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Original Article

The Efficacy of Agrowaste on Cultivation of *Pseudomonas fluorescens* - A Potential Biocontrol Agent

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Abstract

Agricultural residues rich in carbohydrates can be utilized in fermentation process to produce microbial protein which in turn can be used to determine the factors influencing cell biomass production *Pseudomonas fluorescens* was cultivated using banana peel out, watermelon skin, and Cane molasses showed that the strain was capable of meeting its components required for growth. The organism was capable of growth at 37^{o}C, when supplemented with agricultural wastes in different concentration mixed with agar. The number of colony forming unit were more when compared with nutrient agar.
Key words: *Pseudomonas fluorescens*, Banana peel out, Water melon, Cane molasses.

Introduction

Increasing world population and the resultant food crises has shifted emphasis to the availability of 'waste' products of agriculture that could be utilized for all alleviating food shortage (Bhattacharjee, 1970). In many of the developing countries where major nutritional problems exist, excess of materials rich in carbohydrates are produced. These materials can be utilized in fermentation processes to produce microbial protein which in turn can be used to upgrade both human food and animal feeds. Traditional protein sources are relatively more expensive and, the absence of well-developed technology facilities have contributed to losses incurred through spoilage of even the limited available sources. Consequently, there is need to explore alternative ways of meeting the protein demands. Compared to the developed countries (mainly the U.S.A, U.K and Japan) the less developed countries appear to have more abundant supply of agro-waste substrate raw materials that could be converted to additional protein sources (Moo-Young,1977).

Various factors are known to influence fermentation processes. These include carbon and energy source requirements, oxygen demand and supply, temperature, pH, Nitrogen, Phosphorus and potassium (Pirt, 1975). In this study some factors affecting the yield of cell biomass production from agro-wastes by *P.fluorescens* are ascertained.

Materials and Method

Isolation and identification of strain

Rhizosphere soil samples of Potato, Tomato and Tomato root soil were collected from Palladam, Coimbatore, South India. Serially diluted with sterile distilled water upto 10^{-7}, where the dilution was started from 10^{-2} (1g of sample in 100ml-distilled water). From the above dilutions 10^{-2}, 10^{-3} and 10^{-4} were adopted for pour plate technique and the plates were incubated at 37˚C for 24hrs. The colonies were confirmed by biochemical method (Bergey's,1994).

Preparation of Substrate & Inoculation

The agro-waste (Banana waste, Water melon, and Cane molasses) were collected, 10gm of each sample was weighed and the extract was prepared with 100ml of sterile distilled water by crushing in a mortar and pestle. The extract was filtered first with what man No.1 filter paper. The basal media for the growth studies of the test strain were prepared using agro-waste. A range of waste concentrations of 5-25% were prepared by suspending variable amounts of the wastes in 100ml of de-ionized water and pH adjusted and required amount of Agar added as described by Cruickshank *et al*., 1975). The media were autoclaved and poured in petridish. After solidification 1ml of *P.fluorescens* suspension (10^{-5}, 10^{-6} & 10^{-7}) were inoculated separately and incubated at 37 ºC for 24 hours.

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Results and Discussion

Isolation and Identification

From the different groundnut rhizosphere soil, *Pseudomonas fluorescens* was isolated. Serial dilution of rhizosphere soil was done and plated in nutrient agar. The suspected colonies from the nutrient agar plates were streaked on King's B medium for further confirmation. On King's B medium, the colonies appeared as bluish green color which indicates *Pseudomonas fluorescens*. Organism of Gram-negative rods and motile. Based on the results of conventional biochemical tests the cultures were confirmed as *P.fluorescens* where they positive to citrate, oxidase and negative to Indole, MR and VP (Bergey's Manual of Determinative Bacteriology, 1994).

Substrate concentrations

The Colony forming Unit of selected *P.fluorescens* showed variations in different concentration of modified medium. The total viable count, was highest for the cane molasses at 25%. On the other hand, the growth levels were approximately equal at 25% water melon waste concentration and least at banana peel out. The results also showed that with increased concentration of substrate, Colony Forming Unit were also increased. It is evident that an increase in viscosity of the medium was noticed as substrate concentration was progressively increased. The substrate concentration and therefore medium viscosity would influence the growth of the test strain. Solomons (1983) has also reported that substrate concentration affects the yield of *Saccharomyces cerevisiae* when grown on an assimilable carbohydrate such as glucose or sucrose.

Physical and chemical parameters of Banana peel out, Water melon juice and cane molasses were checked. The cane molasses, Water melon and Banana peel out (Cruickshank *et al*., 1975) were used as Carbon sources. The Nitrogen analysis (Charles *et al*., 1982) showed only and slight change in Water melon and Banana peel out sources, when compared to cane molasses, and potassium (Phillips *et al*., 1960) content is very high in cane molasses which may be responsible for enhanced growth of *P.fluorescens*.

The protein and carbohydrate contents (Sadasivam and Manikam,1997) are high in Banana peel out. In water melon juice, dissolved oxygen is higher than the cane molasses. Highest number of Colony forming Unit was found in 25% cane molasses followed by water melon and Banana peel out respectively (Table 1, 2&3).

Table -1: Components present in Agro-waste

S.No	Components	Cane Molasses	Banana Peel Out	Water Melon Juice
1	Protein	1.35mg/ml/min	1.96mg/ml/min	0.47mg/ml/min
2	Carbohydrate	0.8mg/ml/min	1.17mg/ml/min	1.23mg/ml/min
3	Nitrogen	13.69mg/ml	8.1mg/ml	7.1mg/ml
4	Potassium	15mg/ml	12mg/ml	6mg/ml
5	Phosphorous	19.2mg/ml	10.5mg/ml	7.5mg/ml
6	Dissolved oxygen	80mg/litre	65mg/litre	95mg/litre
7	Dissolved solids	0.000000012mg/litre	0.00000010mg/litre	0.0000000012mg/litre

Table -2: Number of colony forming unit in different concentration of Cane molasses + Agar medium

S.No	Dilution	Colony forming Unit in Nutrient Agar	Percentage of Cane molasses & CFU of *P.fluorescens*				
			5%	10%	15%	20%	25%
1	10^{-5}	299	76	110	126	169	TNTC
2	10^{-6}	183	69	82	112	143	194
3	10^{-7}	175	64	71	94	128	162

Growth determination of microbial load in substrate at different concentrations

Standard plate count method

Common medium is used to check the Colony forming Unit of microorganisms. The number of colonies formed in Nutrient agar is 299,183 and 175 at different dilution 10^{-5}, 10^{-6} and 10^{-7} respectively. Initially, the substrate Cane molasses taken in different concentrations (5% 10%, 15%, 20% and 25%) with the inoculation of *Pseudomonas fluorescens* incubated at 37ºC for 24 hours and

colony forming unit were counted. Colony forming Unit in 5% Cane molasses modified Agar medium showed less number of colonies such as 76, 69 &64 in the bacterial dilution of 10^{-5}, 10^{-6} and 10^{-7} respectively. The colonies were too numerous to count were seen in 25% concentration. The differences in colony forming unit in different concentrations shown in Table -2. The substrate Banana peel out taken in different concentrations (5% 10%, 15%, 20% and 25%) with the inoculation of *Pseudomonas fluorescens* in each concentration and incubated at 37ºC for 24 hrs. The colony forming units were varied in different concentrations. In banana peel out modified agar, 5% concentration showed less number of colonies in bacterial dilutions 10^{-5}, 10^{-6} and 10^{-7} such as 45, 42 and 38 respectively. As concentration of banana peel out increased, the colony forming unit also increased. The results were interpreted in Table -3.

Table -3: Number of colony forming unit in different concentration of banana peel out + Agar medium

S.No	Dilution	Colony forming Unit in Nutrient Agar	Percentage of banana peel out CFU of *P. fluorescens*				
			5%	10%	15%	20%	25%
1	10^{-5}	299	45	72	78	120	231
2	10^{-6}	183	42	64	72	112	187
3	10^{-7}	175	38	50	54	98	146

The substrate Water melon taken in different concentrations (5% 10%, 15%, 20% and 25%) with the inoculation of *Pseudomonas fluorescens* in each concentration incubated at 37ºC for 24 hours. The colony forming units were varied in different concentrations. In water melon modified Agar medium, 5% concentration showed less number of colonies in bacterial dilutions (10^{-5}, 10^{-6} and 10^{-7}) such as 42, 38 and 32.As concentration of water melon juice increased, the colony forming unit also increased. The results were interpreted in Table 4.

Table- 4: Number of CFU in different concentration of water melon juice + Agar medium

S.No	Dilution	Colony forming Unit in Nutrient Agar	% of water melon juice CFU of *P.fluorescens*				
			5%	10%	15%	20%	25%
	10^{-5}	299	42	71	78	168	TNTC
	10^{-6}	183	38	56	73	142	267
	10^{-7}	175	32	41	68	130	196

Conclusion

The agro waste generated can be properly utilized for the cultivation and mass multiplication of bacteria. The nutritive components present in Agro waste helps to grow the bacteria and reduce the cost of cultivation. Agro waste are also easily degradable and does not cause much pollution to environment.

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