. (2007). Effect of carrier, sterilization method and incubation on survival of *Bradyrhizobium japonicum* in soybean (*Glycine max* L.) inoculants", *Enzyme and Microbial Technology*. *41*, 780-784.

Sy, A., Giraud, E., Jourand, P., Garcia, N., Willems, A., de Lajudie, P., Prin, Y., Gillis, M., Boivin-Masson, C., & Dreyfus, B. (2001). Methylotrophic *Methylobacterium* bacteria nodulate and fix nitrogen in symbiosis with legumes. *J. Bacteriol*., *183*, 214–220.

Vendan, R. T., & Thangaraju, M. (2006). Development and standardization of liquid formulation for *Azospirillum* bioinoculant. *Ind. J. Microbiology., 46*(4), 135-138.