PLANT SCIENCE TODAY
ISSN 2348-1900 (online)
Vol 11(sp4): 01–10
https://doi.org/10.14719/pst.5094

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REVIEW ARTICLE

Harnessing ethylene-producing bacteria for fruit bio-ripening: a comprehensive review

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ARTICLE HISTORY
Received: 16 September 2024
Accepted: 11 October 2024
Available online
Version 1.0 : 11 December 2024

Additional information

Peer review: Publisher thanks Sectional Editor and the other anonymous reviewers for their contribution to the peer review of this work.

Reprints & permissions information is available at https://horizonepublishing.com/journals/index.php/PST/open_access_policy

Publisher's Note: Horizon e-Publishing Group remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

Indexing: Plant Science Today, published by Horizon e-Publishing Group, is covered by Scopus, Web of Science, BIOSIS Previews, Clarivate Analytics, NAAS, UGC Care, etc See https://horizonepublishing.com/journals/index.php/PST/indexing_abstracting

CITE THIS ARTICLE
Brunda Y, Muthuvel I, Kavitha PS, Karthikeyan M, Ramesh D. Harnessing ethylene-producing bacteria for fruit bio-ripening: a comprehensive review . Plant Science Today.2024;11(sp4):01-10. https://doi.org/10.14719/pst.5094

Abstract

Fruit ripening is a complex biological process regulated by various endogenous and exogenous factors, with ethylene playing a pivotal role as a gaseous hormone. Traditionally, ethylene for commercial use has been derived from environmentally hazardous processes. However, the discovery of ethylene-producing bacteria presents a sustainable alternative for fruit ripening. This review explores the mechanisms and potential of utilizing ethylene-producing bacteria in the bio-ripening of fruits. Through an analysis of current research, we elucidate the synthesis pathways of ethylene in microorganisms, optimization techniques to enhance ethylene production, and the compatibility among microbial strains for the development of effective microbial consortia. Recent studies have demonstrated the efficacy of ethylene-producing bacteria, such as *Bacillus* and *Pseudomonas* species, in bio-ripening fruits like kiwifruit, plums, bananas, and apples. The optimization of growth conditions and the development of microbial consortia aim to maximize ethylene production efficiency While minimizing environmental impacts. This review underscores the importance of ethylene-producing bacteria in revolutionizing fruit ripening technology and advancing sustainable agricultural practices. By providing insights into the opportunities and challenges associated with bio-ripening fruits by ethylene-producing bacteria, this review seeks to guide future research and innovation in the field. Ultimately, using ethylene-producing bacteria offers a promising avenue for achieving safe, environmentally friendly, and efficient fruit ripening methods in agricultural practices.

Keywords

Bacillus and *Pseudomonas* ; microbial consortia; compatibility; sustainable

Introduction

Fruit ripening is a complex biological process influenced by numerous internal and external factors. Ethylene, a gaseous hormone, regulates fruit ripening, ageing, and stress responses (1). Traditionally, ethylene for commercial use is obtained through steam cracking, a method linked to environmental risks and reliance on non-renewable resources. However, ethylene-producing bacteria have emerged as a safer, eco-friendly alternative for ripening fruits (2).

Utilizing ethylene-producing bacteria in bio-ripening marks a breakthrough in agricultural technology. These bacteria generate ethylene through various metabolic processes and offer a sustainable approach to artificial fruit ripening (3). By optimizing growth conditions and developing microbial consortia, researchers are working to enhance ethylene production, ensuring adequate fruit ripening with reduced environmental impact (4).

This review explores the mechanisms behind fruit bio-ripening facilitated by ethylene-producing bacteria. It covers the pathways through which microorganisms synthesize ethylene, techniques for optimizing ethylene production, and the compatibility of different microbial strains to form effective microbial consortia. By reviewing recent studies and advances, we aim to highlight the potential of ethylene-producing bacteria as a sustainable, eco-friendly solution for fruit ripening.

Through an in-depth literature analysis, we emphasize the role of ethylene-producing bacteria in transforming fruit ripening technology and promoting sustainable agriculture. This review outlines the promising opportunities and challenges in bio-ripening, paving the way for future research and innovation.

Importance of fruits - Current Scenario

Fruits have been a fundamental part of the human diet since before the development of grain cultivation. They are rich in phytonutrients like vitamins, polyunsaturated fatty acids, and carotenoids, all vital to human health (5). With a deeper understanding of food safety, there is a growing emphasis on production practices that prioritize physical and physiological safety (6).

Data from the National Horticulture Board show a steady increase in fruit production and cultivation area, with fruits accounting for around 31.5% of all horticultural crops. This growth highlights concerns about fruit safety and quality. Artificial ripening methods, while common, can alter the attributes and quality of fruits, posing health risks and reducing consumer preference.

Climacteric fruits undergo a sharp rise in respiration rate during ripening, known as the climacteric rise, which either coincides with or follows an increase in ethylene production. These fruits respond to external ethylene exposure with a logarithmic surge in ethylene production. Conversely, non-climacteric fruits ripen without releasing ethylene or experiencing respiration spikes and do not need ethylene to facilitate ripening. The respiration rate of fruits, measured by tracking CO_2 concentration changes over time in a sealed environment, offers insights into their ripening process (7).

History of the Plant Hormone Ethylene

The discovery of ethylene as a plant hormone dates to 1896, when Dimitry Neljubow observed unique changes in laboratory-grown pea plants, such as reduced radial swelling, elongated hypocotyls, shorter roots, and enhanced apical hook formation. In 1934, Richard Gane quantified and confirmed ethylene production in ripening apples. Later, Yang and Adams (1980) advanced understanding by detailing ethylene's biosynthesis and signalling processes.

Ethylene plays a crucial role in fruit ripening and the withering of vegetative tissue (8). The degreening effect, noted in citrus fruits exposed to warm, enclosed environments with kerosene heaters (9), was later linked to ethylene gas. By the 1920s, ethylene, already produced commercially for other uses, was identified as the agent causing this ripening effect. Studies in the 1930s confirmed that plants naturally produced ethylene, which matched the composition of ethylene emitted by kerosene heaters (1).

Further research showed that ethylene influences plants in multiple ways and is classified as a plant hormone or growth regulator. Additionally, it was discovered that fruit exposed to ethylene displayed similar quality and health benefits as fruit that ripened naturally (10). This occurs because ethylene induces ripening changes in both naturally ripened and ethylene-treated fruit, with the fruit needing to reach its physiological green maturity stage before responding to external ethylene.

Ripening Physiology

Fruit ripening is a multifaceted process that involves alterations in colour, texture, flavour, aroma, and nutrient composition (11). The cell walls of fruits, rich in cellulose, hemicelluloses, and pectin, are typically less lignified (12). Cellulose polymers are connected by B-1,4 glucose bonds, much like hemicelluloses such as homogalacturonan (HMG), rhamnogalacturonan I (RhaI), and rhamnogalacturonan II (Rha-II). The RhaI polymer, composed of 12 sugars and approximately 20 glucosidic linkages, is a crucial component. Nearly all fruits contain the pectin polymer xylogalacturonan (13).

Ethylene plays a central role in regulating fruit development and the stages of plant growth. It controls transcriptional changes in both climacteric and non-climacteric fruits. Climacteric fruits, such as tomatoes, bananas, and mangoes, undergo ripening triggered by ethylene, which activates the expression of ripening-related genes. In contrast, non-climacteric fruits like citrus, grapes, and strawberries do not depend on transcriptional factors for ripening, although external ethylene can influence their environmental response (14).

The fruit life cycle consists of three stages: fruit set, fruit development, and fruit ripening. Ripening marks the beginning of fruit senescence, a genetically programmed process that transitions the fruit from an unripe to a ripe state, resulting in a visually appealing and edible fruit (15). This irreversible process involves physiological, biochemical, and organoleptic changes (16), including shifts in carbohydrate content, increased sugar levels, and alterations in colour, texture, aroma, flavour, phenolic compounds, and organic acids.

Respiration, the process of breaking down complex substances into simpler molecules for energy and cellular functions, indicates metabolic activity in cells. The patterns of respiration change throughout fruit development, ripening, and senescence (17). Ethylene, closely linked to fruit ripening, triggers ripening and senescence processes. Fruits are categorized as climacteric or non-climacteric based on how their ripening is regulated.

Climacteric fruits experience a marked increase in respiration and ethylene production during ripening. In contrast, non-climacteric fruits show stable or decreasing respiration rates leading upto senescence, with only minimal ethylene production (18). While climacteric fruits can continue to ripen after being harvested with exogenous ethylene, non-climacteric fruits generally mature while still attached to the parent plant (19). Contrary to traditional views, studies suggest that some ripening changes in climacteric fruits can occur independently of ethylene, and some non-climacteric fruits may also undergo ethylene-dependent changes.

Ethylene biosynthesis occurs in two distinct systems: System 1 produces ethylene during the pre-climacteric phase and throughout the ripening of non-climacteric fruits, while System 2, which is auto-stimu-lated, generates high levels of ethylene during the ripening of climacteric fruits (20).

Global Scenario of Ethylene Production

The U.S. Energy Information Administration (EIA) projected in its 2018 Annual Energy Outlook (AEO2018) that natural gas production from shale resources would more than double over the coming decades. While natural gas production demand remained relatively low until 2011, it has experienced a substantial rise, increasing from 2% in 2000 to a peak of 27%.

Natural gas, which consists of various hydrocarbons, shows significant variation in composition, particularly in shale-derived sources. Among the natural gas liquids (NGs') found, such as pentane, ethane, propane, butane, and isobutene, ethane is primarily used as a feedstock in ethylene production. The EIA notes that global ethane cracking typically requires about 90,000 barrels per day for ethylene production. In Saudi Arabia, ethane accounts for approximately 62% of ethylene production, with propane contributing 25% and naphtha 10.8%. Projections indicate global ethylene consumption will reach 300 million metric tons by 2038. Between 2008 and 2017, global ethylene production capacity grew by 31%, from 130 million to over 170 million metric tons. Northeast Asia and the Asia-Pacific regions are expected to play a significant role in this growth (21).

Ethylene Cycle in Plant

The biosynthesis of ethylene, a crucial plant hormone, originates from L-methionine and progresses to methyl-thio-butyric acid. The Yang cycle of ethylene production revealed the intricate ethylene cycle in plants. The enzyme ACS (Amino Cyclo Propane Carboxylase Synthase) significantly enhances ethylene production within this cycle. Initially, S-adenosyl methionine synthase catalyzes the conversion of methionine and adenosine triphosphate into S-adenosyl-methionine, initiating the cycle. Subsequently, S-adenosyl-L-methionine cleavage produces 5-methylthio-adenosine and 1-Amino- cyclopropane-carboxylate (ACC). ACC serves as the direct precursor for ethylene synthesis, yielding ethylene along with CO_2, HCN, and H_2O, catalyzed by the enzyme ACC oxidase.

Further extension of the cycle involves the synthesis of 5-methylthioribose from 5-methylthioadenosine, mediated by methylthio nucleosidase. The subsequent synthesis of 5'- Methylthio-ribose-1-phosphate, catalyzed by methylthioribose kinase, ultimately produces a keto-y-methylthio butyric acid, facilitated by transaminase. N-malonyl transferase inhibits ACC's ethylene production by malonylating ACC to malonyl-ACC.

The Role of Ethylene in Ripening

Ethylene is an essential hydrocarbon in agriculture, playing three critical roles in plant systems: ripening, senescence, and stress response. During ripening, ethylene promotes fruit softening, triggers colour changes, and primarily converts stored starches and acids into sugars, which boosts the fruit's sweetness. Senescence refers to the natural cell death process that leads to abscission. In response to stress, plants increase ethylene production to manage environmental challenges (22).

The Role of Ethylene in Plants

Ethylene is a crucial plant hormone that regulates various processes such as growth, flowering, senescence, ageing, fruiting, and ripening (23). It is known to improve plant yields, though its effects on growth are complex and depend on its concentration.

Ethylenes' impact on leaf growth and development

Ethylene, in combination with transcriptional regulators and other phytohormones, plays a role in leaf growth (24). Ethylene response factors are vital for plant growth and their response to ethylene, with much of this research focused on Arabidopsis (25). The concentration of ethylene influences how leaves respond, with both auxin and ethylene being essential for initiating leaf growth in plants (26). Ethylene exposure in plants, such as potatoes, can cause symptoms like yellowing and chlorophyll loss (27).

Ethylene in flower development

Ethylene is crucial for floral initiation, signalling the plant's transition from vegetative to reproductive (28). During flower development in Arabidopsis, genes such as eto 1, etr 1, ein 2-1, and ein 3-1 become active, highlighting ethylene's regulatory role.

Ethylene signal transduction in fruit ripening

Ethylene receptors (ETR) on the endoplasmic reticulum membrane detect ethylene and rely on their interaction with the Green-ripe (GR) protein. In the absence of ethylene, ETRs act as negative regulators of ethylene responses by activating constitutive triple-response 1 (CTR1), which keeps ethylene insensitive 2 (EIN2) inactive. As a result, EIN2 cannot stabilize EIN3/ethylene insensitive 3-like (EIN3/EIL) transcription factors, which are degraded by EBF proteins, thus inhibiting the transcription of ethylene-responsive genes. When ethylene binds to ETRs, it deactivates the receptors and inhibits CTR1. This leads to EIN2 activation, stabilizing EIL transcription factors and triggering the expression of ethylene-responsive genes, including ethylene response factors (ERF), critical regulators of ethylene-related gene expression (29).

Ethylene in fruit development and senescence

Fruit development, a fundamental physiological process, involves the growth of the ovary after fertilization and the formation of seeds within the fruit. Ethylene, a volatile organic compound, is crucial in promoting fruit development. The biochemical changes in fruit, such as sugar breakdown and alterations in colour and aroma, become more pronounced with increased ethylene production. Fruits are categorized as climacteric or non-climacteric based on their responses to ethylene. Climacteric fruits undergo autocatalytic ripening, characterized by a peak in ethylene production during the early stages of ripening.

On the other hand, non-climacteric fruits are less responsive to external ethylene exposure. The ripening process is regulated by ACO and ACS genes, with the ethylene-dependent tomato serving as a model plant for study (30). The polygalacturonase enzyme helps break down cell wall components, while phytoene synthase contributes to pigment development in flowers and other essential plant organs, such as fruits (31). Fruit firmness, which affects its appearance during senescence, is influenced by ethylene concentration and autocatalytic ethylene biosynthesis. Enzymes like pectate lyase (PL), B-galactosidase, polygalacturonase (PG), and expandins (EXP) play a role in fruit softening and the eventual process of senescence. The levels of ethylene in various fruit crops are detailed in the Table 1.

Table 1. Based on the level of ethylene production in fruit crops (32).

Level of ethylene	Rate μl/kg/hr)	Example
Very low	<0.1	Grape, citrus (0.1-2.0)
Low	0.1-1.0	Pineapple (0.2-0.4)
Medium	1-10	Mango, Banana (3), Guava, Fig (1-10) Litchi (1-5)
High	10-100	Apple, Papaya, Avacado, Plum, Ber (100), Pear (75-100)
Very high	>100	Passion fruit, Sapota, Apple, cherimoya (219)

Applications of Bio-ripening

Traditional techniques

Various traditional techniques have been used to induce ripening in unripe fruits since the inception of commercial fruit cultivation. One standard method for ripening bananas in Maharashtra and Tamil Nadu is smoke treatment. In this process, straw, leaves, and cow dung are burned in a closed chamber, with banana bunches arranged in a heap. The treatment lasts 18-24 hours in summer and 48 hours in winter. This smoke treatment method is widely used for ripening bananas and mangoes (33). The smoke in the chamber releases ethylene, acetylene, and other gases, which trigger the ripening process and cause the fruit's surface colour to change. Many fruit traders use this technique to achieve more uniform ripening, particularly for bananas and mangoes. However, a significant disadvantage of this method is that the fruits often fail to develop a consistent colour and flavour. Additionally, the lingering smoky odour on the fruit negatively affects its quality. The key drawbacks of this technique are the lack of uniformity in colour and flavour, along with the persistent smoky odour that diminishes the fruit's overall quality (34).

Paddy straw and banana leaves are commonly used for fruit ripening in rural areas where commercial ripening facilities are unavailable. Bananas and mangoes are typical fruits that are ripened using this method. In this process, cushioning and wrapping materials help to trap heat and ethylene produced by the fruits. Fruits are often placed in wheat, paddy straw, or similar materials to encourage ripening (35). Arranging unripe fruits in layers over paddy husk or wheat straw allows them to ripen within a week (34). To reduce the cost of chemicals, some ethylene-releasing fruits like papayas and bananas can be stored together in the same room. Since ethrel, a plant hormone accelerates the ripening process by releasing ethylene into the atmosphere, this practice is considered one of the safest methods (36).

Treated banana fruits with *Adhatoda vasica* (basooti) leaves and observed that the fruits began to soften within five days. They reported that using these indigenous plant materials could extend the shelf life of bananas by 2-3 days compared to those treated with ethephon (37). Farmers in the Mandi district (H.P.) use twigs of basooti to ripen bananas. The bananas are wrapped in basooti foliage and placed in bamboo baskets in a dry area. This method is believed to generate heat, which accelerates ripening, typically within 3-4 days. To further speed up the ripening process, farmers in Kerala use the leaves of Kanikonna (*Cassia fistula*). By placing the leaves between the banana bunches, the fruits ripen within a day (38).

Effect of ethylene

Ethylene is a simple gaseous plant hormone that plays a crucial role in various physiological processes in plants, including fruit ripening (39). Fruit ripening is a complex, genetically programmed event that significantly changes the fruits' fleshs' colour, flavor, aroma, texture, and nutritional value (40). Ethylene gas activates a range of enzymes in banana fruits, leading to alterations in colour, texture, and flavour (41). It is believed to regulate ripening by coordinating the expression of genes involved in processes such as increased respiration rate, ethylene production, chlorophyll breakdown, carotene synthesis, starch conversion to sugar, and the activity of enzymes that degrade the cell wall. Ethylene is also naturally emitted by fruits and other plant tissues when injured during handling. In addition, it is artificially applied to ripen and colour various fruits, including bananas, pears, mangoes, tomatoes, and citrus fruits (42).

Bananas are typically harvested at the mature-green stage for transportation to non-banana-producing countries. Due to their early harvest at this green stage, bananas often lose their ability to ripen naturally or exhibit uneven ripening. Commercial ripening using ethylene in storage rooms is a common practice to ensure uniform ripening before distribution to the consumer market. Ethylene plays a crucial role in various physiological and developmental processes, including germination, growth, flower initiation and opening, senescence of leaves and flowers, organ abscission, and fruit ripening (43).

Ethylene exposure leads to several changes in fruits, including the loss of chlorophyll, colour development, softening of tissues, removal of tannins, flavour enhancement, increased sweetness, and early activation of proteolytic enzymes. Ethaphon is one of the most used chemicals for generating ethylene in postharvest treatments. It promotes pre-harvest ripening in crops such as top fruit, soft fruit, tomatoes, sugar beet, fodder beet, coffee, and many others. Additionally, it aids in fruit and berry harvests, accelerates postharvest ripening (e.g., bananas), and prevents lodging in cereals, maize, and flax (44).

The effects of ethrel in aqueous solutions at concentrations of 250, 500, and 1000 ppm, as well as the ethylene released from three at these concentrations, on the ripening of white and pink-fleshed guavas. They found that ripening was accelerated in all treated fruits of both guava types at all concentrations. The ripening rate increased progressively with higher concentrations. They also observed that ethyl released from ethrel was more effective in triggering ripening than dipping the fruits in an aqueous ethrel solution. Depending on the concentration, ripening occurred 2-6 days faster in fruits treated with Ethrel solution and 6-9 days earlier in fruits treated with ethylene released from Ethrel, compared to untreated fruits. The ripening effect was indicated by an enhanced climacteric peak in respiration, improved peel colour, increased total soluble solids, and reduced flesh firmness (45).

One batch of banana fruits was exposed to 100 ppm ethylene gas for 24 hours, while another batch was treated with an aqueous ethephon solution at concentrations of 250, 500, 750, and 1000 ppm for 5 minutes. The fruits were packed in plastic crates and stored in a ripening chamber at 16-18°C and 90-95% relative humidity. They found that bananas treated with 100 ppm ethylene gas and 500 ppm ethephon ripened adequately within 4 days, displaying uniform colour, pleasant flavour, desirable firmness, acceptable quality, and improved shelf life. In contrast, untreated control fruits remained hard, with poor colour and quality (46).

Dipping banana fruits in 1000 ppm ethephon for 5 minutes was highly effective for ripening, with fruits beginning to soften within 3 days and becoming ready to eat in 5 days, with a shelf life of up to 8 days (33). Treated Robusta bananas harvested at 75–80% maturity with various ethrel concentrations (250–1000 ppm) for 5 minutes (47). They observed that bananas treated with 500 ppm ethrel ripened uniformly without affecting taste or flavour. At the same time, untreated control fruits remained shrivelled green and did not ripen evenly after 8 days in storage. Bananas treated with 500 ppm ethrel ripened fully in 6 days at 20±1°C. Treated mango fruits with different concentrations of ethrel (100, 200, and 300 ppm), while other mangoes were left in the laboratory for natural ripening. They noted that untreated fruits showed incomplete ripening, resulting in sourness and making them unsuitable for consumption. Conversely, mangoes treated with 100, 200, and 300 ppm ethrel ripened on the 13th, 11th, and 9th days, respectively, with a uniform colour, whereas the control fruits were poor in colour and quality (48).

Effect of calcium carbide

Calcium carbide is an organic compound widely used to ripen fruits such as bananas, mangoes, papayas, apples, peaches, pears, avocados, and sapodillas, often known by the trade name "masala." Upon hydrolysis, calcium carbide releases acetylene gas, which has a ripening effect like ethylene (49). Ripening is a natural process that makes fruit edible and nutritious after a certain period of undisturbed development (50). Today, many fruits are ripened with calcium carbide to achieve faster and more uniform ripening (51,52). However, calcium carbide negatively impacts fruit quality and leaves toxic residues that pose health risks to consumers. Despite a long-standing ban under the PFA Act of 1955, traders still use calcium carbide due to its low cost, availability, and ease of use.

The effects of calcium carbide at varying doses (0–0.7 g) on kiwifruit ripened at room temperature (22°C) over 7 days (53). They observed a decrease in titratable acidity and vitamin C content, while fruit juice's and water-soluble solids' pH increased during ripening. Additionally, studies showed that treating bananas and mangoes with calcium carbide at 2–4 g/kg of fruit resulted in accelerated and uniform ripening (54-63). Papaya developed a flavour when ripened with calcium carbide, unlike when treated with Ethrel or ethylene (64). Calcium carbide poses several health risks and has carcinogenic effects due to trace amounts of arsenic and phosphine (65). Optimized calcium carbide doses for ripening mature green mangoes, where fruits packed with calcium carbide and moistened with water were sealed with newspaper to prevent acetylene leakage. These mangoes ripened within eight days, and fruits (4-5 kg) ripened with 2g of calcium carbide were found to have the most desirable taste and flavour (56).

Potential Health Risks

Calcium carbide, which releases acetylene like ethylene, poses health risks such as reduced oxygen supply to the brain and gastrointestinal irritation (66, 51). Direct exposure to industrial-grade calcium carbide can lead to skin damage, weakness, nausea, vomiting, and respiratory issues (51).

Significance of Food Safety

Artificial ripening agents like calcium carbide (CaC_2) release acetylene, a harmful gas that can cause neurological disorders and other health problems in humans. Calcium carbide also contains traces of arsenic and phosphorus. Fruits ripened with calcium carbide lose their natural antioxidant properties, nutritional value, and ability to absorb iron (67). According to the Food Safety and Standards Act of 2013, regulations were implemented to control food processing and ensure access to safe food. The Prevention of Food Adulteration (PFA) Act of 1954 and its 1955 regulations prohibit using artificial ripening agents. Finding safer, physiologically acceptable methods for artificially ripening fruits is crucial.

Microorganisms Producing Ethylene

Considering the health hazards associated with artificial ripening chemicals, eco-friendly approaches involve the utilization of ethylene-producing microorganisms. It is well -established that ethylene is a byproduct of the metabolism of certain organisms. For instance, *Penicillium digitatum* utilizes the a-ketoglutarate route to synthesize ethylene. Several bacterial strains belonging to genera such as *Azotobacter*, *Bacillus*, and *Pseudomonas* have been identified for their ethylenogenic characteristics, converting methionine into ethylene (68). Previous studies have demonstrated that *Aeromonas hydrophila* can produce up to 10,320 nmol of ethylene per litre of media (69). It has been identified various bacteria capable of ethylene production, including Enterobacter cloacae (142 nmol/66h), Pseudomonas indigofera (6,195 nmol/66h) and Arthrobacter sp. (800 nmol/66h) (51). Therefore, microbes have the potential to produce ethylene in significant quantities, which could be applied in various agricultural contexts (Tables 2 and 3).

Table 2. List of bacteria, yeast and moulds producing ethylene Bacteria (68,69).

Bacteria	
Micrococcus luteus	*Enterobacter aerogene*
Acinetobacter calcoaceticus	*Chromobacteriu violaceum*
Klebsiella pneumonia	*Corynebacterium aquaticum*
Aeromonas hydrophila	*Citrobacter* sp.
Escherichia coli	*Pseudomonas syringae* pv. *glycinea*
Arthrobacter sp.	*Xanthomonas campestris*
Erwinia herbicola	*Pseudomonas syringae* pv. *mori*
acillus mycoides	*Pseudomonas syringae* pv. *phaseolicola*
Bacillus subtilis	*Pseudomonas solanacearum*
Enterobacter cloaceae	*Thiobacillus ferroxidans*
Brevibacterium linens	*Rhizobium trifoli*
Serratia liquefaciens	*Serratia marcesens*
Staphylococcus aureus	*Thiobacillus novellus*
Yeast	
Schizosaccharomyces octosporus	*Cryptococcus laurentii*
Saccharomyces cerevisiae	*Cryptococcus albidys*
Moulds	
Tyromyces palustris	*Botrytis spectabilis*
Thielavia alata	*Aspergillus ustus*
Agarius bisporus	*Gloeophyllum trabeum*
Alternaria solani	*Hirshioporus abietinus*
Sclerotinia laxa	*Aspergillus variecolor*
Aspergillus flavus	*Penicillum digitatum*

Importance of Microbial Ethylene

The technique of producing ethylene using *E. coli* cell-free extracts (70). Certain ethylene-producing strains have been shown to synthesize ethylene from L-methionine (71). Investigated the growth and ethylene production capacity of ethylenogenic microorganisms when fed with 2-Keto-4-methylthiobutyric acid (KMBA) and L-methionine (72).

Table 3. Ethylene production by soil-borne bacteria (68).

Organism	No of isolates	Ethylene produced in n mol (mg dry wt^{-1} 66 h^{-1})
Citrobacter sp.	02	045-082
Enterobacter cloacae	04	111-142
Escherichia coli	06	091-374
Klebsiella pneumoniae	13	043-546
pneumoniae subsp. *oxytoca*	01	341
Klebsiella ozaenae	01	146
Serratia liquefaciens	01	078
Aeromonas hydrophilra	12	040-722
Pseudomonas sp.	10	600-1095
Pseudomonas indigofera	02	650-6195
Arthrobacter sp.	04	082-800

The ethylene production system purified the ethylene-forming enzyme in vitro from *P. syringae* pv. *phaseolicola* PK2. Fruit ripening can be effectively achieved through the ethylene-forming mechanism of microorganisms. This approach holds promise as a source of bioethylene and as a substitute for chemically produced ethylene (73).

Ethylene is a well-known exogenous plant hormone that plays a role in various physiological processes in plants, such as fruit ripening. Even at low concentrations (10 to 100 nl/L), ethylene is active and effective (1). Traditionally, most commercial ethylene is produced through hazardous steam cracking, which relies on non-renewable energy sources. In contrast, microbial ethylene production is safe and advantageous for fruit ripening. For bio-ripening fruits like kiwifruit, plums, bananas, and apples, *E. coli* engineered with the ethylene-forming enzyme system from *Pseudomonas syringae* pv. *phaseolicola* has been used (74).

Ethylene Synthesis Cycles in Microorganisms

Microorganisms employ distinct pathways for ethylene synthesis, differing from those in plants. The two primary mechanisms involved in ethylene production as a byproduct of microbial metabolism are the 2-keto-4-methylthiobutyric acid pathway and the 2- oxoglutarate process.

Ethylene formation from the 2-keto-4-methylthiobutyric acid (KMBA) pathway

Two pathways for ethylene biosynthesis, highlighting KMBA as a potential intermediate in E. coli ethylene production (75). The KMBA pathway involves the formation of keto-4-methylthibutyric acid, an intermediate molecule synthesized in methionine-supplied microorganisms like E. coli (72). The mechanism of ethylene formation, as described by (76), relies on NADH and involves the conversion of Fe^{3+} to Fe^{2+} via NADH. Molecular O$_2$ oxidizes Fe^{2+} EDTA, while anion superoxide prevents a reaction with Fe^{2+} that produces hydroxyl radical (OH). Hydroxyl radicals then convert KMBA oxidizer to ethylene. Conducted investigations into transaminases in bacteria, providing further insights (28). Underscored the methionine dependency of the ethylene-forming system in ethylogenic microorganisms.

The 2-oxoglutarate pathway in microorganisms

The anaerobic methionine salvage pathway (MSP), described by (77), utilizes the available sulfur supply to produce ethylene. This pathway, typically employed by anaerobic microorganisms like *Rhodospirillum rubrum* and *Rhodopseudomonas palustris*, requires oxygen for ethylene production at the end of the cycle. The cycle of metabolite events in the oxoglutarate pathway involves several steps, starting with 5 methylthioadenosine and culminating in ethylene production from 5 methylthioadenosine. Enzymes such as methylthiophosphorylase (mtnp), MTRU-IP isomerase (mtnA), 5-(methylthio) ribose-IP aldolase (ald2), and probable alcohol dehydrogenase catalyze this cycle of events.

Anaerobic genes like rip 1 and met 17 are implicated in this route of ethylene synthesis, highlighting the complexity of microbial ethylene production.

Optimization of Media for Improved Ethylene Production

Optimizing the variables in enhancing ethylene production is crucial, and response surface methodology (RSM) is an effective tool for this purpose (78). utilized central composite design and response surface approach to process wastewater, indicating its applicability in optimization studies (79). In a survey of *P. digitatum,* ethylene generation increased by approximately 60 nl/g/h under optimal conditions. This highlights the potential for significant improvement in ethylene production through proper variable optimization. Applying response surface methodology allows for systematically exploring the interaction between variables, leading to enhanced ethylene yields.

Quantitative of Ethylene Production

The quantification of ethylene production will be carried out by gas chromatography, and a syringe will be used to collect approximately 1 millilitre of gas from the air-sealed serum vial. The sample was then analyzed using a Thermo Scientific Gas Chromatogram 700 equipped with a flame ionization detector and a porapak Q column. The injector port will be kept at 80 °C, while the temperature of the column and detector will be kept at 200 °C. As fuel gas, hydrogen flow rate is 45 millilitres per minute, while nitrogen gas flow rate is 30 millilitres per minute as the carrier gas. This will allow for the determination of ethylene gas presence in the sample. A standard injection of 0.5 ml of 99.95% pure ethylene gas will be made, and the corresponding peak retention duration will be noted. The gas evolved will be given in terms of n moles per litre of the medium, and the ethylene synthesis by the bacterial isolates will be measured every 24 hours up to 96 hours. To calculate the quantity of ethylene, the Response factor (RF) has to be calculated, then the amount of ethylene is calculated.

$$RF = \frac{\text{Peak Area of Analyte}}{Concentration\ of\ analyte}$$

$$\text{Concentration} = \frac{\text{Peak Area of Analyte}}{RF}$$

The bacteria *Bacillus mycodies, Aeromonas culicicol, Bacillus subtilis* sp. *Subtitles*, *Lactobacillus pentoses*, *Pseudomonas syringae* pv. *tomato* produces 254.45 n mol/l, 322.12 n mol/l, 461.63 n mol l^{-1}, 512.45 n mol/l and 436.78 n mol/l(80).

Compatibility of Ethylene-Producing Microorganisms

When developing an effective ethylene-producing microbial consortium, assessing the compatibility among different isolates is crucial. The cross-streak method is a proven and efficient technique for evaluating the compatibility of microbial strains. For example, the cross-streak method was used to examine the compatibility of *Bacillus* species in probiotic development (81). Similarly, it has been applied to investigate the ability of *Streptomyces* species to produce bioactive compounds (82). By utilizing the cross-streak method, researchers can systematically assess how different ethylene-producing microorganisms interact with each other. This approach provides valuable information on the synergies and interactions between microbial strains, aiding in creating effective and robust ethylene-producing consortia for fruit bio-ripening applications.

Ethylene-Producing Microbial Consortium for Bioripening of Fruits

The efficacy of utilizing two distinct species of microorganisms, Bacillus and Pseudomonas, effectively degrading diesel-contaminated soil (83). Their study thoroughly investigated the efficiency of combined strains, highlighting the significant role of microbial consortia in achieving synergistic effects. The advantages of microbial consortia include shared metabolic activities, enhanced benefits when cultivated together, and product creation (83). The microbial consortia employed in fermentation contribute to increased hydrogen generation (84). As ethylene gas remains the sole naturally occurring method of fruit ripening, microbial ethylene presents the safest and most environmentally friendly approach (2). Reports of aerobic heterotrophs capable of producing ethylene date back to 1940 (85). Furthermore, microorganisms utilizing precursors such as L-methionine and 2-keto-4- methylthiobutyric acid have been identified to possess ethylenogenic properties (75).

Conclusion

The bio-ripening of fruits through ethylene-producing bacteria offers a promising and environmentally sustainable approach to fruit ripening. Through a comprehensive literature review, we have highlighted the various mechanisms by which ethylene-producing microorganisms contribute to fruit ripening, including the synthesis pathways, optimization techniques, and microbial consortia. Ethylene, a natural plant hormone, plays a pivotal role in fruit ripening, senescence, and stress response. Using ethylene-producing bacteria is a safe and efficient alternative to traditional chemical methods, minimizing artificial ripening agents' health risks and environmental impacts. Moreover, the compatibility among microbial strains and the optimization of growth conditions are crucial factors for enhancing ethylene production and ensuring adequate fruit ripening. Developing microbial consortia and utilizing advanced techniques such as response surface methodology contribute to maximizing ethylene production efficiency.

By harnessing the potential of ethylene-producing bacteria, we can achieve an ideal for fruit ripening and contribute to sustainable agricultural practices and food security. Further research in this field promises to unlock new insights and innovations in fruit ripening technology, paving the way for a healthier and more environmentally conscious approach to food production and consumption.

Acknowledgements

I want to express my sincere gratitude to Tamil Nadu Agricultural University. My heartfelt thanks to my Chairperson (Muthuvel I) for their continuous guidance and encouragement. Finally, I must thank all the authors who helped improve this manuscript.

Authors' contributions

MI conceptualized the review and defined the structure of the manuscript. BY, KPS, KM, and RD helped to improvise language and content. All authors read and approved the final version of the manuscript.

Compliance with ethical standards

Conflict of interest: The authors declare no conflict of interest.

Ethical issues: None

References

1. Abeles FB, Morgan PW, Saltveit Jr ME. Ethylene in plant biology. Academic Press; 2012.
2. Dhillion SS, Anderson RC. Production on burned and unburned sand prairies during drought and non-drought years. Vegetation. 1994;115:51-9.
3. Silva WB, Silva GM, Santana DB, Salvador AR, Medeiros DB, Belghith I, da Silva NM, Cordeiro MH, Misobutsi GP. Chitosan delays ripening and ROS production in guava (*Psidium guajava* L.) fruit. Food Chem. 2018;242:232-8. https://doi.org/10.1016/j.foodchem.2017.09.052
4. Brenner K, You L, Arnold FH. Engineering microbial consortia: a new frontier in synthetic biology. Trends Biotechnol. 2008;26(9):483-9. https://doi.org/10.1016/j.tibtech.2008.05.004
5. Seymour GB, Colquhoun IJ, Dupont MS, Parsley KR, Selvendran RR. Composition and structural features of cell wall polysaccharides from tomato fruits. Phytochem. 1990;29(3):725-31. https://doi.org/10.1016/0031-9422(90)80008-5
6. Canavari M, Bazzani GM, Spadoni R, Regazzi D. Food safety and organic fruit demand in Italy: a survey. Brit Food J. 2002;104(3/4/5):220-32. https://doi.org/10.1108/00070700210425688
7. Watkins CB. The use of 1-methylcyclopropene (1-MCP) on fruits and vegetables. Biotechnol adv. 2006;24(4):389-409. https://doi.org/10.1016/j.biotechadv.2006.01.005
8. Crocker WE. Similarities in the effects of ethylene and the plant auxins. Contributions. Boyce Thompson Instit Plant Res, 1935;7:231-48.
9. Denny FE. Effect of ethylene upon respiration of lemons. Botanical Gazette. The UChicago press J 1924;77(3):322.
10. Chace EM. Health problems connected with the ethylene treatment of fruits. Am J Public Health Nations Health. 1934 ;24(11):1152-6.
11. Rose WC. The nutritive significance of the amino acids. Physiol. Rev.1938;18(1):109. https://doi.org/10.1152/physrev.1938.18.1.109
12. Toivonen PM, Brummell DA. Biochemical bases of appearance and texture changes in fresh-cut fruit and vegetables. Postharvest Biol Technol. 2008;48(1):1-4. https://doi.org/10.1016/j.postharvbio.2007.09.004
13. Ralet MC, Dronnet V, Buchholt HC, Thibault JF. Enzymatically and chemically de-esterified lime pectins: characterization, polyelectrolyte behaviour and calcium binding properties. Carbohydr. Res. 2001;336(2):117-25. https://doi.org/10.1016/S0008-6215(01)00248-8
14. Liu M, Pirrello J, Chervin C, Roustan JP, Bouzayen M. Ethylene control of fruit ripening: revisiting the complex network of transcriptional regulation. Plant Physiol. 2015 ;169(4):2380-90. https://doi.org/10.1104/pp.15.01361
15. Perotti VE, Moreno AS, Podestá FE. Physiological aspects of fruit ripening: the mitochondrial connection. Mitochondrion. 2014 Jul 1;17:1-6. https://doi.org/10.1016/j.mito.2014.04.010
16. Joshi H, Kuna A, Lakshmi MN, Shreedhar M, Kumar AK. Effect of stage of maturity, ripening and storage on antioxidant content and activity of Mangifera indica L. var. Manjira. Int J Food Sci. Nutr. 2017;2(3):1-9.
17. Tripathi K, Pandey S, Malik M, Kaul T. Fruit ripening of climacteric and non climacteric fruit. J Environ Appl Biores. 2016;4(1):27-34.
18. Bouzayen M, Latché A, Nath P, Pech JC. Mechanism of fruit ripening. Plant Develop Biol-Biotechnol Perspectives: Volume 1 2009.
19. Pathak S, Sriramulu S, Thandavan SP, Jothimani G, Banerjee A, Marotta F. Enhancement of shelf life of the climacteric fruits. A review on application of CRISPRi technology. Trends Tech Sci Res. 2018;1(2):23-9. https://doi.org/10.1093/jxb/erf072
20. Alexander L, Grierson D. Ethylene biosynthesis and action in tomato: a model for climacteric fruit ripening. J Exp Bot. 2002;53(377):2039-55. https://doi.org/10.1093/jxb/erf072
21. Faramawy S, Zaki T, Sakr AE. Natural gas origin, composition, and processing: A review. J Nat Gas Sci Eng. 2016;34:34-54. https://doi.org/10.1016/j.jngse.2016.06.030
22. Bradford KJ. Shang Fa Yang: Pioneer in plant ethylene biochemistry. Plant Sci. 2008;175(1-2):2-7. https://doi.org/10.1016/j.plantsci.2008.01.005
23. Khan NA, Mir MR, Nazar R, Singh S. The application of ethephon (an ethylene releaser) increases growth, photosynthesis and nitrogen accumulation in mustard (*Brassica juncea* L.) under high nitrogen levels. Plant Biol. 2008;10(5):534-8. https://doi.org/10.1111/j.1438-8677.2008.00054.x
24. Bar M, Ori N. Leaf development and morphogenesis. Development. 2014;141(22):4219-30. https://doi.org/10.1242/dev.106195
25. Dubois M, Van den Broeck L, Claeys H, Van Vlierberghe K, Matsui M, Inzé D. The ethylene response factors ERF6 and ERF11 antagonistically regulate mannitol-induced growth inhibition in Arabidopsis. Plant Physiol. 2015;169(1):166-79. https://doi.org/10.1104/pp.15.00335
26. Reinhardt D, Mandel T, Kuhlemeier C. Auxin regulates the initiation and radial position of plant lateral organs. Plant Cell. 2000;12(4):507-18. https://doi.org/10.1105/tpc.12.4.507

27. Beyer Jr EM. A potent inhibitor of ethylene action in plants. Plant Physiol. 1976 ;58(3):268-71. https://doi.org/10.1104/pp.58.3.268

28. Ogawara T, Higashi K, Kamada H, Ezura H. Ethylene advances the transition from vegetative growth to flowering in *Arabidopsis thaliana*. J Plant Physiol. 2003;160(11):1335-40. https://doi.org/10.1078/0176-1617-01129

29. O'Malley RC, Rodriguez FI, Esch JJ, Binder BM, O'Donnell P, Klee HJ, Bleecker AB. Ethylene-binding activity, gene expression levels, and receptor system output for ethylene receptor family members from *Arabidopsis* and tomato. Plant J.2005;41(5):651-9. https://doi.org/10.1111/j.1365-313X.2004.02331.x

30. Smith CJ, Watson CF, Bird CR, Ray J, Schuch W, Grierson D. Expression of a truncated tomato polygalacturonase gene inhibits expression of the endogenous gene in transgenic plants. Molecular General Genetics MGG. 1990;224:477-81. https://doi.org/10.1007/BF00262443

31. Fray RG, Grierson D. Identification and genetic analysis of normal and mutant phytoene synthase genes of tomato by sequencing, complementation and co-suppression. Plant Mol Biol. 1993;22:589-602.

32. Burg SP, Burg EA. Role of ethylene in fruit ripening. Plant physiology. 1962;37(2):179. https://doi.org/10.1104/pp.37.2.179

33. Ram HB, Singh SK, Singh RV, Surjeet Singh SS. Effect of Ethrel and smoking treatment on ripening and storage behaviour of banana cv. Harichhal. Progressive Hort. 1979. https://doi/full/10.5555/19800384520

34. Selvarajan M. Technology for ripening fruits as important as marketing them. [internet]. India : The Hindu 2011. [updated 2024 25 Nov].

35. Parmar C. 2010. Use of chemicals in fruits ripening [Internet]. 2010 India : Tribune . Available from: www.tribuneindia.com/july2010.

36. Thamaraiselvi SP. 2011 Technology for ripening fruits as important as marketing them. [internet]. India : The Hindu 2011. [updated 2024 25 Nov].

37. Ram R. Openness, country size, and government size: Additional evidence from a large cross-country panel. J Pub Econo. 2009;93(1-2):213-8. https://doi.org/10.1016/j.jpubeco.2008.04.009

38. Swapna TS. Salt stress-induced changes on enzyme activities during different developmental stages of rice (*Oryza sativa* Linn.). Indian Journal of Biotechnology. 2003;2:251-58 https://doi/full/10.5555/20033120247

39. Guo H, Ecker JR. The ethylene signaling pathway: new insights. Current Opinion in Plant Biology. 2004;7(1):40-9. https://doi.org/10.1016/j.pbi.2003.11.011

40. Giovannoni JJ. Genetic regulation of fruit development and ripening. The plant cell. 2004;16(suppl_1):S170-80. https://doi.org/10.1105/tpc.019158

41. Gierson D, Kader AA. Fruit ripening and quality. In: Atherton JG, Rudich J, editors. The tomato crop: A scientific basis for improvement. Dordrecht: Springer Netherlands;1986. p. 241-80.

42. Saltveit ME. Effect of ethylene on quality of fresh fruits and vegetables. Postharvest Biology and Technology. 1999;15(3):279-92. https://doi.org/10.1016/S0925-5214(98)00091-X

43. Pelayo C, de B Vilas-Boas EV, Benichou M, Kader AA. Variability in responses of partially ripe bananas to 1-methylcyclopropene. Postharvest biology and technology. 2003;28(1):75-85. https://doi.org/10.1016/S0925-5214(02)00124-2

44. Harvey HW. Biological chemistry and physics of seawater. The University Press; 1928.

45. Mohamed NIA, Abu GAA. Effect of ethrel in aqueous solution and ethylene released from ethrel on guava fruit ripening. Agriculture and Biology Journal of North America. 2010;1(3):232-7.

46. Mahajan BV, Kaur T, Gill MI, Dhaliwal HS, Ghuman BS, Chahil BS. Studies on optimization of ripening techniques for Banana. Journal of Food Science and Technology. 2010;47:315-9. https://doi.org/10.1007/s13197-010-0050-0

47. Kulkarni SG, Kudachikar VB, Keshava Prakash MN. Studies on physico-chemical changes during artificial ripening of banana (*Musa* sp) variety Robusta. Journal of Food Science and Technology. 2011; 48:730-4. https://doi.org/10.1007/s13197-010-0133-y

48. Venkatesan T, Tamilmani C. Effect of ethrel on phenolic changes during ripening of off-season fruits of mango (*Mangifera indica* L. var. Neelum). Current Botany. 2010; 1:22-28.

49. Deshi VV, Siddiqui MW, Homa F, Lata D, Singh DR. CaC_2-induced ripening: Unveiling the bitter truth behind sweet fruit. Food Chemistry. 2024;140097.

50. Siddiqui MW, Dhua RS. Eating artificially ripened fruits is harmful. Current science. 2010; 25:1664-8.

51. Wills RB, McGlasson WB, Graham D, Joyce DC. Postharvest: an introduction to the physiology and handling of fruit, vegetables and ornamentals University of New South Wales Press Ltd. Sydney, Australia. 2007. https://doi/full/10.5555/19980301716

52. Siddiqui MW. Studies on some aspects of mango ripening. Department of Post-Harvest Technology of Horticultural Crops. Mohanpur, Nadia, India. 2008.

53. Bal E, Kök D. Effects of different calcium carbide doses on some quality criteria of kiwifruit (*Actinidia deliciosa*). Journal of Tekirdag Agricultural Faculty. 2006; 3(2): 213-9. https://doi/full/10.5555/20063120772

54. Nagaraj P, Ramana KV, Prasad BA, Mallikarjunaradhya S, Patwardhan MV, Ananthakrishna SM, Rajpoot NC, Subramanyan L. Effect of calcium carbide on ripening and quality of Alphonso mangoes. Journal of Food Science and Technology. 1985; 21 (5). 278-82. https://doi/full/10.5555/19850330655

55. Mann SS. Effect of ethylene and acetylene on the ripening of mango fruits. Acta Hortic. 1985;158:409-12. https://doi.org/10.17660/ActaHortic.1985.158.47

56. Chacón SI, Víquez F, Chacón G. Artificial ripening of bananas: effect of two ripening agents: calcium carbide and ethylene in solution. https://doi/full/10.5555/19900394227

57. Tauqir Abbas TA, Javaid Iqbal JI, Musahib-ud-Din Khan MU. Effect of calcium carbide on physical changes in mango fruits (cvs. Desi and Dusehri) during ripening. https://doi/full/10.5555/19920317126

58. Kumar A, Dhawan SS. Effect of post harvest treatments on the enhancement of ripening of mango (*Mangifera indica*) fruit cv. Dashehari. https://doi/full/10.5555/19970300939

59. Padmini S, Prabha TN. Biochemical changes during acetylene-induced ripening in mangoes (var. Alphonso). Tropical Agriculture. 1997; 74(4):1-5. https://doi/full/10.5555/19990303267

60. Guha D, Bhuiyan MA. Effect of ripening materials on mango. Annals of Bangladesh Agriculture. 1997;7(1):73-5.

61. Pal RK. Ripening and rheological properties of mango as influenced by ethrel and calcium carbide. Journal of Food Science and Technology-Mysore.1998.https://doi/full/10.5555/19990301261

62. Amarakoon R, Illeperuma DC, Sarananda KH. Effect of calcium carbide treatment on ripening and quality of Velleicolomban and Willard mangoes. Tropical Agricultural Research. 1999;11:54-60.

63. Joon MS, Kumar JK, Sharma RK, Singhrot RS. Comparison of calcium carbide and ethephon on ripening of mango. Haryana Journal of Horticultural Sciences. 2001;30(3-4):181-82

64. Jayawickrama F, Wilson Wijeratnam RS, Perera S. The effect of

selected ripening agents on organoleptic and physico-chemical properties of papaya. In: IV International Conference on Postharvest Science 553 2000 Mar 26 (pp. 175-178). https://doi.org/10.17660/ActaHortic.2001.553.37

65. Ur-Rahman A, Chowdhury FR, Alam MB. Artificial ripening: what we are eating. Journal of Medicine. 2008;9(1):42.

66. Fattah SA, Ali MY. Carbide ripened fruits-a recent health hazard. Faridpur Medical College Journal. 2010;5(2):37-.

67. Dhembare AJ. Bitter truth about fruit with reference to artificial ripener. Arch Appl Sci Res. 2013;5(5):45-54.

68. Primrose SB, Dilworth MJ. Ethylene production by bacteria. J Gen Microbiol. 1976;93(1):177-81.

69. Billington DC, Golding BT, Primrose SB. Biosynthesis of ethylene from methionine. Isolation of the putative intermediate 4-methylthio-2-oxobutanoate from culture fluids of bacteria and fungi. Biochem J. 1979;182(3):827-36. https://doi.org/10.1042/bj1820827

70. Ince JE, Knowles CJ. Ethylene formation by cell-free extracts of *Escherichia coli*. Arch Microbiol.1986;146:151-8. https://doi.org/10.1007/bf00402343

71. Shipston BN, Bunch AW. The physiology of L-methionine catabolism to the secondary metabolite ethylene by *Escherichia coli*. Microbiol.1989;135(6):1489-97. https://doi.org/10.1099/00221287-135-6-1489

72. Mansouri S, Bunch AW. Bacterial ethylene synthesis from 2-oxo-4-thiobutyric acid and from methionine. Microbiol.1989;135(11):2819-27. https://doi.org/10.1099/00221287-135-11-2819

73. Nagahama K, Ogawa T, Fujii T, Tazaki M, Tanase S, Morino Y, Fukuda H. Purification and properties of an ethylene-forming enzyme from *Pseudomonas syringae* pv. *phaseolicola* PK2. Microbiol.1991;137(10):2281-6. https://doi.org/10.1099/00221287-137-10-2281 77

74. Digiacomo F, Girelli G, Aor B, Marchioretti C, Pedrotti M, Perli T, Tonon E, Valentini V, Avi D, Ferrentino G, Dorigato A. Ethylene-producing bacteria that ripen fruit. ACS Synthetic Biol. 2014;3(12):935-8. https://doi.org/10.1021/sb5000077

75. Primrose SB. Evaluation of the role of methional, 2-keto-4-methylthiobutyric acid and peroxidase in ethylene formation by *Escherichia coli*. Microbiol. 1977;98(2):519-28. https://doi.org/10.1099/00221287-98-2-519 79

76. Fukuda H, Ogawa T, Tanase S. Ethylene production by microorganisms. Adv Microbial Physiol. 1993;35:275-306. https://doi.org/10.1016/S0065-2911(08)60101-0

77. North JA, Miller AR, Wildenthal JA, Young SJ, Tabita FR. Microbial pathway for anaerobic 5′-methylthioadenosine metabolism coupled to ethylene formation. Proceedings National Academy Sci.2017;114(48):E10455-64. https://doi.org/10.1073/pnas.1711625114

78. Bezerra MA, Santelli RE, Oliveira EP, Villar LS, Escaleira LA. Response surface methodology (RSM) as a tool for optimization in analytical chemistry. Talanta. 2008;76(5):965-77. https://doi.org/10.1016/j.talanta.2008.05.019

79. Ahmadi M, Vahabzadeh F, Bonakdarpour B, Mofarrah E, Mehranian M. Application of the central composite design and response surface methodology to the advanced treatment of olive oil processing wastewater using Fenton's peroxidation. J Hazard Mater. 2005;123(1-3):187-95. https://doi.org/10.1016/j.jhazmat.2005.03.042

80. Induja R, Gopal NO, Anandham R. Isolation and screening of ethylene producing bacteria. Madras Agricultural Journal. 2019;106.

81. Lertcanawanichakul M, Sawangnop S. A comparison of two methods used for measuring the antagonistic activity of *Bacillus* species. Walailak J Sci Technol. 2008;5(2):161-71.

82. Peela S, Kurada VB, Terli R. Studies on antagonistic marine actinomycetes from the Bay of Bengal. World J Microbiol Biotechnol. 2005 ;21:583-5. http://dx.doi.org/10.1007%2Fs11274-004-3493-5

83. Silva DD, de Lima Cavalcanti D, de Melo EJ, dos Santos PN, da Luz EL, de Gusmão NB, de Queiroz MD. Bio-removal of diesel oil through a microbial consortium isolated from a polluted environment. Int Biodeter Biodegr. 2015;97:85-9.

84. Maintinguer SI, Fernandes BS, Duarte IC, Saavedra NK, Adorno MA, Varesche MB. Fermentative hydrogen production by microbial consortium.Int J Hydrogen Energy. 2008;33(16):4309-17. https://doi.org/10.1016/j.ijhydene.2008.06.053

85. Miller HJ, Henken G, Veen JV. Variation and composition of bacterial populations in the rhizospheres of maize, wheat, and grass cultivars. Canadian J Microbiol. 1989;35(6):656-60. https://doi.org/10.1139/m89-106