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Advances in Applied Science Research, 2011, 2 (2): 156-160

Pelagia Research Library

ISSN: 0976-8610
CODEN (USA): AASRFC

Production of *Pseudomonas fluorescence* from agricultural wastes and its application in the preservation of selected vegetables

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ABSTRACT

The main aim of the study is about to increase the production of Pseudomonas fluorescence from agricultural waste and its application in preservation of green leafy vegetables. The seven agricultural waste combinations were analyzed, out of which, combination containing rice straw, rice husk, wheat husk, cow dung, coconut water was found to be suitable for the optimum production of Pseudomonas fluorescence. The Pseudomonas fluorescence obtained from above combinations was used for the preservation of some of the green vegetables. The nutritive values for the above green vegetables were determined using one category with Pseudomonas fluorescence preservation and another category without preservation of the same. It was found out that the nutritive value was almost maintained.

Keywords: *Pseudomonas fluorescence*, Green vegetables, Agricultural waste.

INTRODUCTION

The genus pseudomonas is one of the most diverse Gram negative bacterial genera, isolated from sources ranging from plants to soils and water. This genus is straight or slightly curved rods, motile by means of polar flagella. *Pseudomonas* is characterized by their ability to grow in simple media at the expense of a great variety of simple organic compounds, without needing organic growth factors [1, 2]. The genus *Pseudomonas* contains more than 140 species, most of which are saprophytic. More than 25 species are associated with human. Most of them are known to cause disease in human are associated with opportunistic infection, these include *P. aeruginosa, P.fluorescens, P.putida, P.cepacia, P.stutzeri* and *P.putrefaciens* etc. [3]. Certain

strains of the genus *Pseudomonas* have the ability to suppress a range of plant disease caused by soil born plant pathogenic fungi.

The antibiotics 2, 4- diacetylphloroglucinol, Pyoluteorin and pyrrolnitrin etc. are produced by various *Pseudomonas* strains [4]. Leaves either them or along with tender stem constitute the leafy vegetable. Despite of their nutritive and medical value, they are the less exploited class of vegetables. Storage of green leafy vegetables is the major obstacle in the popularization of these important groups of vegetables. The moisture retaining capacity of green leafy vegetable is very less making it difficult to transport from place to place. By the time they have reached to the consumer, it might be unfit for consumption. The present study was undertaken to exploit the bio-control efficiency of *Pseudomonas fluorescence* in the preservation of green leafy vegetables and analysis of their nutritional quality.

MATERIALS AND METHODS

Organism

The test culture of *Pseudomonas fluorescence* was collected from the Department of Microbiology, Kerala Agricultural University, Vellayani, Trivandrum, India. The *Pseudomonas fluorescence* culture was grown on Kings B-broth for 24-48 h, which was used as inoculum. The cell count was determined (2×10^8 cfu/ml) using serial dilution and plate count method.

Vegetables and agricultural wastes

All the agricultural wastes were collected from local area and the vegetables were bought from Tamilnadu Agricultural University Garden, Coimbatore, Tamilnadu, India and from local market.

Agricultural waste combinations

The seven different combinations of agricultural waste were used to detect the increased production of *Pseudomonas fluorescence* as follows: 1. Sugarcane waste + Tapioca waste + Rice husk + Wheat husk, 2. Carrot waste + Tomato waste + Pineapple waste + Yeast, 3. Coconut water, 4. Rice straw + Rice husk + Wheat husk + Cow dung + Coconut water, 5. Cow dung + Wheat husk + Coffee husk, 6. Rice straw + Tapioca waste + Cow dung + Maida, 7. Saw dust + Rice husk + Coconut water.

Production of *Pseudomonas fluorescence* from above combinations

All the combinations were first boiled in 1 L of water for 30-45 minutes and cooled. The pH was adjusted to appropriate range (pH 7.0±2.0). 250 ml of the extract was alone collected from each combination and placed separately in series of conical flasks. Then the media was autoclaved at 121^oC at 15 lbs for 20 minutes. The duplications were also prepared. 5 ml of inoculum (*Pseudomonas fluorescence*) was inoculated into the cooled extract under aseptic condition and incubated at 37^oC±2 for 48-72 h. After the incubation period the cell count (*Pseudomonas fluorescence*) was determined by serial dilution and plate count method [5] using Kings B media.

Biochemical estimations

Ascorbic acid was estimated according to [6]. Total carbohydrate and protein was estimated by [7] and [8]. Calcium and iron was estimated by [9], [10] modified by [11].

Determination of moisture content [12]

For determination of moisture content, the sample materials were taken in a flat-bottom dish and kept overnight in a hot air oven at 100 to 110°C and weighed. The loss in weight was regarded as a measure of moisture content. It was calculated as follows:

$$\text{Moisture content} = \frac{\text{Initial weight} - \text{Final weight}}{\text{Weight of the sample}} \times 100$$

RESULTS AND DISCUSSION

It was found that increased growth of *Pseudomonas fluorescence* was found at combination IV (Rice straw + Rice husk + Wheat husk + Cow dung + Coconut water). The highest cell count was detected rather than all other combinations experimented. This combination was taken for further studies. The rich combination of carbohydrate, sugar in rice and other micronutrients like nitrogen, phosphorous in cow dung gave a positive influence for the luxurious growth of *Pseudomonas fluorescence* in the medium. The nutrient combination has also favored a high rate of green pigment production in the above medium. *Pseudomonas fluorescence* growth in combination VI (Rice straw + Tapioca waste + Cow dung + Maida) was also found to be good but the pigmentation production was comparatively lesser than growth combination IV (Table 1). Hence, the IV combination is best suitable medium for increased production for *Pseudomonas fluorescence*. The other combinations found to be not suitable medium for the growth of *Pseudomonas fluorescence.* This study proved that the nature and relative concentration of carbon, nitrogen and other nutrient sources in culture media are important for the growth and production of microbial growth.

Table 1: Production of *Pseudomonas fluorescence* using different agricultural combinations

Combinations	Cell count cfu/ml*				
	10^5	10^6	10^7	10^8	10^9
1	131	48	18	Nil	Nil
2	172	72	19	Nil	Nil
3	638	312	66	Nil	Nil
4	1022	890	321	182	32
5	106	35	14	Nil	Nil
6	212	110	40	3	Nil
7	19	3	Nil	Nil	Nil
8	Nil	Nil	Nil	Nil	Nil

*cfu – colony forming units, *Values are mean of replicates*

The table 2 shows the bio-control property of *Pseudomonas fluorescence* is effective for the preservation of vegetables, which on other hand perished fast if unpreserved. From the study it

was found that storage period of cabbage could be extended to 30 days with the control exhibiting only 8 days preservation at room temperature. The cauliflower samples treated with *Pseudomonas fluorescence* exhibited 24 days and the control spoiled on 7th day. Curry leaves treated with *Pseudomonas fluorescence* was fresh for use for 9 days but control for only 2 days. Stored amaranthus varieties exhibited 6 days preservation of treatment with *Pseudomonas fluorescence*.

Table 2: Preservation period of stored and control samples

S.No	Green leafy vegetables	Number of preserved days	
		Control*	Sample*
1	Cabbage	8	30
2	Cauliflower	7	24
3	Curry leaf	3	9
4	Amaranthus (red)	2	6
5	Amaranthus (green)	2	5

**Values are mean of replicates*

The data presented in the table 3 shows that there was only a slight difference in nutrient value in stored and fresh samples for all the vegetables tested. The bio-control efficiency of *Pseudomonas fluorescence* is mainly due to the production of several antibiotics and volatile compounds like 2, 4- diacetylphloroglucinol, Pyoluteorin and pyrrolnitrin etc. having broad spectrum of activity. Several hormones like IAA, Gibberlic acid etc. produced by *Pseudomonas fluorescence* contributes to its preservative properties.

Table 3: Nutritional study of control and preserved vegetables using *Pseudomonas fluorescence*

S. No	Estimations	Cabbage		Cauliflower		Curry leaf		Amaranthus (red)		Amaranthus (green)	
		C*	P*	C*	P*	C*	P*	C*	P*	C*	P*
1	Ascorbic acid (mg)	40.6	38.6	46.4	43.9	18	16	33	30	36	34
2	Calcium (mg)	20	17	22	20	102	90	800	785	840	825
3	Iron (mg)	0.5	0.2	0.6	0.3	0.9	0.6	22.9	22.2	25	24.3
5	Protein (g)	1.6	1.0	2	1.4	0.5	0.3	3	2.5	4	3.6
8	Total carbohydrate (g)	4	3.3	5	4.4	2	1.6	7	6.4	7.5	7.0
9	Moisture content (in %)	74	71	73	70	52	50	72	70	64	62

*C – Control sample, P – Preserved sample. *Values are mean of replicates*

CONCLUSION

It is concluded that the economically cheap medium could be defined for the production of *Pseudomonas fluorescence*, and making it available at a low cost and thus renewing the wastes, otherwise it will be a burden for the environment. Preservation studies of green leafy vegetables shows the bio-control efficiency of *Pseudomonas fluorescence*, which could be exploited for their preservation. Preservation of green leafy vegetables would be beneficial in the popularization of its consumption among consumers.

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