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## Arsenic respiration and detoxification by purple non-sulphur bacteria under anaerobic conditions



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## ABSTRACT

Two arsenic-resistant purple non-sulphur bacteria (PNSB), Q3B and Q3C, were isolated (from industrial contaminated site and paddy fields) and identified by SSU rRNA gene sequencing as *Rhodospirillum* and *Rhodospirillaceae* species, respectively. Maximum arsenic reduction by these PNSB was observed in anaerobic conditions. *Rhodospirillum* sp. Q3B showed 74.92% (v/v) arsenic reduction while *Rhodospirillaceae* sp. Q3C reduced arsenic up to 76.67% (v/v) in anaerobic conditions. *Rhodospirillaceae* sp. Q3C was found to contain highest carotenoid content up to 5.6 mg·g<sup>-1</sup>. Under anaerobic conditions, the isolates were able to respire arsenic in the presence of lactate, citrate, and oxalate. *Rhodospirillum* sp. Q3B and *Rhodospirillaceae* sp. Q3C were also found to produce hydrogen gas. Such diverse bacteria can be useful tools for bioremediation purposes. These bacteria can be further exploited and optimized to treat wastewater containing arsenic along with bio-hydrogen production.

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### 1. Introduction

Arsenic (As) is one the most toxic metals and its contamination occurs due to natural processes as well as human activities [1]. This toxic metalloid can find its way to water bodies causing drinking water contamination. Arsenic, therefore, can find its way in many foods such as rice, which is a staple food in many countries [2]. Long-term As exposure leads to arsenicosis that causes major health problems such as cancers of different body parts [3]. Arsenite [As(III)] and arsenate [As(V)] are the most predominant forms of As, and As(III) is known to be 100 times more toxic than As(V) [4]. Arsenic is a metal that

is toxic for all life forms, including plant, animal, bacteria, and human [5].

Many microorganisms have the ability to resist and detoxify As and other such toxic metals through mechanisms such as exclusion [6], compartmentalization [7], formation of complexes [6], biosorption [8], and enzymatic detoxification [9]. The genes responsible for As resistance and detoxification are located in the *ars* operon [10]. On the other hand, biosorption is a physiochemical process of removing toxic heavy metals from environment with the help of living organisms [11]. Biosorption utilizes dead or living biomass to chelate heavy metals from aqueous solutions. In this process, sorbate is any metal ion or molecule that is to sorb on a sorbent that is any solid surface, thus forming a sorbate-sorbent complex. A variety of functional groups is present at the bacterial cell surfaces that serve as binding sites for metallic ions [12].

Purple non-sulphur bacteria (PNSB) are one of the most diverse group of bacteria, capable of adapting a variety of

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metabolic life styles depending on the type of nutrients available in their habitat and on the presence or absence of oxygen as well as light. They can grow photoheterotrophically in anoxic light environment, oxidizing various organic carbon sources while respiring difference chemical compounds, thus reducing them in the process. Many PNSB are also known to resist and detoxify many toxic metals and elements such as As. PNS bacteria are also known to produce hydrogen when cultured anaerobically in the presence of incandescent light, which is a clean and renewable fuel and is widely accepted as a potential substitute for fossil fuels [13].

The objective of the present study was to check PNSB tolerance towards As and to determine whether these bacteria can detoxify As by respiring it. PNSB could be one of the ideal organisms for the bioremediation of As and such toxicants due to their versatile metabolic nature [14].

## 2. Materials and methods

### 2.1. Samples collection

Two samples were collected separately. One sample was taken from Quaid-e-Azam Industrial Estate, Kot Lakhpat, Lahore, Pakistan. The other sample was taken from paddy fields, University of the Punjab, Lahore, Pakistan. The samples were collected in such a way that they contained both water and the corresponding sediments. Both samples were taken from a depth of 2–2.5 feet.

### 2.2. Enrichment and isolation

Arsenic-resistant PNSB were enriched by inoculating the samples in a Mineral Salt Succinate (MSS) broth [15] containing 1.0 mM of As(V) ( $\text{Na}_2\text{HAsO}_4 \cdot 7 \text{H}_2\text{O}$ ). The medium was prepared anaerobically in 500-mL Schott bottles and the enrichment cultures were incubated under continuous incandescent light of  $\sim 3000$  lux at  $30^\circ\text{C}$  (Memmert model ICP, Germany) for two weeks. After incubation, different serial dilutions of the enrichment cultures were plated on the MSS medium both with and without As(V). The plates were incubated in an anaerobic jar (OXOID AG0025, England) containing an anaerobic sachet (Thermo Scientific Oxoid AnaeroGen, England) placed under continuous incandescent light at  $30^\circ\text{C}$  for two weeks. Morphologically diverse colonies were picked and purified by several rounds of quadrant streaking. Gram reaction, catalase, and oxidase tests were performed. All experiments were performed in triplicates if not mentioned otherwise.

### 2.3. Determination of metal resistance in the isolates

An MSS broth containing 1.0, 2.5 and 5.0 mM of As(III) and 1.0, 2.5, 5.0 and 10 mM As(V) was prepared separately. In the case of Cu, Hg, Co, Ni, Se, Pb, and Cr, a concentration of 0.5 mM was used in the MSS broth. The tubes were inoculated with fresh MSS broth cultures anaerobically and aseptically. The tubes were placed in continuous incandescent light at  $30^\circ\text{C}$  for 1 week.

### 2.4. Determination of As-respiration

MSS agar was prepared and the carbon source, i.e. sodium succinate, was replaced with other carbon sources such as citrate, oxalate, lactate, acetate, and propionate. Such media were prepared both with and without 0.5 mM As(V). The medium was inoculated with the bacterial isolates and incubated in anaerobic jars under continuous incandescent light (3000 lux) at  $30^\circ\text{C}$  for 1.0 week.

### 2.5. Estimation of pigment production

#### 2.5.1. Estimation of photopigment

The MSS broth was inoculated with a fresh bacterial culture and incubated under anaerobic conditions. The bacterial cells were harvested at 1844 g for 20 min. The supernatants were discarded, and the pellets were resuspended in bovine serum albumin with 0.25% w/v. Absorbance was taken at 495 nm, 591 nm, and 864 nm with an UV-Vis spectrophotometer (CECIL CE 7200, Aquarius, UK).

#### 2.5.2. Estimation of dry cell weight & carotenoid content

Bacterial cells were harvested by centrifugation at 6869 g for 20 min at  $4^\circ\text{C}$  (Sigma 2K30 Laborzentrifugen, Germany) and resuspended in a phosphate buffered saline 1.0X at pH 7.4. Each cell suspension was divided into two sets by shifting half of the cell suspensions into fresh tubes. The cells were harvested by centrifuging them again. Supernatants were discarded, and pellets were washed thrice with autoclaved water. One set of pellets was dried by placing them in a hot-air oven at  $105^\circ\text{C}$  for 24 hours. The dry cell weight was calculated by the following formula taken from Al-Azad, Soon, and Ransangan [16]:

$$\text{dry cell weight (g}\cdot\text{L}^{-1}) = (\text{final weight} - \text{initial weight}) / V \times 1000$$

where

initial weight = weight of the tube (g)

final weight = weight of the tube (g) + dry pellet weight (g)

$V$  = volume of the sample (mL).

The other set of pellets was resuspended in an acetone-methanol solution (2:7 v/v). The suspensions were covered with an aluminium foil and incubated at  $4^\circ\text{C}$  for 30 min. Following this the suspensions were centrifuged again and the optical density of the supernatants was measured at 480 nm using an UV-Vis spectrophotometer (CECIL CE 7200, Aquarius, UK). The carotenoid content was calculated using the formula given by Jalal et al. [17]:

$$C = D \times V \times F (2500) / \text{dry weight of the sample (g)}$$

where

$C$  = total carotenoid content ( $\text{mg}\cdot\text{g}^{-1}$ )

$D$  = absorbance at 480 nm

$V$  = total volume of the sample used

$F$  = dilution factor of the sample

2500 = average extinction coefficient for carotenoids.

### 2.6. Determination of As oxidation/reduction

A bacterial inoculum was prepared aerobically in an MSS broth. The cells were harvested by centrifugation at 1428 g. The pellets were resuspended in 10 mL of 0.09%

(v/v) normal saline. Then the cell suspension was distributed in different tubes that were then supplemented with 0.5 mM of As(V) or 0.1 mM of As(III). Controls were also set up that were devoid of any As. Half of the tubes were incubated aerobically while the other half was incubated anaerobically, both for 5 h at 30 °C. After incubation, the suspensions were further divided into two equal halves. The suspensions were centrifuged at 1844 g for 10 min. Supernatants and pellets of one half of the tubes were separated and stored at –20 °C till further analysis. The pellets of the other half of the tubes were resuspended in 0.5 M EDTA. EDTA cell suspensions were again centrifuged at 1844 g for 10 min, and the supernatant was then transferred to fresh tubes and stored at –20 °C till further analysis.

### 2.6.1. Qualitative analysis of As oxidation/reduction

As oxidation/reduction in the above-mentioned stored samples was determined following the method of Salmassi et al. [18]. One hundred and sixty-six microliters of each sample was added in the wells of a microtiter plate along with 5 µL of 0.01 M KMnO<sub>4</sub>, and colour change was observed. Negative controls (not containing any arsenate) and positive controls (arsenate standards of known concentrations) were also used. Purple to yellowish colour change indicates As(V) reduction. The selected isolates were investigated further for extent of As oxidation/reduction through quantitative As assay.

### 2.6.2. Quantitative analysis of As oxidation/reduction

The extent of As oxidation/reduction was estimated following the method of Cummings et al. [19] with minor modification, as reported by Shafique et al. [20]. Absorbance was taken at 865 nm using a spectrophotometer (CECIL CE 7200, Aquarius, UK). The standard curve was made by treating the standards similarly. The concentration of oxidized/reduced As was calculated using the formula of the standard curve.

### 2.7. Detection of hydrogen gas production

For detection of hydrogen gas production, the syringe displacement method was used [21]. For this purpose, an MSS broth medium was prepared with some modifications. The medium contained 0.33 g of KH<sub>2</sub>PO<sub>4</sub>, 0.33 g of MgSO<sub>4</sub>·7 H<sub>2</sub>O, 0.33 g of NaCl, 0.5 g of NH<sub>4</sub>Cl, 1.0 g of sodium succinate, 20 mg of yeast extract, 0.5 g of K<sub>2</sub>HPO<sub>4</sub>, 0.4 g of L-glutamine, 1.0 mL of trace salt solution, 500 µL of 0.0001% (v/v) FeSO<sub>4</sub>·7 H<sub>2</sub>O, and 1.0 mL of vitamin solution per litre. The solution of vitamins contained 10 mg of biotin, 35 mg of niacin, 30 mg of thiamine dichloride, 20 mg of *p*-aminobenzoic acid, 10 mg of calcium pantothenate, 5 mg of vitamin B12, 10 mg of pyridoxine hydrochloride per 100 mL of distilled water. The solution of vitamins was sterilized by filtration and added to the medium aseptically after autoclaving. The medium was prepared anaerobically and dispensed in autoclaved serum bottles aseptically. The bottles were sealed with rubber stoppers and aluminium crimps. The inoculum was prepared in LB-broth. Syringe needles (3 mL) were inserted in serum bottles through the rubber stoppers

aseptically so that the tip of the needle was dipped in the medium. The bottles were incubated under continuous incandescent light at 30 °C for 1.0 week. The displacement of the syringe plungers was taken as an indication of gas production.

### 2.8. SSU rRNA gene sequencing and phylogenetic analysis

PCR was performed to amplify the SSU rRNA gene by using 27f (AGAGTTTGATCCTGGCTCAG) and 1522r (AAGGAGGTGTGCCARCCGCA) primers. The selected isolates were sequenced for SSU rRNA gene from Macrogen Inc. (South Korea). The sequences obtained by this Sanger sequencing method were checked for base call quality through Finch TV and were cleaned. The NCBI BLAST tool was used to find homology between the isolates and other bacteria.

### 2.9. Statistical analysis

Statistical analysis was performed in Microsoft Excel 2013. The results were displayed as the mean values and the standard deviation was displayed as error bars.

## 3. Results

### 3.1. Isolation, characterization and identification of As-resistant PNSB

The temperature of the sample taken from Quaid-e-Azam Industrial Estate was 34 °C, and its pH was 8.0. The temperature of the sample taken from paddy fields, University of the Punjab, was 29 °C; its pH was 7.5. Five isolates were selected based on arsenic resistance, reduction potential, and biosorption. Three of the isolates (Q3C, Q3B, and Q2B) belonged to Quaid-e-Azam Industrial Estate, Kot Lakhpat, Lahore, Pakistan, whereas the rest of the two isolates (PFI and PFII) were from paddy field, University of the Punjab, Lahore, Pakistan. Maroon colonies of PNSBs were obtained on the MSS agar plates, which were then purified by many rounds of quadrant streaking. All the isolates were gram-negative rods, and catalase and oxidase positive. SSU rRNA gene sequence analysis and NCBI BLAST results revealed the identity of Q3B as *Rhodospirillum* species (95% homology) and of Q3C as *Rhodospirillaceae* sp. (98% homology). The sequences were submitted in Genbank under accession numbers MH807574 and MH807575 for Q3B and Q3C, respectively.

### 3.2. Resistance of toxic metals in PNSB

*Rhodospirillum* sp. Q3B, *Rhodospirillaceae* sp. Q3C and PFII showed resistance up to 10 mM As(V). *Rhodospirillaceae* sp. Q3C was able to resist 5 mM As(III) (Table 1). Se and Ni (0.5 mM) were resisted by all five isolates. Co (0.5 mM) was resisted by all isolates except isolate H1. All isolates showed resistance to Pb (0.5 mM), except *Rhodospirillaceae* sp. Q3C. Cu (0.5 mM) was resisted by all the isolates, except *Rhodospirillaceae* sp. Q3C and isolate H2 (Table 2).

**Table 1**  
Arsenic resistance by PNSB.

Bacterial isolates	Arsenic resistance						
	As(V) 1.0 mM	As(V) 5.0 mM	As(V) 10 mM	As(III) 0.5 mM	As(III) 1.0 mM	As(III) 2.5 mM	As(III) 5 mM
<i>Rhodospirillum</i> sp. Q3B	+++	+++	+++	+++	+++	–	–
<i>Rhodospirillaceae</i> sp. Q3C	+++	+++	++	+++	+++	++	++
PFII	+++	+	+	+++	+++	+	+
H1	+++	–	–	–	+++	–	–
H2	+++	+	–	+	–	–	–

PNSB: purple non-sulphur bacteria.

+++; maximum growth; ++; intermediate growth; +; slight growth; –; no growth.

**Table 2**  
Resistance to toxic metals by PNSB.

Bacterial isolates	Hg 0.5 mM	Pb 0.5 mM	Ni 0.5 mM	Se 0.5 mM	Cr 0.5 mM	Co 0.5 mM	Cu 0.5 mM
<i>Rhodospirillum</i> sp. Q3B	–	++	+	++	–	++	++
<i>Rhodospirillaceae</i> sp. Q3C	–	–	+	++	–	+++	–
PFII	–	++	+	+	–	++	+
H1	–	+	+	++	–	–	+++
H2	–	+	+	+	–	+	–

PNSB: purple non-sulphur bacteria; Hg: mercury; Pb: lead; Ni: nickel; Se: selenium; Cr: chromium; Co: cobalt; Cu: copper.

+++; maximum growth; ++; intermediate growth; +; slight growth; –; no growth.

### 3.3. As-respiration by PNSB

Anaerobic fermentation of the carbon sources by the bacterial isolates was analysed in the absence of As. Acetate was fermented by *Rhodospirillaceae* sp. Q3C, PFII and isolate H2, as growth was observed by these bacteria under anaerobic conditions in media containing only this carbon source. Lactate was fermented only by PFII. Citrate and oxalate were fermented by *Rhodospirillum* sp. Q3B and PFII, while propionate was fermented by *Rhodospirillum* sp. Q3B, PFII and H2 (Table 3).

Those isolates that did not ferment different carbon sources were provided with As(V) to check whether these bacteria can use these carbon sources as electron donors and As(V) as electron acceptor, thus respiring As. All the isolates were able to respire As(V) in the presence of lactate, citrate, and oxalate, except PFII. All the isolates except H1 were able to respire As(V) in the presence of acetate. In the presence of propionate, all the isolates showed As(V) respiration except isolate H2 (Table 3).

### 3.4. Photopigment production by PNSB

All isolates showed maximum absorption at 864 nm, indicating the presence of bacteriochlorophyll a [22] (Table S1).

### 3.5. Dry cell weight and carotenoid content measurements

*Rhodospirillum* sp. Q3B, *Rhodospirillaceae* sp. Q3C and PFII showed 8.33, 1.67 and 16.6 g·L<sup>-1</sup> of dry cell weight, respectively. *Rhodospirillaceae* sp. Q3C was found to contain highest carotenoid content up to 5.6 mg·g<sup>-1</sup>. Isolate PFII contained 0.18 mg·g<sup>-1</sup> and *Rhodospirillum* sp. Q3B contained 1.1 mg·g<sup>-1</sup> of carotenoid content (Table S1).

### 3.6. As oxidation/reduction by PNSB

In qualitative assays, all the isolates showed colour change from purple to yellowish brown, indicating the reduction of As(V) to As(III). The colour change was more

**Table 3**  
Carbon source utilization and As-respiration by PNSB.

Strains	Carbon sources Fermentation [without As(V)]					Carbon sources Respiration [with As(V)]				
	Acetate	Lactate	Citrate	Propionate	Oxalate	Acetate	Lactate	Citrate	Propionate	Oxalate
<i>Rhodospirillum</i> sp. Q3B	–	–	–	+	++	++++	+++	++++	+++	+
<i>Rhodospirillaceae</i> sp. Q3C	++	–	–	–	–	++++	++	++++	+	+++
PFII	++	+	++	++	++	++++	++	++	++++	++
H1	–	–	–	–	+	–	+++	++++	++	+
H2	+	–	–	–	–	++++	+	+	–	+

PNSB: purple non-sulphur bacteria.

++++; maximum growth; +++; better growth; ++; intermediate growth; +; slight growth; –; no growth.

pronounced in anaerobic conditions as compared to aerobic conditions. In quantitative assays, *Rhodospirillaceae* sp. Q3C was found to show 7.92% (v/v) biosorption aerobically and 11.675% (v/v) anaerobically. As(V) reduction by this bacterium was 47.42% (v/v) aerobically and 76.675% (v/v) anaerobically. *Rhodospirillum* sp. Q3B showed 14.43% (v/v) biosorption aerobically and 19.27% (v/v) anaerobically. This bacterium showed 56.67% (v/v) As(V) reduction aerobically and 74.92% (v/v) As(V) reduction anaerobically (Fig. 1).

3.7. Detection of hydrogen gas production by PNSB

*Rhodospirillum* sp. Q3B and *Rhodospirillaceae* sp. Q3C showed gas production under anaerobic conditions in the presence of incandescent light, as indicated by the displacement of syringe plungers. No gas production or

displacement of the syringe plunger was detected in the control serum bottles (Fig. 2).

4. Discussion

Being metabolically diverse, PNSB hold great potential as tools for biotechnology purposes such as wastewater treatment, bioremediation, and bio-hydrogen production. They can prove a very inexpensive way to deal with many environmental problems as they can easily grow in a variety of environments such as rice fields, industrial effluents, marine water, coastal lagoons, fish ponds, etc. [23,24]. Metal-resistant PNSB have potential use for remediation of heavy metal contaminated sites [2,25]. The present study focuses on the ability of PNSB to detoxify and respire As along with photopigment, carotenoid, and hydrogen production.

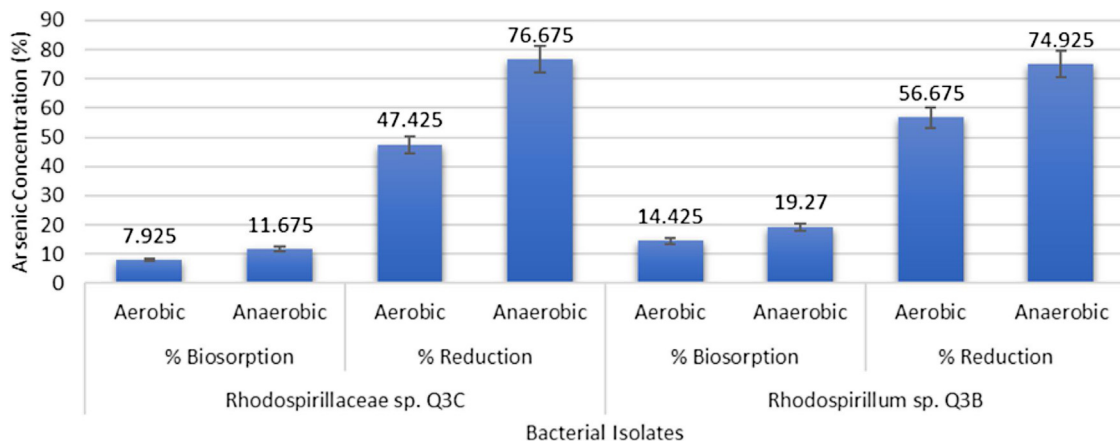


Fig. 1. Arsenic reduction and biosorption by *Rhodospirillum* sp. Q3B and *Rhodospirillaceae* sp. Q3C.

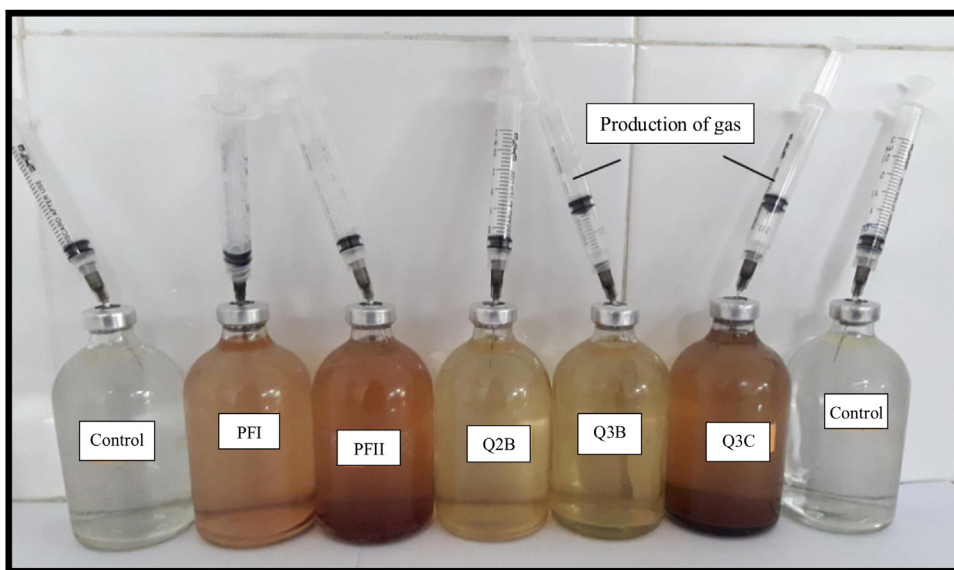


Fig. 2. Hydrogen gas production demonstrated by purple non-sulphur bacteria (PNSB) isolates *Rhodospirillum* sp. Q3B and *Rhodospirillaceae* sp. Q3C under anaerobic conditions.

Arsenic detoxification by the bacteria was initially determined by qualitative method using  $\text{KMnO}_4$  in which colour changes from purple to yellowish brown indicated reduction of As(V) to As(III). The colour change was more pronounced in anaerobic samples of *Rhodospirillaceae* sp. Q3C, *Rhodospirillum* sp. Q3B and H1. These isolates were further analysed for the extent of As- reduction and biosorption [9]. Bacteria-mediated metal reduction is a process in which bacteria transforms the valence state of the metal, affecting its toxicity. This is followed by extrusion of the metal out of the bacterial cell [6]. *Rhodospirillaceae* sp. Q3C was highly efficient and reduced 76.67% (v/v) As(V) in anaerobic conditions, while in aerobic conditions *Rhodospirillum* sp. Q3B reduced the highest As(V) concentration up to 56.67% (v/v). Bacteria possess this reduction potential due to the presence of *ars* operon and other related detoxification mechanisms [26]. Batool et al. [27] reported 62.9% (v/v) As(V) reduction by *R. palustris* strain CS2 ( $6.29 \pm 0.24$  mM). These purple non-sulphur bacteria were also found to resist other heavy metals. Sakpirom et al. [28] reported *R. palustris* strain TN110 to reduce Cd up to  $84.21 \pm 0.28\%$  (v/v) and Zn up to  $54.83 \pm 0.70\%$  (v/v).

Many bacteria are known to oxidize organic compounds anaerobically by reducing As, a process that is called As-respiration [29]. Many As-transforming PNSB of the present study were found to grow anaerobically only when provided with As(V). Thus these bacteria obtained energy by respiring As(V). Huber et al. [30] reported that *Pyrobaculum arsenaticum* (type strain PZ6), a strict anaerobic archaeon isolated from hot spring, utilizes As(V) as a terminal electron acceptor in respiration. Yamamura et al. [31] reported dissimilatory arsenic reduction by *Bacillus* sp. strain SF-1. The bacterium was also found to reduce selenate under anaerobic conditions. Though many bacteria are known to respire arsenic, reports of PNSB involved in arsenic respiration are, however, scarce. Nookongbut et al. [32] reported possible arsenic respiration by *Rhodopseudomonas palustris* strain C1.

Maximum carotenoid content was observed in *Rhodospirillaceae* sp. Q3C up to  $5.6 \text{ mg}\cdot\text{g}^{-1}$ . There is an indirect correlation between dry cell weight and carotenoid content. Heat from light source is trapped by high cell density, thus affecting the carotenoid content as it is heat sensitive [33]. Jalal et al. [34] reported highest carotenoid content by *Rhodopseudomonas* sp. IIUM-JHR2 up to  $4.08 \pm 2.21 \text{ mg}\cdot\text{g}^{-1}$  after three days of incubation.

The bacteria were identified by SSU rRNA gene sequence classification via NCBI BLAST. Q3B showed 95% homology with *Rhodospirillum rubrum*. *R. rubrum* is an important PNSB that has showed significant potential in hydrogen and bioplastic production, as mentioned by Reslewic et al. [35]. Q3C showed 98% homology with *Rhodospirillaceae* species. The family *Rhodospirillaceae* is composed of 18 species placed in the four genera *Rhodopseudomonas*, *Rhodospirillum*, *Rhodocyclus*, and *Rhodomicrobium*, many of which are known to produce hydrogen [36].

Under anoxygenic light conditions, the gas produced by PNSB is hydrogen gas [37]. PNSB have the ability to produce hydrogen gas when suitable conditions and

environment are provided. Bianchi et al. [21] reported *Rhodopseudomonas palustris* AV33 as one of the best candidates to produce hydrogen gas (up to 1.2 L per litre of culture). Basak and Das [38] reported *Rhodobacter sphaeroides* strain O.U.001 to produce hydrogen in bioreactor up to  $4.5 \pm 0.05$  mol. *Rhodopseudomonas palustris*, with some mutation in its gene that constitutively express nitrogenase genes, has the ability to produce specifically hydrogen gas when supplied with media containing ammonium as a nitrogen source [39,40]. In PNSB, hydrogen production occurs by process called photofermentation that utilizes the breakdown of small-chain fatty acids into  $\text{H}_2$  and  $\text{CO}_2$  [41]. The enzyme responsible for hydrogen production in PNSB is nitrogenase. In the absence of nitrogen, the nitrogenase enzyme of PNSB is fully dedicated to  $\text{H}_2$  production [42]. However, minor amounts of other gases such as  $\text{CO}_2$  can also be produced when PNSB perform photofermentation [43].

In the light of all above-mentioned experiments, it is clear that metabolically diverse PNSB hold potential solutions for environmental problems. The present study utilizes PNSB for As reduction and adsorption along with oxidation of organic compounds as well as hydrogen production. This gives a comprehensive view of how such diverse bacteria can be used to achieve multiple goals simultaneously. Hydrogen gas produced by these bacteria can be used for energy purposes. However, the production of hydrogen needs to be further evaluated and estimated at different conditions. Moreover, these processes need to be operated and optimized simultaneously to achieve maximum results along with field trials.

### Contribution of the authors

HS and QUK contributed equally in the experimental work. HS drafted the manuscript. YR planned and supervised the work as well as he reviewed and finalized the manuscript.

### Compliance with ethical standards

The current study does not involve any experiments performed on human beings or animals.

### Funding

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### Research involving human participants and/or animals

Not applicable.

### Informed consent

Not applicable.

### Disclosure of interest

The authors declare that they have no competing interest.

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## Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at <https://doi.org/10.1016/j.crv.2019.02.001>.

## References

- J.P. Maity, S. Kar, J.-H. Liu, J.-S. Jean, C.-Y. Chen, J. Bundschuh, S.C. Santra, C.-C. Liu, The potential for reductive mobilization of arsenic [As (V) to As (III)] by OSBH2 (*Pseudomonas stutzeri*) and OSBH5 (*Bacillus cereus*) in an oil-contaminated site, J. Environ. Sci. Health, Pt. A: Toxic/Hazard. Subst. Environ. Eng. 46 (2011) 1239–1246.
- S. Nookabkaew, N. Rangkadilok, C. Mahidol, G. Promsuk, J. Satayavivad, Determination of arsenic species in rice from Thailand and other Asian countries using simple extraction and HPLC–ICP–MS analysis, J. Agric. Food Chem. 61 (2013) 6991–6998.
- B.K. Mandal, K.T. Suzuki, Arsenic round the world: a review, Talanta 58 (2002) 201–235.
- G. Tam, S. Charbonneau, F. Bryce, C. Pomroy, E. Sandi, Metabolism of inorganic arsenic (74As) in humans following oral ingestion, Toxicol. Appl. Pharmacol. 50 (1979) 319–322.
- C. Jain, I. Ali, Arsenic: occurrence, toxicity and speciation techniques, Water Res. 34 (2000) 4304–4312.
- B.P. Rosen, Families of arsenic transporters, Trends Microbiol. 7 (1999) 207–212.
- M. Ghosh, J. Shen, B.P. Rosen, Pathways of As (III) detoxification in *Gacharomyces cerevisiae*, Proc. Natl. Acad. Sci. U S A 96 (1999) 5001–5006.
- Saba, R. Andreasen, Y. Li, Y. Rehman, M. Ahmed, R.L. Meyer, A.N. Sabri, Prospective role of indigenous *Exiguobacterium profundum* PT2 in arsenic biotransformation and biosorption by planktonic cultures and biofilms, J. Appl. Microbiol. 124 (2018) 431–443.
- B.P. Rosen, Biochemistry of arsenic detoxification, FEBS Lett. 529 (2002) 86–92.
- C. Jackson, K. Harrison, S. Dugas, Enumeration and characterization of culturable arsenate resistant bacteria in a large estuary, Syst. Appl. Microbiol. 28 (2005) 727–734.
- M. Fomina, G.M. Gadd, Biosorption: current perspectives on concept, definition and application, Bioresour. Technol. 160 (2014) 3–14.
- J.B. Fein, C.J. Daughney, N. Yee, T.A. Davis, A chemical equilibrium model for metal adsorption onto bacterial surfaces, Geochim. Cosmochim. Acta 61 (1997) 3319–3328.
- M. Azwar, M. Hussain, A. Abdul-Wahab, Development of bio-hydrogen production by photobiological, fermentation and electrochemical processes: a review, Renew Sust. Energ. Rev. 31 (2014) 158–173.
- M. Kis, G. Sipka, E. Asztalos, Z. Rázga, P. Maróti, Purple non-sulfur photosynthetic bacteria monitor environmental stresses, J. Photochem. Photobiol. 151 (2015) 110–117.
- N. Pfennig, K.D. Lippert, On the B<sub>12</sub> needs of phototrophic sulfur bacteria, Arch. Microbiol. 55 (1966) 245–256.
- S. Al-Azad, T.K. Soon, J. Ransangan, Effects of light intensities and photoperiods on growth and proteolytic activity in purple non-sulfur marine bacterium, *Affifella marina* strain ME (KC205142), Adv. Biosci. Biotechnol. 4 (2013) 919–924.
- K. Jalal, Z.A. ZA, M. Rahman, B. Kamaruzzaman, N. Faizul, Carotenoid contents in anoxygenic phototrophic purple bacteria, *Marichromatium* sp. and *Rhodospseudomonas* sp. of tropical environment, Malaysia, Oriental J. Chem. 30 (2014) 607–613.
- T.M. Salmassi, K. Venkateswaren, M. Satomi, D.K. Newman, J.G. Hering, Oxidation of arsenite by *Agrobacterium albertimagni*, AOL15, sp. nov., isolated from Hot Creek, California, Geomicrobiol. J. 19 (2002) 53–66.
- D.E. Cummings, F. Caccavo, S. Fendorf, R.F. Rosenzweig, Arsenic mobilization by the dissimilatory Fe(III)-reducing bacterium *Shewanella alga* BrY, Environ. Sci. Technol. 33 (1999) 723–729.
- M. Shafique, A. Jawaid, Y. Rehman, Redox biotransformation of arsenic along with plant growth promotion by multi-metal resistance *Pseudomonas* sp. MX6, C.R. Biologies 340 (2017) 330–338.
- L. Bianchi, F. Mannelli, C. Viti, A. Adessi, R. De Philippis, Hydrogen-producing purple non-sulfur bacteria isolated from the trophic lake Averno (Naples, Italy), Int. J. Hydrogen Energy 35 (2010) 12216–12223.
- R. Croce, H. Van Amerongen, Natural strategies for photosynthetic light harvesting, Nat. Chem. Biol. 10 (2014) 492–501.
- R.S. Poretsky, Finding a niche: the habits and habitats of purple non-sulfur bacteria, Microb. Divers. Athens, GA 30602 (2003) 1–10.
- R. Shapawi, T.E. Ting, S. Al-Azad, Inclusion of purple non-sulfur bacterial biomass in formulated feed to promote growth, feed conversion ratio and survival of Asian Seabass Lates calcarifer Juveniles, J. Fish. Aquat. Sci. 7 (2012) 475–480.
- P. Nookongbut, D. Kantachote, K. Krishnan, M. Megharaj, Arsenic resistance genes of As-resistant purple non-sulfur bacteria isolated from As-contaminated sites for bioremediation application, J. Basic Microbiol. 57 (2017) 316–324.
- S. Silver, L.T. Phung, Genes and enzymes involved in bacterial oxidation and reduction of inorganic arsenic, Appl. Environ. Microbiol. 71 (2005) 599–608.
- K. Batool, F. tuz Zahra, Y. Rehman, Arsenic-redox transformation and plant growth promotion by purple non-sulfur bacteria *Rhodospseudomonas palustris* CS2 and *Rhodospseudomonas faecalis* SS5, BioMed. Res. Int. 2017 (2017) 8.
- J. Sakpirom, D. Kantachote, T. Nunkaew, E. Khan, Characterizations of purple non-sulfur bacteria isolated from paddy fields, and identification of strains with potential for plant growth promotion, greenhouse gas mitigation and heavy metal bioremediation, Res. Microbiol. 168 (2017) 266–275.
- J.F. Stolz, R.S. Oremland, Bacterial respiration of arsenic and selenium, FEMS Microbiol. Rev. 23 (1999) 615–627.
- R. Huber, M. Sacher, A. Vollmann, H. Huber, D. Rose, Respiration of arsenate and selenate by hyperthermophilic archaea, Syst. Appl. Microbiol. 23 (2000) 305–314.
- S. Yamamura, M. Ike, M. Fujita, Dissimilatory arsenate reduction by a facultative anaerobe, *Bacillus* sp. strain SF-1, J. Biosci. Bioeng. 96 (2003) 454–460.
- P. Nookongbut, D. Kantachote, M. Megharaj, Arsenic contamination in areas surrounding mines and selection of potential As-resistant purple non-sulfur bacteria for use in bioremediation based on their detoxification mechanisms, Ann. Microbiol. 66 (2016) 1–11.
- P. Prasertsan, W. Choorit, S. Suwanno, Isolation, identification and growth conditions of photosynthetic bacteria found in seafood processing wastewater, World J. Microbiol. Biotechnol. 9 (1993) 590–592.
- K. Jalal, Z.A. ZA, M. Rahman, B. Kamaruzzaman, N. Faizul, Carotenoid contents in anoxygenic phototrophic purple bacteria, *Marichromatium* sp. and *Rhodospseudomonas* sp. of tropical environment, Malaysia, Orient. J. Chem. 30 (2014) 607–613.
- S. Reslewic, S. Zhou, M. Place, Y. Zhang, A. Briska, S. Goldstein, C. Churas, R. Runnheim, D. Forrest, A. Lim, Whole-genome shotgun optical mapping of *Rhodospirillum rubrum*, Appl. Environ. Microbiol. 71 (2005) 5511–5522.
- M. Madigan, S.S. Cox, R.A. Stegeman, Nitrogen fixation and nitrogenase activities in members of the family *Rhodospirillaceae*, J. Bacteriol. 157 (1984) 73–78.
- F.E. Rey, E.K. Heiniger, C.S. Harwood, Redirection of metabolism for biological hydrogen production, Appl. Environ. Microbiol. 73 (2007) 1665–1671.
- N. Basak, D. Das, Photofermentative hydrogen production using purple non-sulfur bacteria *Rhodobacter sphaeroides* OU 001 in an annular photobioreactor: a case study, Biomass Bioenergy 33 (2009) 911–919.
- E.K. Heiniger, Y. Oda, S.K. Samanta, C.S. Harwood, How posttranslational modification of nitrogenase is circumvented in *Rhodospseudomonas palustris* strains that produce hydrogen gas constitutively, Appl. Environ. Microbiol. 78 (2012) 1023–1032.
- A. Adessi, J.B. McKinlay, C.S. Harwood, R. De Philippis, A *Rhodospseudomonas palustris* nifA<sup>+</sup> mutant produces H<sub>2</sub> from NH<sub>4</sub><sup>+</sup>-containing vegetable wastes, Int. J. Hydrogen Energy 37 (2012) 15893–15900.
- N. Basak, A.K. Jana, D. Das, D. Saikia, Photofermentative molecular biohydrogen production by purple non-sulfur (PNS) bacteria in various modes: the present progress and future perspective, Int. J. Hydrogen Energy 39 (2014) 6853–6871.
- D. Muzziotti, A. Adessi, C. Faraloni, G. Torzillo, R. De Philippis, H<sub>2</sub> production in *Rhodospseudomonas palustris* as a way to cope with high light intensities, Res. Microbiol. 167 (2016) 350–356.
- D.D. Simeonova, D. Lievremon, F. Lagarde, D.A. Muller, V.I. Groudeva, M.-C. Lett, Microplate screening assay for the detection of arsenite-oxidizing and arsenate-reducing bacteria, FEMS Microbiol. Lett. 237 (2004) 249–253.