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Jinnamu Aresh
Research Scholar, Department of Pomology and Post Harvest Technology, Faculty of Horticulture, Uttar Banga Krishi Viswavidyalaya, Pundibari, West Bengal, India

Prodyut Kumar Paul
Professor, Department of Pomology and Post Harvest Technology, Faculty of Horticulture, Uttar Banga Krishi Viswavidyalaya, Pundibari, West Bengal, India

Mutum Preema Devi
Assistant Professor, Department of Pomology and Post Harvest Technology, Faculty of Horticulture, Uttar Banga Krishi Viswavidyalaya, Pundibari, West Bengal, India

Ashok Chaudhury
Professor, Department of Soil Science and Agricultural Chemistry, Faculty of Agriculture, Uttar Banga Krishi Viswavidyalaya, Pundibari, West Bengal, India

Surajit Khalko
Assistant Professor, Department of Plant Pathology, Faculty of Agriculture, Uttar Banga Krishi Viswavidyalaya, Pundibari, West Bengal, India

Corresponding Author:
Jinnamu Aresh
Research Scholar, Department of Pomology and Post Harvest Technology, Faculty of Horticulture, Uttar Banga Krishi Viswavidyalaya, Pundibari, West Bengal, India

Microbial isolate used as delay ripening agent as postharvest management in banana

Jinnamu Aresh, Prodyut Kumar Paul, Mutum Preema Devi, Ashok Chaudhury and Surajit Khalko

Abstract
Recent study investigated the biological depletion of ethylene for delay ripening by using microbial isolates *i.e., Pseudomonas sp*. The experiment was carried out in a factorial completely randomization design with nine treatments and four replications at the Laboratory of the Department of Pomology and Postharvest Technology, UBKV, Pundibari. The matured unripe banana was treated with different combinations of *Pseudomonas sp.* isolated from vermicompost (S_1), orchard (S_2) and forest soil (S_3) and carrier media *i.e.*, Peat soil + cotton (T_1), cotton (T_2) and peat soil (T_3) are T_1S_1, T_2S_1, T_3S_1, T_1S_2, T_2S_2, T_3S_2, T_1S_3, T_2S_3 and T_3S_3. Fruits treated with microbial isolates of forest soil having carrier media peat soil (T_3S_3) showed less ethylene evolution rate as compared to orchard and vermicompost, followed by T_2S_3 in the storage condition. The highest ethylene evolution rate was perceived in control followed by T1S1. Microbial isolates from forest soil and having carrier media as a peat soil showed the best result among the different treatments combinations in removing ethylene from the surroundings as evidenced by a slow rate of increase in TSS, least ethylene concentration or higher ethylene depletion, less yellowing of the peel and higher phenol content in the fruit. Therefore, using *Pseudomonas sp*. in storage facilities can reduce the ethylene concentration and can be used as a postharvest treatment tool for delayed ripening.

Keywords: Microbial isolates, ethylene, depletion, carrier media

1. Introduction
The banana (*Musa acuminata*), one of the most popular fruits consumed worldwide, is a rich source of vitamins and bioactive substances (Singh *et al*., 2016) [17]. Banana is the fourth most cultivated crop in the world and it covers an area of approximately 10 million hectares in more than 130 countries of the tropical and subtropical regions (Netshiheni *et al.,* 2019) [11]. It is one of the largest producing fruit in the world just next to citrus, with a contribution of about 16 % of the world's fruit production. India is the largest producer of bananas, contributing to 27 % of the world's banana production (Mohapatra *et al.,* 2010) [10]. The banana is a climacteric fruit harvested at mature and green stage, kept in climate-controlled spaces and given an ethylene treatment before being marketed (Ahmed and Palta, 2016) [3]. Ripening is an inevitable process that brings a series of biochemical and physical changes which are responsible for the change of colour, pigment formation, starch breakdown, textural changes, volatile and aroma development and finally abscission of fruits (Jiang *et al*., 1999) [7]. According to Val'erie Passo Tsamo *et al*., (2014) [19], ethanol treatment caused a series of physiological and biochemical alterations in banana fruit that resulted in the production of distinctive soft flesh tissue that affects consumer appeal. These changes are responsible for a short shelf life of 3–7 days at ambient temperatures.
Ethylene production frequently spikes during the postharvest ripening of banana fruit after the increase in respiration rate (Gamrasni *et al*., 2020) [6]. In climacteric fruits, the ripening beginning is significantly regulated by ethylene. Exogenous 1-methyl cyclopropane (1-MCP) therapy as a competitive ethylene receptor inhibitor greatly retarded the ripening of various climacteric fruits, whereas exogenous ethylene treatment significantly accelerated the ripening of various climacteric fruits (Gamrasni *et al*., 2020; Pongprasert, Srilaong, & Sugaya, 2020; Zhang *et al*., 2020) [6, 14, 20]. The present study focuses on the isolated microbial isolate i.e., Pseudomonas *sp*. treated with banana fruits for delayed ripening in the storage conditions.

2. Materials and Methods
2.1 Raw Material
Banana (*Musa acuminata*, cv Amritsagar) fingers of the same shape and weight from the

middle hands on the bunches and with no visual defects were collected at the green mature stage (75 % to 80 % maturity) from the University farm and immediately transported to Pomology and Postharvest laboratory at Uttar Banga Krishi Viswavidyalaya, Pundibari. The fruit fingers with uniform shape, colour, and size were selected for experiments. The source of *Pseudomonas sp.* was isolated from vermicompost (S_1), orchard soil (S_2) and forest soil (S_3). The carrier media for the *Pseudomonas sp* was peat soil and cotton (1:1) (T_1), cotton (100 %) (T_2), and peat soil (100 %) (T_3) along with desiccant. All the treatments and determinations were conducted in four biological replicates.

2.2 Time to yellowing

The fruits for different treatments were placed in the glass desiccator and observed periodically for colour changes in the peel.

2.3 Total Soluble Solids (°Brix)

The total soluble solids (TSS) of the banana were determined by using a hand refractometer. The hand refractometer was washed with double distilled water and cleaned with tissue paper. Before the measurement of the sample, the instrument was calibrated and then used for sample measurement at ambient temperature. Juice from banana fruits was extracted by staining of pulp with a muslin cloth or cotton. Then one or two drops of juice were poured on the prism of the refractometer in the centre. Adjustment of total soluble solids was carried out at 20 ºC minimum to 30 ºC maximum. The instrument was washed with distilled water after recording each sample. The corresponding reading was shown as Brix (%).

2.4 Ethylene depletion

For determining the efficacy of the treatments in depleting ethylene from the storage environment, ethylene depletion was measured by using a portable CID - CI 900 Bioscan ethylene analyser. Mature green bananas (cv. Amritsagar) were procured from the university farm. The bananas were stored in an ambient environment for determining ethylene depletion on different days of storage. For the first day, mature green bananas were placed in a closed chamber of an ethylene analyser and incubated for 60 min and then the ethylene concentration (ppm) in the chamber was measured. This reading was recorded as control. Then the fruits from the same hand was incubated for 60 min along with a 20×10^6 population of *Pseudomonas sp.* inoculated in carrier media as per the treatment combination. Ethylene concentration was recorded after 60 min of incubation.

2.5 Estimation of total phenol content in pulp and peel

Using a spectrophotometer, readings were taken at 740 nm after mixing diluted banana pulp and peel extract with the Folin-Ciocalteau reagent to create a blue-coloured complex (UV-Visible 1800 spectrophotometer, Shimadzhu, Kyoto, Japan). Results were expressed as after utilising a calibration curve to measure the total phenolic contents.

3. Result and Discussion

3.1 Time to 50% yellowing

The fruits were treated with microbial isolates with carrier media as peat soil and cotton as a source of inoculation to observe the changes in the colour of the peel. The fruits treated with microbial isolates from the orchard and having carrier media cotton as (T_2S_2) showed the early yellowing of the fruit in 54.75 hours when compared to other treatments. From this study, it was observed that the fruits inoculated with microbial isolates from forest soil having carrier media as peat soil shown the less yellowing on the fruit. The treatment T_3S_3 has recorded the best result of more than 72 hrs for yellowing of the peel followed by the microbial isolates from the orchard soil and inoculated on the fruit with peat soil has a carrier media source T_3S_2. This study observed that the microbial isolates had restricted the development of the colour on the peel of the banana fruits. Ahmad *et al*., 2007 reported the peel colour changes in the fruit were influenced by the ethylene concentration in the surroundings. Thus, the delayed yellowing of the fruits was due to ethylene depletion by *Pseudomonas* sp. The fruits were stored at different temperatures and the fruits stored at room temperature had shown more yellowing in less time period whereas the fruits stored at low temperature at 16-18 ºC with an enclosed system has the best result in their study. Santosa *et al*., 2010 [16] has reported that the usage of clay as a potassium permanganate carrier for delayed ripening significantly reduced the peel colour of the banana.

3.2 Total Soluble Solids in inoculated fruit

The effect of microbial isolates of *Pseudomonas sp.* from different sources are inoculated with fruit using carrier media peat soil and cotton at the aseptic condition and stored at ambient temperature are shown below in Table 1. The results revealed that the value of TSS gradually increased from normal fruit on the initial day to TSS after 4th day. The fruits which were not treated with any microbial isolates had recorded a high TSS content of nearly 21.5 °Brix which was considered a control. The fruits treated with microbial isolates from different sources had recorded less TSS when compared to the control. TSS was observed on the 4th day from all the treatments and was noted that among all the microbial isolate treated fruit, the least TSS was observed in treatment T_3S_3 (17.25 °B) having microbial isolates from forest soil having carrier media as peat soil which was considered as the best of having more storage life when compared to other treatments. The second best result was observed from the vermicompost soil and having peat soil as a carrier media source for microbial inoculation with fruit.

Among the different treatments, the highest TSS was observed in the T_1S_1, having microbial isolates from the vermicompost and carrier media sources such as cotton and peat soil. This study revealed that the fruits having less TSS due to delay in ethylene induced ripening related changes owing to ethylene depletion from surrounding by the *Pseudomonas* sp. It is to further conclude that the strain isolated from forest soil had better efficacy to metabolize ethylene. Patil and Shanmugasundaram, (2015) [12] have reported the TSS of the fruit gradually increases. The result obtained from their study was TSS increased from 3 to 22° Brix in 4 days of storage. Rashid *et al*., (2012) [15] got a similar result in their study, the untreated fruit has high TSS when compared to treated fruits with chemicals and packaging material. Kim (2006) [8] also demonstrated the ethylene removal by *Pseudomonas sp.*

3.3 Phenol content on pulp after microbial isolates inoculated on fruit

Phenols are secondary metabolites which impart a role in the bitterness, flavour, taste and aroma of fruits. The effect of fruit pulp on phenolic compounds after the microbial inoculation was investigated and results are presented in table 1. The control has less phenol content in the pulp with compare to the other treatments. The results obtained from the study were fruit inoculated with a microbial isolate from vermicompost and having carrier media as peat soil and cotton showed less phenol content in the pulp with 37.37 mg/100 g. The fruit inoculated with microbial isolates from the forest having carrier media as cotton has more phenol content in the pulp is considered as the best from different treatments maintaining more shelf life compare to other treatments. The second best result was observed from the forest and vermicompost isolates having carrier media as peat soil with 49.95 mg/100 g. The fruits treated with microbial isolates from forest soil and having carrier media as cotton and peat soil are significantly best by having more phenol content in the pulp. This study was revealed that more phenol content in the pulp had shown the less ripening of the fruit and having storage life with compare to other treatments. Aquino *et al*., 2016 [5] has obtained the average value of 50 mg/ 100 g phenolic content in mature fruit.

3.4 Phenol content on peel after microbial isolates inoculated on fruit

The effect of a banana peel on total phenols was evaluated and mean values of different treatments are presented in table 1. It was concluded that the highest total phenol content was observed in T3S3 has a microbial isolate from the forest soil with carrier media has peat soil followed by the microbial isolates of vermicompost having peat soil having a carrier media of 57.93 mg/100 g. The fruits treated with microbial isolates from vermicompost having peat soil and cotton have a carrier media source for microbial isolates showing less phenol content in the peel of the banana among the different treatments. The minimum value of total phenols was noticed in the control. The microbial isolates from different sources observed different results but the microbial isolates from the forest soil considered as the best with having more phenol content in the peel when compared to orchard soil and vermicompost. The interaction between the microbial isolates of different sources and carrier media as source inoculation on the fruit for absorbing ethylene evolution from fruits and reducing the ethylene concentration inside the storage system was significant. Hence, by this study, it was revealed that phenolic contents were higher in mature fruit treated with the inoculates of forest soil and gradually declined with the advancement of fruits ripening during storage. Sulaiman *et al*. (2011) [18] observed contents of phenolic compounds in banana cultivars of AAA, AAB, AA and ABB ranging from 0.09 to 20.47 mg gallic acid equivalents/ 100 g, in the fully ripe pulp and peel, respectively. Alothman *et al*., (2009) [4] reported average values of 27.0 to 72.2 mg gallic acid equivalents /100g in the ripe banana fruit.

3.5 Ethylene evolution and depletion after the inoculation of microbial isolates

The mature green banana fruits were treated with microbial isolates from different sources. The ethylene emission was observed periodically in the ethylene analyser each day with carrier media as peat soil and cotton. The fruits were inoculated with microbial isolates from forest soil, vermicompost and orchard soil having peat soil and cotton as carrier media to observe the ethylene emission from the fruits. The ethylene emission was observed from control fruit and among different treatments. The ethylene emission from the control banana fruit was nearly 8.98±0.05 ppm. Among the different treatments, ethylene emission was observed less in microbial isolates from forest soil having peat soil has carrier media T_3S_3 and second less emission was observed from forest soil having cotton as carrier media and maintaining aseptic condition. Among different treatments, T_1S_1 microbial isolates from vermicompost having peat soil and cotton as the source of carrier media observed the highest ethylene emission rate on day 1. The ethylene emission on day 2 was observed from the different sources, highest ethylene emission was observed from the control with 9.15±0.09 ppm. The control fruits were not treated with any microbial isolates and maintained in aseptic conditions. The fruits treated with microbial isolates had shown less ethylene emission from the fruit when compared to control. The ethylene emission from the fruit treated with microbial isolates of the forest soil having carrier media as peat soil showed the less ethylene emission. On day 3 the fruits treated with microbial isolates from forest soil having peat soil having carrier media observed the less ethylene emission when compared to other treatments. The fruits inoculated with the microbial isolates of the forest soil having peat soil has a carrier media has the least ethylene emission rate from the fruit on the day 4. Jiang *et al*. 1999 [7] has used 1-MCP and observed the result in delay ripening of the fruit and reduced the ethylene emission of the fruit. This study revealed that the ethylene emission concentration is reduced by using microbial isolates from the different sources. The microbial isolates *i.e.*, *Pseudomonas sp*. has an ability to reduce the ethylene from the stores. The similar result was obtained by the Kim in 2006 [8] and he used *Pseudomonas* strains in reducing ethylene concentration below the threshold level of plant hormone. Moghadam *et al*. 2015 [9] has used the *Pseudomonas putida* in reducing the ethylene from the fruit and maintaining the shelf life of the fruit. Pierce *et al*., 2014 [13] has utilized the *Rhodococcus rhodochrous* DAP 96253 cell in delay ripening by suppressing the enzymatic activing of the fruits.

Table 1: Effect of microbial isolates on time to 50 % yellow, TSS, Pulp and Peel Phenol

	Time to 50 % yellow (Hours)			TSS (°Brix)			Pulp Phenol (mg/100 g)			Peel Phenol (mg/100 g)		
	S_1	S_2	S_3	S_1	S_2	S_3	S_1	S_2	S_3	S_1	S_2	S_3
T_1	57.75	58.50	61.00	20.28	19.10	19.13	39.92	42.86	44.45	37.37	47.51	49.82
T_2	59.50	54.75	58.75	18.78	18.48	17.30	45.71	46.59	49.97	51.91	53.70	54.74
T_3	66.00	67.75	73.00	17.78	18.00	17.25	49.95	48.23	49.95	57.93	55.42	59.17
	T	**S**	**T×S**	**T**	**S**	**T×S**	**T**	**S**	**T×S**	**T**	**S**	**T×S**
SE(m)	0.81	0.81	1.40	0.16	0.16	0.28	0.09	0.09	0.16	0.34	0.34	0.59
CD	2.35	2.35	NA	0.46	0.46	NA	0.27	0.27	0.47	0.99	0.99	1.71

Table 2: Ethylene Depletion (ppm)

	Day -1			Day -2			Day -3			Day -4		
	S_1	**S_2**	**S_3**	**S_1**	**S_2**	**S_3**	**S_1**	**S_2**	**S_3**	**S_1**	**S_2**	**S_3**
T_1	0.86	1.74	2.46	0.87	1.72	2.46	0.82	1.83	2.50	0.73	1.76	2.58
T_2	2.73	3.74	4.56	2.70	3.72	4.57	2.60	3.85	4.51	2.50	3.75	4.52
T_3	2.98	4.90	5.30	2.97	4.96	5.17	2.93	4.87	5.12	2.95	4.78	5.05
	T	**S**	**T×S**	**T**	**S**	**T×S**	**T**	**S**	**T×S**	**T**	**S**	**T×S**
SE(m)	0.05	0.05	0.08	0.06	0.06	0.10	0.07	0.07	0.13	0.09	0.09	0.15
CD	0.13	0.13	0.28	0.17	0.17	0.29	0.22	0.22	0.37	0.26	0.26	0.54

Depletion calculated based on the difference between untreated and treated sample: Value of ethylene evolution in untreated sample:- Day-1= 8.98±0.05 ppm; Day-2=9.15±0.09; Day- 3=9.32±0.06; Day-4=9.63±0.07ppm.
Sources are S1: Vermicompost, S2: Orchard Soil S3: Forest soil.
Treatments are T1: Peat soil and cotton, T2: Cotton, T3: Peat Soil

4. Conclusion

From this study, it was concluded that microbial isolates *i.e.*, *Pseudomonas sp*. has an efficiency and ability to reduce the concentration of ethylene, which helps in delay of ripening of the fruit and to maintain high shelf life of the produce. Microbial isolates from forest soil and having carrier media as a peat soil had shown the best result among the different treatments combinations in removing ethylene from the surrounding and it was evident from slow rate of increase in TSS, least ethylene concentration or higher ethylene depletion, less yellowing of the peel and higher phenol content in the fruit. Based upon the results observed in this experiment, it was concluded that the use of *Pseudomonas sp*. can reduce the ethylene concentration and can be used as a post- harvest treatment tool in horticultural storage facilities.

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