Science World Journal Vol. 19(No 2) 2024 https://dx.doi.org/10.4314/swj.v19i2.9
www.scienceworldjournal.org
ISSN: 1597-6343 (Online), ISSN: 2756-391X (Print)
Published by Faculty of Science, Kaduna State University

MOLECULAR IDENTIFICATION OF *RHODOPSEUDOMONAS PALUSTRIS* ISOLATED FROM SOIL IN WASTE DUMP SITE IN MINNA METROPOLIS

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ABSTRACT
Rhodopseudomonas palustris, is a purple non-sulphur bacterium with properties of extraordinary metabolic versatility, carbon source diversity and metabolite diversity. Due to its biodetoxification and biodegradation properties, *R. palustris* has been traditionally applied in wastewater treatment and bioremediation, and also in agriculture. This study aimed to isolate and identify *R. palustris* from waste dump site in Minna, Nigeria, using conventional and molecular techniques. Soil samples were collected at 3 cm depth from different points at waste dump sites in Minna, Niger State, Nigeria. Inoculation was made on Nutrient agar plates and incubated at 30 ºC under 60 W incandescent light for 7days. The suspected bacterial isolate, based on cultural and morphological characteristics as well as biochemical reactions was subjected to molecular ribotyping. A PCR product of approximately 789 bp DNA maker was detected by agarose gel electrophoresis using 1500 bp Lamda maker. The DNA fragments of the isolates were obtained from DNA sequencing and were deposited in the NCBI-GenBank database. Based BLASTn search in GenBank using the fragments as the query, showed that the 16S rDNA of EG-4 share 99% sequence identity with *Rhodopseudomonas palustris*. Results of this study indicate the presence of *R. palustris* in waste dump site in Minna.

Keywords: *Rhodopseudomonas palustris*, waste dump, molecular ribotyping, photosynthetic bacteria, PCR.

INTRODUCTION

Background to the study

Rhodopseudomonas palustris is a purple non-sulphur photosynthetic bacterium (PNSB) in the class, Alpha-proteobacteria and phylum, Proteobacteria (Su *et al.,* 2019). It is a bacterium with the properties of extraordinary metabolic versatility, carbon source diversity and metabolite diversity. Given its adaptable metabolism, *R. palustris* has been studied and used in an extensive variety of applications such as examining metabolic tradeoffs for environmental perturbations, biodegradation of aromatic compounds, environmental remediation, biofuel production, agricultural biostimulation, and bioelectricity production (Brown *et al*., 2022). *R. palustris* is rich in various metabolites, contributing to its application in agriculture, aquaculture and livestock breeding as additives. It is widely distributed in nature, mainly in anaerobic water environments with sufficient light, such as lakes, soils, swamps, and the sea (Li *et al.,* 2022). As a result of its metabolic versatility, it grows by any one of the four modes of metabolism that support life: it can grow as photoautotrophic or photosynthetic (energy from light and carbon from carbon dioxide), photoheterotrophic (energy from light and carbon from organic compounds), chemoheterotrophic (carbon and energy from organic compounds) and chemoautotrophic (energy from inorganic compounds and carbon from carbon dioxide). *R. palustris* enjoys exceptional flexibility within each of these modes of metabolism. It grows with or without oxygen and uses many alternative forms of inorganic electron donors, carbon and nitrogen. It degrades plant biomass and chlorinated pollutants and generates hydrogen as a product of nitrogen fixation (Larimer *et al*., 2003; Venkidusamy & Megharaj, 2016). Thus *R. palustris* is a model organism to probe how the web of metabolic reactions that operates within the confines of a single cell adjusts and reweaves itself in response to changes in light, carbon, nitrogen and electron sources that are easily manipulated experimentally (Larimer *et al*., 2003; Venkidusamy & Megharaj, 2016). The aim of this study was to isolate and genetically characterize *R. palustris* from waste dump sites in Minna, Nigeria.

MATERIALS AND METHODS

Study Area

The study was carried out in Minna metropolis, Niger State, Nigeria. Minna, the capital of Niger State is located between Longitude 3˚30' E and 7˚20' N and Latitudes 8˚20' N and 11˚30' N. Minna is about 135km away from the Federal Capital Territory and 300 km away from Kaduna city. The town lies on a relatively high land with a site height of between 240m - 270m above sea level (Popoola et al., 2016).

Collection of Samples

Soil samples were collected using hand trowel, at 3cm depth from different points at waste dump sites. The samples were collected into clean polythene bags and transported to the Microbiology laboratory for the isolation of bacteria.

Isolation of bacteria from Waste dump soil

One gramme of soil was serially diluted in sterile distilled water and mL of the suspension was inoculated in Nutrient Agar in duplicates, using pour plate method. The inoculated Nutrient agar (NA) plates were incubated at 30 ºC under 60 W incandescent light for 7days according to Mehrabi, (2001). Several orange colonies appeared after incubation and the most dominant ones were subcultured into screw cap tubes filled with nutrient broth medium and was incubated under the light at room temperature for enrichment.

Characterization and identification of isolates

The suspected bacterial isolates were characterized based on their cultural and morphological characteristics as well as biochemical

reactions. Gram staining and biochemical tests were carried out according to the methods outlined by Oranusi *et al*. (2013). The identities of the potential isolate were confirmed by comparing the characteristics with those of known taxa using Bergey's Manual of Determinative Bacteriology (Buchanan and Gibbons, 1994). The molecular ribotyping of the bacterial isolate was performed to further confirm the identities of the isolate.

Molecular ribotyping
Molecular ribotyping of the bacterial isolate was carried out using the partial gene sequence of 16S rRNA (Green & Sambrook, 2012).

Extraction of genomic DNA
The bacterial isolate on solid medium was scraped by adding 1000µl of 1× phosphor buffer saline (PSB), using wire loop. The content was transferred into a 1.5 mL tube. To the content, 400µl of lysis buffer and 25µl proteinase K were added, mixed gently and incubated at 60 °C for 1h. To the same tube, 400µl of phenol chloroform (1:1) was added to the lysate, votexed briefly and spun at 10000rpm for 10minutes to separate the phases. The supernatant was carefully transferred into a fresh sterile 1.5ml tube using sterile cut tip. Equal volumes of 100% ethanol and 20µl of 3M sodium acetate were added to the supernatant and mixed by inverting the tube several times. This was incubated at -20 ºC overnight. The tube was spun at 14,000rpm for 10 minutes (in refrigerated centrifuge) in the same orientation. The ethanol was extracted using micro pipette and another 400µl of 70% ethanol was added. This was spun at 14,000rpm for 5 minutes at 4 ºC (in refrigerated centrifuge). The last step was repeated twice to make sure that the salt (sodium acetate) was all gone. The tube was spun for 30sec at 13,000rpm and all traces of ethanol were removed as much as possible. The DNA was dried out by leaving the tube open for 30 minutes. The DNA pellet was resuspended in 100µl deionized water (Green & Sambrook, 2012).

Polymerase Chain Reaction (PCR)
PCR was performed in a thermal cyder (Eppendorf master cycler personal) (Green & Sambrook, 2012).

PCR mix composition
Deionized water - 90µl, Enzyme buffer - 2µl, Forward primer (Ribose 1 – 5´GGA CTA CA GGG TAT CT AAT´3) and Reverse primer (Ribose 2- 5´AGA GTT TGA TCC TGG´3), Sample DNA-2µL.

PCR amplification condition
This involves initial denaturation 95°C (5 min.) (1cycle), Denaturation 94°C (1 min), Annealing 52°C (I min.), Primer extension 72°C (1 min.), Final extension 72°C (7 min.) (35 cycles) (Green & Sambrook, 2012).

Agarose gel electrophoresis
Agarose gel (1.5g) was mixed with 100µL of 1× Triacetate. The agarose solution was heated in a microwave until agarose was completely dissolved. This was allowed to cool in a water bath set at 50 ºC. A gel casting tray was prepared by sealing ends of gel chamber with tape. Two combs were placed in the gel tray. A quantity of 5µl of ethidium bromide was added to the cooled gel and poured into gel tray. This was allowed to cool for 30 min at room temperature. The combs were removed from the gel tray and placed in electrophoresis chamber and covered with buffer. The DNA and standard marker (Ladder) as well as the negative control (8µL each) were loaded onto the gel and set for electrophoresis at 94 volts for 26minutes. The DNA bands were visualized using gel imaging system (Green & Sambrook, 2012).

DNA sequencing
Nucleotide sequences of the PCR amplicon were determined by using the ABI Prism 310 genetic analyzer, using the big dye Terminator kit (Applied Biosystems) at BioServe India Ltd., Hyderabad. The identity of the sequences determined was established by a comparison with the sequences obtained with the gene sequences available in the database using NCBI - BLAST (Begum & Alimon, 2020).

RESULTS

Identification of bacterial isolates
The bacterial growths on Nutrient agar were orange-coloured colonies (Plate I). The Gram reaction showed Gram negative rods. Biochemical tests which included citrate, catalase, mannitol, sucrose, starch hydrolysis and others were carried out and the results are presented in Table 1.

Table 1: Cultural, morphological and biochemical characteristics of bacteria isolated from waste dump

Characteristics	Results
Isolate	GE-4
Colony characteristics	Orange-coloured
Cell shape	Rod
Gram's reaction	-
Motility	+
Presence of spore	-
Glucose	+
Fructose	+
Lactose	-
Sucrose	+
Maltose	-
Arabinose	-
Galactose	-
Mannitol	-
M R	-
V P	-
Citrate	-
Catalase	-
Indole	-
Amylase	+
Oxidase	+
Starch	-
Urease	-
Casein	-
H_2S	-

+ = present; - = not present; M R= methyl Red; V P= Voges-Proskeur

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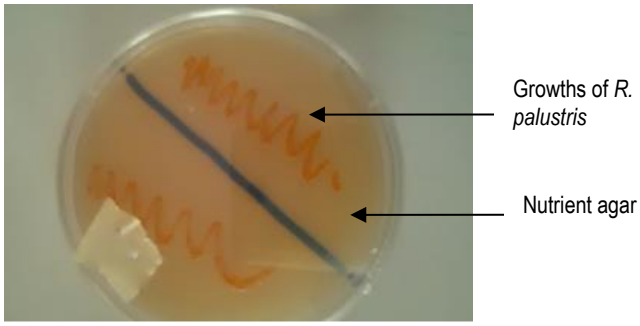

Plate I: *Rhodopseudomonas palustrus* on Nutrient agar

The identities of *Rhodopseudomonas palustris* confirmed by ribotyping showing the PCR amplicon of 16S rRNA gene using gel electrophoresis in PLATE II and partial gene sequence of 16S rRNA, which was amplified and sequenced, as shown in Fig 1. The primer generated amplicon at 789 base pair (Plate II).

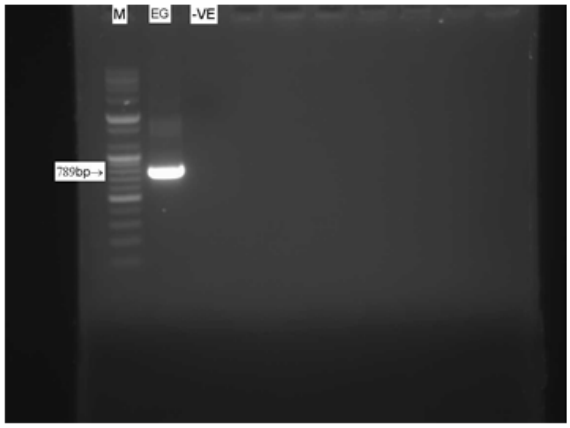

Plate II: The PCR amplicon of 16S rRNA gene obtained from *Rhodopseudomonas palustris*
Lane M: Lamda DNA marker
Lane EG: Amplified 16S rRNA gene of isolate.

The DNA fragments of the isolates were obtained from DNA sequencing and were deposited in the NCBI-GenBank database. Based BLASTn search in GenBank using the fragments as the query showed that the 16S rDNA of EG-4 shared 99% sequence identity with *Rhodopseudomonas palustris*.

DISCUSSION

Rhodopseudomonas palustris is a soil-borne phototrophic bacterium and possesses a metabolic versatility, an endowment that enables it to grow in diverse environmental conditions. Lignocellulosic biomass hydrolysates can be degraded by *R. palustris* (Li *et al.*, 2022), hence its presence in waste dump site, which is composed of many wastes of plant origin, containing lignin and celluloses. *R. palustris* thrives in such environments because it metabolizes structurally diverse compounds found as components of degrading plant and animal wastes (Larimer *et al.*, 2003).

Rhodopseudomonas palustris was reportedly isolated from oil-contaminated soil by Nalvothula *et al.* (2022) under anaerobic conditions, in contrast with the present study, in which the bacterium was isolated under aerobic conditions. *R. palustris* is a Purple non-sulphur Bacteria (PNSB), which constitute a group of versatile organisms in which most exhibit four modes of metabolism: photoautotrophic, photoheterotrophic, chemoheterotrophic and chemoautotrophic, switching from one mode to another depending on conditions available (Larimer *et al*., 2003). PNSB can capture CO_2 under anaerobic conditions, a photoautotrophic mechanism very different from cyanobacteria (Grattieri, 2020). Ribulose 1,5-bisphosphate (RuBP) carboxylase/oxygenase (RubisCO) is responsible for CO_2 fixation by catalyzing the carboxylation of RuBP *via* the CBB cycle (Li *et al*., 2022). *R. palustris* can use inorganic matters as the carbon source, such as CO_2, which is fixed by the Calvin Bassham Benson (CBB) cycle to participate in cell growth metabolism using thiosulfate as the electron donor (Huang *et al*., 2010).

This metabolic versatility allows *R. palustris* to use light, inorganic, and organic compounds as its carbon and energy sources under anaerobic or aerobic conditions. Photosynthesis genes enable the use of light as an energy source by cyclic photophosphorylation under anaerobic conditions (Larimer *et al*., 2003; Venkidusamy & Megharaj, 2016). Hsu *et al*. (2021) reported the isolation of two strains of *R. palustris* from paddy fields. Kim *et al.* (2004), also reported the isolation of *R. palustris* from eutrophicated ponds. Purple photosynthetic bacteria are a major component of microbial populations found in wastewater treatment facilities exposed to sunlight. In the report of Jiao *et al.* (2005) and that of Del Socorro *et al.* (2013), the criteria used to validate the bacterial isolate as *Rhodopseudomonas palustris* agree with the results of this study.

Conclusion

In conclusion, the results of this study shows that *R. palustris* can be isolated from waste dump site with particular attention to light source. Further molecular research of *R. palustris* for its application and characterization, exploring more genetic editing tools is necessary.

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