CHEMICAL ENGINEERING TRANSACTIONS

VOL. 88, 2021

A publication of AIDIC
The Italian Association of Chemical Engineering
Online at www.cetjournal.it

Guest Editors: Petar S. Varbanov, Yee Van Fan, Jiří J. Klemeš

ISBN 978-88-95608-86-0; **ISSN** 2283-9216

DOI: 10.3303/CET2188076

The Potential of Rhodopseudomonas Palustris as a Bio-Fertiliser for Sustainable Agriculture

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Sustainable agriculture is an ongoing research strives for meeting society's current food demand without compromising the future need and development. Maintaining soil fertility for quality farming is one of the essential parts. However, the wide application of synthetic agrochemicals (e.g., chemical fertiliser) has been a significant contributor to environmental pollution. This review aims to assess the potential of *Rhodopseudomonas palustris* (*R. palustris*), a purple non-sulphur bacterium, as a commercialised bio-fertiliser to sustainably promote plant growth. *R. palustris* is evaluated based on two defined pillars of sustainability, including the effects on plant growth, environmental impact, and feasible production. The effectiveness is based on the improvement of plant growth through the secretion of extracellular metabolites, resistance to abiotic stresses, bioremediation of heavy metals, and mitigation of greenhouse gas emissions. This review suggests the imperative roles of *R. palustris* as an effective bio-fertiliser in agriculture. However, the scalability of production and application deserved more attention. The potential substrates ranging from different waste streams and formulation methods for *R. palustris* production are summarised to discuss environmental and economic sustainability.

1. Introduction

Modern intensive farming has enabled faster and higher yield of crops to meet the ever-increasing global food and agricultural demand but often come at a heavy cost, especially to the natural environment, such as loss of soil fertility and environmental pollution. Bio-fertilisers (i.e., microbial-based fertilisers) containing strains of beneficial microorganisms are emerging as a greener and cost-effective alternative to minimise problems arising from excessive mineral fertilisation because of their positive impacts on plant growth and the environment. The widespread utilisation of plant growth-promoting rhizobacteria (or bacteria) (PGPR/PGPB) as bio-fertilisers has been reported due to their beneficial effects to promote plant growth and yield in many plants (Basu et al., 2021). PGPB are microorganisms that colonise plant roots or free-living organisms that can directly or indirectly enhance plant growth. There is vast potential in exploiting the diverse soil microbial community to increase global crop production; thus, discovering and applying a new promising bacterial genus as a novel bio-fertiliser to enhance plant growth and yield is highly desirable.

Rhodopseudomonas palustris (R. palustris) is a free-living bacterium species under the genus *Rhodopseudomonas*, phylum Proteobacteria. It uses light, inorganic, or organic compounds as substrates for the biosynthesis of energy. *R. palustris* is one of the purple non-sulphur bacteria (PNSB) characterised by a unique versatility and flexibility in switching between four modes of metabolism (Larimer et al., 2004). These metabolisms are chemoautotrophic, photoautotrophic, chemoheterotrophic, and photoheterotrophic, which allow the microbe to grow in various cultivation modes and habitats (Sakarika et al., 2020). Its metabolic versatility has attracted more attention from the research community due to its potential use in biotechnological

Paper Received: 4 May 2021; Revised: 2 September 2021; Accepted: 12 October 2021
Please cite this article as: Sabki M.H., Ong P.Y., Lee C.T., Ibrahim N., Fan Y.V., Klemeš J.J., 2021, The Potential of Rhodopseudomonas Palustris as a Bio-Fertiliser for Sustainable Agriculture, Chemical Engineering Transactions, 88, 457-462 DOI:10.3303/CET2188076

applications, including a promising bio-fertiliser to be utilised in agriculture. Some beneficial functions of *R. palustris* include (i) nitrogen (N) fixation (Wong et al., 2014), (ii) heavy metal remediation (Batool et al., 2017), and (iii) methane (CH_4) emission mitigation in saline paddy fields (Kantha et al., 2015). *R. palustris* is known to produce plant growth-promoting substances like indole-3-acetic acid (IAA) (Wong et al., 2014) and 5-aminolevulinic acid (ALA) (Kantha et al., 2010). *R. palustris* also can secrete exopolymeric substances (EPS), which help to adsorb sodium ions (Na^+) (Nunkaew et al., 2015b). These substances contribute to the stimulation of plant growth and improved resistance to environmental stresses. Another beneficial feature of *R. palustris* is that it can be prepared in a low-cost culture medium facilitating large-scale production (Lo et al., 2020). These features make *R. palustris* an appealing candidate as a bio-fertiliser and biocontrol agent for numerous agricultural applications, although an overview on *R. palustris* multi-functional traits is still lacking.

Research has demonstrated that inoculating plants with PGPR as bio-fertilisers can be an effective strategy to stimulate crop growth. Backer et al. (2018) have extensively reviewed and elucidated the roadmap to commercialise PGPR as bio-fertilisers. Basu et al. (2021) also have discussed various aspects of PGPR as bio-fertilisers, including the beneficial applications, mechanisms of actions and commercialisation pathways. Specific PGPR strains have also been focused and covered in different aspects, including *Bacillus* spp. and their role in plant growth development and stress mitigation (Radhakrishnan et al., 2017), the impact of *Azospirillum* spp. in agricultural and environmental applications (Cassán et al., 2020), and the potential applications and possible operational mechanisms of rhizobia (Jaiswal et al., 2021). Most of the highlighted PGPR are from various microbial taxa, with a lack of emphasis on PNSB to showcase and exploit their full potential as bio-fertilisers. The inclusion of PNSB like *R. palustris* as a promising bio-fertiliser is often overlooked, probably due to the lack of commercialisation or limited report on the application for growth promotion of crops compared to other common bacteria. Recent work by Sakarika et al. (2019) paid attention to purple non-sulphur bacteria, providing a comprehensive review on the benefit for plant growth, environmental aspects, and preliminary cost-effectiveness analysis. The roadmap of transitioning from research to implementation has also been discussed focused on the shelf-life issues and application methods. The discussion by Sakarika et al. (2019) is from a relatively macro perspective, covering all the phototrophic microorganisms.

Our review serves as an updated work that highlights the benefits of a specific strain, mainly *R. palustris*, as promising bio-fertilisers based on three defined pillars of sustainability, including the effects on plant growth, environmental impact, and feasible production. The value of *R. palustris* in agriculture is reviewed and discussed, from their benefits on plant growth to commercialisation as low-cost commercial agricultural inputs to promote sustainable agriculture.

2. Methods

Data collection was conducted from the peer-reviewed international journals and web sources such as Google Scholar and ScienceDirect using a combination of keywords like "*Rhodopseudomonas palustris*", "purple non-sulphur bacteria", "PNSB", and "agriculture". In this review, the data collection only focused on studies related to *R. palustris* and its beneficial application in agriculture. Selected studies were published within the year 2010 to 2020.

3. Plant growth promotion by *R. palustris*

Identifying the mechanisms on how *R. palustris* could promote plant growth and yield of different crops is ongoing research. Wong et al. (2014) and Hsu et al. (2021) highlighted the effects of *R. palustris* to improve nutrient uptake and nitrogen (N) use efficiency in Chinese pakchoi cabbage. *R. palustris* has also been reported to improve soil phosphatase enzyme activity for phosphorus (P) solubilisation in acid sulphate soils, contributing to enhanced rice growth and yield (Khuong et al. 2018). Shao et al. (2017) reported enhancing nutrient availability in soils and their uptake in cucumber plants.

R. palustris can be a good source of stimulus metabolites like IAA and ALA that can enhance plant growth and induce plant resistance against abiotic stress, such as salinity and heavy metal stress. These beneficial effects were primarily associated with activating anti-oxidative enzyme activities preventing oxidative damage in plant cells, and increased chlorophyll content, leading to enhanced photosynthesis activity. The production of IAA by *R. palustris* stimulated the growth of upland rice (Mohd Din et al., 2014) and black gram under arsenic contamination (Batool et al., 2017), while ALA has been shown to stimulate growth in Chinese dwarf cherry (Yin et al., 2012) and cucumber under cadmium stress (Ge et al., 2017). Nunkaew et al. (2014) reported that ALA released by *R. palustris* at concentrations of 2.11 – 2.57 µM increased total chlorophyll. *R. palustris* also increased anti-oxidative enzymes such as catalase, ascorbate peroxide, glutathione reductase and superoxide dismutase, which decreased the free radical effect on rice grown under saline stress. ALA production by *R. palustris* bio-fertilisers in a paddy field soil under saline condition (0.25 % sodium chloride addition) was achievable in the range of 1.64 - 2.61 μM ALA, leading to the stimulation of plant growth (Kantha et al., 2015). Su et al. (2017) reported the enhanced germination and growth in tobacco and induced resistance against plant virus due to the release of IAA and ALA by *R. palustris*. *R. palustris* also promoted the growth of cucumber under salinity stress through the synergistic effects of IAA and ALA production, enhanced N-fixing, and nutrient solubilisation (Ge and Zhang, 2018).

R. palustris could produce microbial EPS with the ability to bind Na^+ and help both strains to survive in high salt concentration of up to 6 % sodium chloride (Nunkaew et al., 2015b). One of the essential characteristics of PGPB like *R. palustris* is their ability to secrete EPS that form biofilms or facilitate adhesion to the surfaces of plant roots. EPS are polymers (e.g., polysaccharides, proteins) biosynthesised by several strains of microorganisms into the environment (Costa et al., 2018). Other examples of beneficial effects of *R. palustris* on plants are summarised in Table 1.

Table 1: Plant growth promotions by R. palustris in agriculture

Plant common name	Benefits	Reference
Rice	ALA and EPS enhanced grain yield in both organic and saline flooded paddy fields	Kantachote et al. (2016)
Rice	ALA, IAA, EPS and siderophores stimulated growth, increased chlorophyll content, antioxidant enzymatic and non-enzymatic activities under As stress	Nookongbut et al. (2018)
Rice	Increased fresh weight, minimised plant toxicity under cadmium stress	Xiao et al. (2019)
Rice	EPS stimulated growth and grain yield, induced resistance against fungal pathogens	Nookongbut et al. (2020)
Maize	Improved plant height, increased antioxidant enzymatic activities under salinity stress	Feng et al. (2019)
Potato	Stimulated growth, enhanced immune response to late blight disease	Zhang et al. (2020)

4. Environmental benefits derived from *R. palustris* application in the agricultural context

Previous studies have demonstrated the capability of *R. palustris* to reduce greenhouse gas (GHG) emissions, reduce salt and heavy metal concentration, and improve soil health in various agricultural applications. Kantachote et al. (2016) has demonstrated the ability of *R. palustris* to outcompete methanogens (i.e., methane (CH_4)-producing bacteria) for utilisable substrates (e.g., acetate), contributing to the reduction of CH_4 emissions in both organic and saline flooded paddy fields. *R. palustris* significantly reduced total CH_4 emissions by 24 – 28 % in the organic and saline paddy soils, compared to fields with organic fertiliser treatment.

Batool et al. (2017) reported the reduction of arsenic (As) contamination in mineral medium up to 62.9 % (6.29 ± 0.24 mM) by *R. palustris*. Nookongbut et al. (2018) showcased the ability of *R. palustris* cells to bio-adsorp/absorb As evidenced by the significant decrease of total As concentration in rice nutrient solution containing As species (in the form of As(III) and As(V)) in all *R. palustris*-inoculated sets. These results were concurrent with the productions of ALA, IAA, EPS and siderophores, which significantly reduced As accumulation in the treated rice. Xiao et al. (2019) have demonstrated the potential of bacterial immobilisation of cadmium in the soils by *R. palustris* while Kantachote et al. (2016) reported the decreased toxicity of Na^+ to rice root by entrapping Na^+ in saline soil through the production of EPS.

The application of *R. palustris* as a bio-fertiliser can also reduce the use of chemical fertiliser and promote soil health. Khuong et al. (2018) reported the potential of *R. palustris* as P solubilisers to reduce P fertiliser application by 25 – 50 % while achieving the maximum rice grain yield. Other examples of environmental impact by *R. palustris* in agricultural applications are summarised in Table 2.

Table 2: Environmental impacts derived by R. palustris in agricultural applications

Benefits of application	Reference
Reduced CH_4 and carbon dioxide (CO_2) emissions (up to 88.41 % and 77.32 %) in rice straw broth with saline condition	Nunkaew et al. (2015)
Reduced CH_4 (as high as 86 - 100 %) and CO_2 (ranging 38 - 47 %) emissions in paddy field model under microaerobic light conditions with salt stress treatments	Kantha et al. (2015)
Reduction of soil acidity for agricultural activity	Khamis et al. (2017)
Improved soil health in acid sulphate soils for rice cultivation	Khuong et al. (2018)

5. Production and application of *R. palustris* as a low-cost bio-fertiliser

The commercialisation and application of PGPB as bio-fertilisers, such as *Pseudomonas* spp. and *Bacillus* spp. among many rhizobacteria, has become a significant component of sustainable agriculture practices in many countries (Mustafa et al., 2019). In Europe, the formulation of photosynthetic bacteria as PGPB has been commercialised as EmFarma Plus by a manufacturer called ProBiotics Polska (Mącik et al., 2020).

Nunkaew et al. (2014) have demonstrated the potential of *R. palustris* as a plant growth-stimulating bacterium at a lower cost than commercial ALA. The successful development of an *R. palustris* formulation is mainly dependent on using sustainable materials and application methods, such as utilising horticultural oil as a potential additive for the liquid-based formulation of *R. palustris* (Lee et al., 2016). Lo et al. (2020) have developed a low-cost formulation of medium to facilitate large-scale production of *R. palustris*. These findings suggest that *R. palustris* has the potential to be developed as a safe, cost-effective and easy-to-process microbial formulation that would facilitate its practical use in the field at a large scale. Despite previous studies have successfully determined suitable *R. palustris* formulations using either liquids or solids as carrier materials or efforts to achieve low-cost production, the commercialisation of *R. palustris* inoculant is relatively new and still has a long way to go.

Timmusk et al. (2017) presented the challenges and procedures for the commercial development of PGPR/PGPB product. Backer et al. (2018) have proposed ten steps to develop and commercialise PGPR-based inoculants, while Basu et al. (2021) have discussed the commercialisation pathways and constraints in developing PGPR bio-fertilisers. Recent work by Sakarika et al. (2019) has elucidated the roadmap for research and valorisation of PNSB products used for plant production. Although these reviews have extensively discussed the strategy or gaps to commercialise PGPR- or PNSB-based bio-fertilisers, the labelling and application instruction aspects are not widely discussed. The commercialisation strategy is also hampered as there is no well-known quality check available for bio-fertilisers at the moment (Basu et al., 2021). Labelling essential information on commercial bio-fertiliser products (e.g., quality, shelf-life, safety) is vital as farmers look upon these details for application on crops. Unlike chemical fertilisers, whose consistent nutrient compositions can be easily stated as their quality indicator, the quality of bio-fertiliser is harder to be predicted due to certain factors. These factors include the production process, unpredictability of plant-bacteria interactions under different agro-enviromental conditions, and farmers' practices (Malusà et al., 2016). Low-quality bio-fertilisers may cause inefficient or failed bio-fertilisation in the fields; thus, establishing quality control standards for bio-fertilisers is crucial. A well-accepted quality standard or indicator can help to inform the inoculation efficiency of-bio-fertilisers and gain confidence among consumers, creating a more competitive fertiliser market.

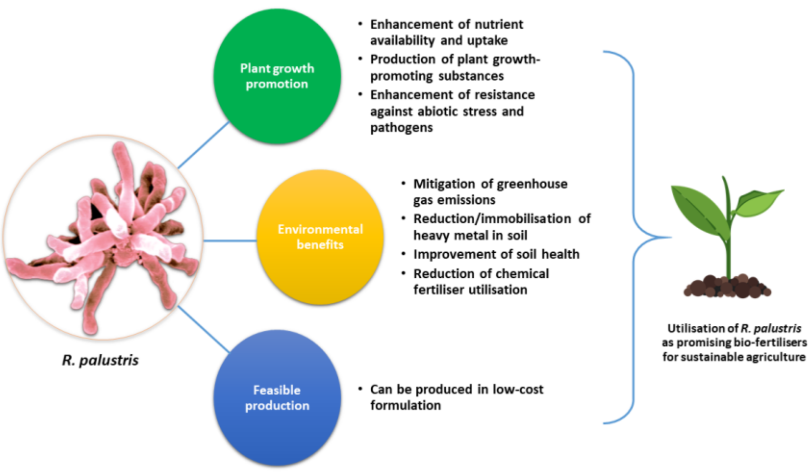

Figure 1: The overview of R. palustris application as bio-fertilisers for sustainable agriculture. Image of R. palustris was retrieved and adapted from Biotech Desk (2014)

6. Conclusion

The application of *R. palustris* as promising bio-fertilisers is evident from previous literature as they can promote plant growth, provide environmental benefits, and be formulated in low-cost and feasible ways. This review highlighted the potential application of *R. palustris* in the agricultural context and discussed the challenges that need to be addressed to facilitate *R. palustris* commercialisation as bio-fertilisers. Although numerous studies have been reported in this aspect, plant growth promoting capability for commercialisation of *R. palustris* as bio-fertilisers is still remains challenge. The in-depth research focusing on crop quality enhancement (e.g., metabolites and nutrients content) should also be widely explored to assess the efficacy of *R. palustris* as bio-fertilisers fully. The inclusion of *R. palustris* application in other fields (e.g., wastewater treatment, biofuel production) can be further reviewed to provide a more comprehensive overview of *R. palustris* as commercial multi-functional bacteria. The inter-disciplinary studies and investments by researcher, industry, and government should be further progressed by focusing on the formulation and expansive commercialisation of *R. palustris* as bio-fertilisers to promote sustainable agriculture.

Acknowledgements

The authors would like to acknowledge the research grants from the Ministry of Education (MOE) Malaysia with grant no. R.J130000.7809.5F147. This research has also been supported by the EU project “Sustainable Process Integration Laboratory – SPIL”, project no. CZ.02.1.01/0.0/0.0/15_003/0000456 funded by European Research Development Fund, Czech Republic Operational Programme Research, Development and Education, Priority 1: Strengthening capacity for quality research, in a collaboration agreement with UTM, Johor Bahru, Malaysia.

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