Complete genome sequence of Shewanella algae strain 2NE11, a decolorizing bacterium isolated from industrial effluent in Peru

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ABSTRACT

Shewanella is a microbial group with high potential to be applied in textile effluents bioremediation due to its ability to use a wide variety of substrates as a final electron acceptor in respiration. The present research aimed to describe a new strain, Shewanella algae 2NE11, a decolorizing bacterium isolated from industrial effluent in Peru. S. algae 2NE11 showed an optimal growth under pH 6-9, temperature between 30-40 °C, and 0-4 % NaCl. It can tolerate high concentrations of NaCl until 10% and low temperatures as 4 °C. It decolorizes azo and anthraquinone dyes with a decolorization rate of 89-97%. We performed next-generation sequencing (Pacific Bioscience®) and achieved its complete genome sequence with a length of 5,030,813bp and a GC content of 52.98%. Genomic characterization revealed the presence of protein-coding genes related to decolorization like azoreductase, dyp-peroxidase, oxidoreductases, and the complete Mtr respiratory pathway. Likewise, we identified other properties such as the presence of metal resistant genes, and genes related to lactate and N-acetylglucosamine metabolism. These results highlight its potential to be applied in the bioremediation of textile effluents and guide future research on decolorization metabolic pathways.

1. Introduction

There are 100,000 different dyes worldwide, mainly used in the textile, plastic, food, and cosmetic industry; among them, azo and anthraquinone dyes are considered the most important, long, diverse, and recalcitrant xenobiotic group mainly used by the textile industry for their high efficiency and low cost [9, 13, 31, 36, 45]. Previous research estimated that approximately 2% of basic dyes and 50% of reactive dyes get lost during the textile industry staining process, percentage that represent a big social concern in contamination [36]. Some dyes are considered harmful to aquatic and human organisms due to their toxicity, mutagenicity, and carcinogenesis [11, 36, 43].

Shewanella genus has over 60 species; they are gram-negative bacilli with a size of 1-3 µm and mobile by a polar flagellum, with a facultative anaerobic metabolism; and they are found in a great diversity of habitats such as sand, water, and marine sediments, coal mines, oil, among others [57]. The genus has a high potential for bioremediation due to its genomic versatility and the capacity for dissimilatory metabolism of a wide diversity of compounds such as toxic elements and insoluble metals [14, 18, 25].

Shewanella has been reported as a potential bioremediation agent for azo and anthraquinone dyes under high salinity, microaerophilic, aerobic, and anaerobic conditions [20, 27, 56]. The decolorization process in bacteria involves dye reduction by enzymes such as azo-educatas, laccases, and peroxidases. Some enzymes, such as manganese peroxidase (MnP), polyphenol oxidase (PPO), tyrosinase, veratryl alcohol oxidase, and lignin peroxidase (LiP), were also associated with decolorization in other microorganisms [30, 42]. Although the biodegradation mechanisms of azo dye by Shewanella species have been studied, genomic characterization focused on identifying the enzymes involved in decolorization of azo dyes by Shewanella strains remains little explored.

This work aims to describe Shewanella algae 2NE11 genomic features and highlight its potential application in decolorization process.

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2. Materials and Methods

2.1. Sampling and Isolation

We isolated *S. algae* 2NE11 from an olive processing company effluent in the city of La Yarada - Los Palos (Tacna - Peru) (18° 12’ 40.1” S 70° 30’ 33.3”). Some physicochemical parameters as DO and DQO (mg/L) were determined during sample collection. The sample was grown in minimal medium broth (g/L: KH₂PO₄:1; Ca(NO₃)₂:0.03; MgSO₄: 0.2; K₂HPO₄:2.77; NH₄NO₃:1) for 48 h at 25°C. Subsequently, bacterial culture was grown in nutritive agar for 48h. Then, colonies were separated based on their morphology and were examined for potential to decolorize dyes efficacy. Finally, we selected strain 2NE11 based on its high decolorization efficiency for further investigation.

2.2. Growth conditions and biochemistry assays

We evaluated the following parameters: temperature (4, 30, 37, 40°C), pH (5, 6, 7, 8, 9, 10, 11) and NaCl concentration (0, 1, 2, 3, 4, 5, 6, 7, 8, 10 %) after 24 h of incubation to determine the optimal growth condition for strain 2NE11 in Luria Bertani broth (LB) modified [39, 50]. The principal biochemical tests were performed as described previously [4, 24]. Besides, we determined the carbohydrates utilization profile in static conditions at 30°C after 72h. We isolated *S. algae* 2NE11 from an olive processing company ATCC 51192T data was taken from different studies [19, 35]. The genome assembly was made with Unicycler using conservative mode [54]. Ab initio gene calling was performed using Prokka and the Rapid Annotation Subsystems Technology (RAST) [2, 40]. Functional categories were predicted through the BLASTKoala web tool using the KEGG database [26]. Genomic islands were expected through Island Viewer 4 server and represented along with the genomic map [3].

2.3. Decolorization kinetics

Decolorization kinetics were performed in 100 mL of ZZ broth pH 7.0 (g/L: (NH₄)₂SO₄: 0.5; KH₂PO₄: 2.66; Na₂HPO₄:2H₂O: 4.32; yeast extract: 5) supplemented with 100 mg/L of dye and inoculated with 5% bacterial culture harvested at mid-log phase (% v/v) [60]. Each flask was homogenized and dispensed in tubes with 5 mL and incubated in static conditions at 30°C during 12 h. After centrifugation at 10,000 rpm for 10 minutes, the absorbance was obtained at a maximum wavelength of each dye evaluated (Direct Blue 71: 574nm; Methyl Orange: 470nm; Bright Blue Remazol: 608nm; Yellow Procion HEXL: 422nm). The assay was performed by triplicate and a negative control group was included in all the experiments.

2.4. DNA extraction and genome sequencing

The strain 2NE11 was inoculated 5% (% v/v) in ZZ broth pH 7.0 supplemented with Direct Blue 71 dye at 100 mg/L and incubated at 30°C for 12h. Then, 5 mL of culture was centrifuged at 10,000 rpm and 7850 rpm for 10 minutes. The pellet obtained was used for total DNA extraction following the standard protocol of the PureLink™ Genomic DNA Mini Kit (Invitrogen™). NanoDrop Lite was used to obtain DNA quantity and quality. DNA integrity was evaluated through an agarose gel electrophoresis. The whole-genome library was generated by a 20kb SMRTbell and sequencing by Single-Molecule Real-Time (SMRT) with RSII and C4-P6, using the Pacific Bioscience® technology at Macrogen (Korea).

2.5. Genome assembly and functional annotation

Genome assembly was made with Unicycler using conservative mode and additional polishing steps with Quiver [51]. The program Quast [16] compared the measure of assemblies quality obtained by Unicycler. A genomic map was depicted with BRIG [1].

The pipeline Prokka and the Rapid Annotation Subsystems Technology (RAST) [2, 40] were used to annotate the complete genome sequence of strain 2NE11. Final genome annotation was obtained by the Prokaryotic genome annotation pipeline (PGAP) [47]. Coding gene and RNA sequences were predicted through Prodigal [23] and Barnnap (http://www.vicbioinformatics.com/software.barnnap.shtml). Functional categories were predicted through the BLASTKoala web tool using the KEGG database [26]. Genomic islands were expected through Island Viewer 4 server and represented along with the genomic map [3].

2.6. Data availability

The genome of *Shewanella algae* 2NE11 was deposited under the NCBI GenBank accession number CP055159. Raw data of sequencing is available in the Sequence Read Archive (SRA) repository of the National Center for Biotechnology Information (NCBI) under the accession number PRJNA547647. Complete information about strain 2NE11 is on the biosample SAMN15232066.

3. Results

3.1. Organism information

3.1.1. Description of *Shewanella algae* 2NE11

The main physiological and biochemical features of *S. algae* 2NE11 were described to understand its metabolism and identify features that allows the strain to enhance its decolorization capacity. Parameters evaluated during sample collection show that it had a DO of 13100 mg/L and a DQO of 36900 mg/L. *Shewanella algae* 2NE11 has a doubling time of 5.03h and grows in optimal conditions at pH 6-9, between 30 and 40°C, and with 0-4% of NaCl. A more detailed description and comparison with the reference strain *S. algae* ATCC 51192 is depicted in Table 1.

3.1.2. Decolorization of synthetic dyes

In this research, we evaluated the decolorization rate of strain 2NE11 under the exposure of various types of dyes to elucidate its potential in bioremediation.

The strain 2NE11 decolorizes azo and anthraquinone dyes like Methyl Orange (95.76%), Bright Blue Remazol (97.29%), Yellow Procion HEXL (91.31%), and Direct Blue 71 (89.24%) at 12 h (Figure 1).

| Table 1 | Physiological and biochemical features of *S. algae* 2NE11 (The reference strain ATCC 51192T data was taken from different studies [19, 35]). |
|---|---|
| S. algae ATCC 51192T | S. algae 2NE11 |
| **Oxidase** | + | + |
| **Catalse** | + | + |
| **Motility** | + | + |
| **Hemolysis** | + | + |
| **Gelatinase production** | + | - |
| **Lysine decarboxylase** | - | - |
| **H₂S production** | + | + |
| **Nitrate reduction** | - | + |
| **Growth at**: | | |
| 4°C | + | - |
| 37°C | + | + |
| 40°C | + | + |
| 0% NaCl | + | + |
| 6% NaCl | + | + |
| 10% NaCl | + | + |
| **Utilization of**: | | |
| Glucose | + | + |
| D-fructose | + | + |
| Maltose | - | + |
| L-arabinose | - | + |
| Citrate | - | + |
| Sucrose | - | + |
| N-acetyl glucosamine | - | + |
| DL-lactate | - | + |
| **% GC** | 52.4 | 52.98 |

*: No growth in 24h; **: Not evaluated
This strain can reduce azo dyes ranging from monoazo dyes such as Methyl Orange and triazo dyes such as Direct Blue 71. It has also been shown to be effective against anthraquinone dyes such as Bright Blue Remazol. However, the time required to decolorize may increase depending on dye complexity, as shown when comparing the decolorization kinetics of Methyl Orange and Direct Blue 71 in our results (Figure 1).

3.2. Genome sequencing information

3.2.1. Chromosome features

We performed whole-genome sequencing and genomic analyses to gain insights into the genome features associated with synthetic dye decolorization. Raw data of strain 2NE11 contains 141,935 subreads with a Subread NS0 of 15,524 and an average subread length of 10,574. The Shewanella algae 2NE11 genome was fully circularized into 5,030,813 bp with 231.29x coverage, and 52.98 %GC content. It was deposited in the GenBank database under accession number CP055159. It was not found evidence of plasmids. Genome features of S. algae 2NE11 and its comparison with other strains are depicted in Table 2. Functional categories analysis revealed genes related to genetic information, signaling, cellular and environmental information processing as the most representative inside the whole genome (Figure 2). Also, it is necessary to mention that it was not possible to conclude the presence of complete prophage regions inside the genome.

Two genomics islands (GI-I and GI-II) were predicted and depicted in Figure 2. GI-I has a length of 25,322 bp and comprises 21 genes, whereas GI-II has a size of 70,550 bp and consists of 64 genes. GI-I contains mainly conjugative transfer proteins, and GI-II has proteins of the type IV secretion system, regulators transcriptional, conjugative transfer proteins, esterases, hydrolases, reductases, nitro reductases, and oxido-reductases. GI-II likely enhances its environmental adaptability because its relationship with many reduction processes and allow greater substrate diversity consumption during its respiration.

The results of the candidate genes search related to decolorization, metal resistance and carbohydrate metabolism are presented in Table 3.

4. Discussion

4.1. Description of Shewanella algae 2NE11

Shewanella algae were isolated in 1990 from a red algae (Jainia spp.), mainly characterized by its ability to tolerate and stimulate its growth in high salt concentrations of up to 12% [35, 41].

S. algae 2NE11 is a mobile microorganism, oxidase, and catalase-positive. It can produce hemolysis and H$_2$S, but does not have gelatinase, lyme decarboxylase, and cannot reduce nitrates. This strain grows in optimal conditions at pH 6-9, with an optimum between 30 and 40°C, and with 0-4% NaCl. Like strain ATCC 51192, it can grow at 4, 37, 40°C, and up to 10% NaCl. However, unlike S. algae ATCC 51192, the strain 2NE11 does not require NaCl to cell growth [19, 35]. S. algae 2NE11 can use a greater variety of carbohydrates such as maltose, L-arabinose, sucrose, N-acetyl glucosamine, and DL-lactate to cell growth.

The physiological description of S. algae 2NE11 suggests that this strain could be used in future bioremediation studies of textile effluents since it tolerates high concentrations of salt and consumes a wide variety of carbohydrates that could be used in the biotechnological process optimization.

4.2. Decolorization of synthetic dyes

Synthetic dyes are classified based on their chemical structure in azo, anthraquinone, and triphenylmethane. Among these, azo dyes are the most used in the textile industry. They are classified in monooazo, diazo, triazo, and polyazo based on the number of azo bonds (N = N) within their chemical structure [11].

Some species inside the genus Shewanella, like S. oneidensis, S. decolorationis, S. putrefaciens, S. xiamenensis, and S. algae, were described for their ability to decolorize under different conditions [20, 27, 29, 34, 53, 55]. Previous reports indicate that S. decolorationis S12 has an efficiency of 99% after 15 h of exposure to Brilliant Blue Remazol dye (50 mg/L) [56]. This efficiency value is higher than the 97.29% obtained after 12 h of incubation of the strain 2NE11. The degradation of other more complex dyes such as Direct Blue 71 has also been previously evaluated in Pseudomonas strains, obtaining up to a little more than 70% efficiency after a broad incubation period of several days [17, 37]. However, with strain 2NE11, we can observe a better decolorization rate of more than 89% after only 12 hours of exposure. Other simple dyes such as methyl orange have been extensively investigated in different species, obtaining almost complete decolorization just as the strain under study [5, 8, 34].

Table 2

| Feature                  | 2NE11 | ATCC 51192 | RQs-106 | KC-Na-R1 | TUM4442 | CECT 5071 | 150735 |
|--------------------------|-------|------------|---------|----------|----------|-----------|--------|
| Genome size (bp)         | 5,030,813 | 4,978,360 | 4,990,025 | 5,036,300 | 4,798,767 | 4,924,764 | 5,070,545 |
| Number of contigs        | 1     | 52         | 1       | 1        | 1        | 1         | 1      |
| Total genes              | 4,475 | 4,505      | 4,430   | 4,715    | 4,264    | 4,400     | 4,528  |
| CDSS                     | 4,334 | 4,392      | 4,296   | 4,582    | 4,130    | 4,264     | 4,392  |
| Protein coding genes     | 4,288 | 4,329      | 4,253   | 4,527    | 4,098    | 4,225     | 4,357  |
| rRNAs                    | 111   | 95         | 105     | 102      | 105      | 107       | 107    |
| tRNAs                    | 25    | 14         | 25      | 25       | 25       | 25        | 25     |
| ncRNAs                   | 5     | 4          | 6       | 4        | 4        | 4         | 4      |
| Pseudo genes             | 46    | 63         | 43      | 55       | 32       | 39        | 35     |
| GenBank accession        | CP055159 | JAAXP0000000000 | CP046378 | CP033575 | AP024610 | CP068230 | CP068229 |
Figure 2. A. Visualization of S. algae 2NE11 genome compared with S. algae KC-Na-R1 (NZ_CP033575.1) and S. algae CCU101 (NZ_CP018456.1). It shows genomic island prediction in two positions (GI-I and GI-II). Starting from the inner circle moving outwards, the following tracks are shown: GC content (Black), GC skew– (Purple), GC skew+ (Green), S. algae KC-Na-R1 genome (light blue), S. algae CCU101 (ed). B. Bar plot showing the principal functional categories of strain 2NE11 according to the KEGG Orthology is depicted.

Table 3
Candidate genes of Shewanella algae 2NE11 related to decolorization and other properties. The letters a, b, and c refer to a link between the words.

| Category                  | Gene name          | Locus tag                        | Description                        |
|---------------------------|--------------------|----------------------------------|------------------------------------|
| Decolorization            |                   |                                  | FMN-dependent NADH-azoreductase     |
|                           | HU689_20695        |                                  | NADPH-dependent oxidoreductase      |
|                           | HU689_04585        | HU689_04700; HU689_21345         | Operon Mtr                         |
|                           | HU689_05310        |                                  | P-type ATPase protein              |
| Metal Resistance          | cadA               | HU689_10830                      | Magnesium and cobalt transport proteinab |
|                           | corA corC          | HU689_12865 (HU689_16615; HU689_20255; HU689_07770)c |
|                           | snmB               | HU689_05170                      | Efflux Zn2+ ion transport proteinab |
| Carbohydrate metabolism   |                    | HU689_06695 HU689_06680          | ATPase protein akArsenate efflux transporter proteina Arsenate reductasea |
|                           |                    | (HU689_06290, HU689_06255) (HU689_06275, HU689_06280, HU689_06285, HU689_06280, HU689_06265, HU689_06260)  |

4.3. Insights from the genome sequence

Table 2 shown clearly that strain 2NE11 has similar genome size with S. algae KC-Na-R1. It shares similar number of rRNAs, tRNAs and ncRNAs with strains RQs-106, KC-Na-R1, TUM4442, CECT 5071, and 150735. Only S. algae ATCC 51192 shown a different number likely due to its partial genome sequencing project. Other features like total genes number and CDS are concordant with the genome size showing a partial correspondence between strains.

4.4. Candidate genes for decolorization

Decolorization by microorganisms has been widely studied for a long time, identifying the main responsible protein of this process. The prediction of genes related to decolorization in strain 2NE11 gives us an overview of how its whole metabolic machinery works in decolorization. Its genome contains protein-coding genes previously associated with decolorization, such as an FMN-dependent NADH-azoreductase gene (HU689_20695), NADPH-dependent oxidoreductase genes (HU689_04585; HU689_04700; HU689_21345), and heme-dependent Dyp peroxidase gene (HU689_05310). The operon Mtr (HU689_08360 - HU689_08395), an electron chain transport previously, encodes two OmcA genes (HU689_08375, HU689_08380) as other strains within the genus Shewanella. Shewanella algae 2NE11 has several genes related to decolorization in other studies, such as the FMN-dependent protein NADH-azoreductase. HU689_20695 gene has a length of 594bp and codify an azoreductase of 197aa that is mainly related with WP_025888486 and WP_025010143 (Shewanella sp.). AzOR (WP_011074045) of the reference specie S. oneidensis is the closer gene intraspecie with only 72.02% of identity, suggesting that azoreductase gene of S. algae could diverge in a different group of the well know AzOR. Azoreductase is widely spread inside the genus algae, such as strains RQs-106 (GMX02_01695), TUM4442 (TUM4442_38770), CECT 5071 (E1N4_020345), KC-Na-R1 (EY24_23150), and 150735 (JKK46_01670). It has been previously described for its ability to bio-transform and detoxify azo dyes and aromatic amines by reducing azo bonds [32]. This mechanism has been related to flavin-dependent enzymes, and it is even considered that flavin could improve the enzyme thermal stability [30]. HU689_05310 gene present a length of 936bp, and it is related with a dyp-peroxidase of 311aa.
mainly anthraquinone dyes. The strain under study 2NE11 would be
mediating the degradation of the Brilliant Blue Remazol dye through the
catalytic activity of the dyp-peroxidase (HU689_05310) found in its
genome. However, the recent discovery of its possible multifunctional
activity as a hydrolytic agent does not allow us to conclude the
biochemical process through which decolorization would perform [12,
30, 44]. Likewise, other potentially enzymes such as NADPH-dependent
oxidoreductases were found with 189aa (30, 44]. Likewise, other potentially
enzymes such as NADPH-dependent biochemical process through which decolorization would perform [12,
44]). They would be involved in
FMNH$_2$-dependent reduction to various metabolites related to stress
response and iron reduction [45].

Other proteins that have also got much relevance within decolor-
ization are the electron transport chain of the operon Mtr [6, 28]. That has
been associated with extracellular decolorization processes and
linked with polar azo dyes. The operon Mtr is composed of proteins in
the inner membrane, outer membrane, and the periplasm that would
allow energy conservation and electrons transport in the cell membrane.
Previous reports indicate that the operon Mtr is highly diverse among
Shewanella species due to gene duplication, acquisition, and loss.
Furthermore, some of its compounds, such as the MtrA, OmcA, and OmcB
genes, play an essential role in electron transport, which they are highly
conserved [21, 59]. The mentioned would support the idea that OmcA
gene duplication in the strain 2NE11, as in other Shewanella algalae
strains, could give it a greater capacity to transfer electrons and improve its
decolorization ability in contrast to other species in the genus.

4.5. Candidate genes for metal resistance

The heavy metal resistance improves a microorganism potential in
the textile effluent bioremediation because they are frequently found as
a component in the textile industry wastewater. Based on genome
analysis of the strain 2NE11, we found that the cadA gene
(HU689_10830) encodes a P-type ATPase protein of 798 aa length,
which has been described previously as a determinant that allows
resistance to cadmium through a decrease in the intracellular accumu-
lization of this cation, mediated by active transport [48, 49]. The
HU689_10830 gene is mainly related with S. algae TUM17384
(APO24616) and strain 150735 (CP068229) with an identity of 99.58
and 99.42%.

Magnesium is a cation widely used by living organisms because it
allows various biological functions such as genome stabilization, a cofactor
for ATP hydrolysis, and DNA replication [22]. We found that strain
2NE11 also contains genes related to magnesium and cobalt transport
such as corA (HU689_12865) and corC (HU689_16615; HU689_20255;
HU689_07770), widely associated with the exterior and interior flux of
Mg$^{2+}$ and Co$^{2+}$ ions in gram-negative and positive bacteria [15].
Resistance to other ions such as zinc was also evaluated, allowing
identify an efflux Zn$^{2+}$ ion transport encoded by the mntB gene
(HU689_05170), closely related to strain 18064-CSB-B-B (CP047422)
and ATCC 49138 (AP024609) with 99.17% identity. This would allow
maintenance of zinc concentration narrow under the limits necessary for
living cells [52]. Previous study in Shewanella sp. strain ANA-3 have
shown that arsenate resistance occurs via two different pathways:
detoxification and arsenate respiratory reduction [33]. Nevertheless,
none of them could be found completely in S. algalae 2NE11. Only, some
arsenate resistance genes such as ATPase protein arcs (HU689_08030),
arsenate efflux transporter arsb (HU689_02860), and arsenate reductase
arsC (HU689_10495) were found in the genome of strain 2NE11; how-
ever, their lack of operon-shaped structure prevents us from concluding
that they are expressed or functional.

4.6. Candidate genes for carbohydrate metabolism

Carbohydrates have been widely studied in the optimization of dyes
degradation process due it represents a carbon source which improves
cell growth. According to genomic annotation, we assume that DL-
lactate metabolism in S. algae 2NE11 could be associated with an L-
lactate permease activity (HU689_06695) and a lactate utilization pro-
tein (HU689_06680). Additionally, we found genes that could be related
to the catalytic pathway of N-acetylglucosamine, involved in processes
such as transport (HU689_06290, HU689_06255), catalysis
(HU689_06275, HU689_06270), regulation (HU689_06250), and com-
plementary processes (HU689_06285, HU689_06280, HU689_06265,
HU689_06260) due their highly similarity to S. oneidensis MR-1 Nag
genes. They have been found in almost all genomes of Shewanella genus
and many isolated strains have been able to grow in N-acetylglucos-
amine as sole carbon source, ability also shared by 2NE11 [38]. More-
ever, Nag genes has been recently related to glucose metabolism
of Shewanella oneidensis MR-1 for enhanced pollutant degradation [10].
Likewise, it has been reported in multiple investigations that lactate can
be used as electron acceptor in cellular respiration, allowing to improve
the dye degradation process required [7, 56].

Although the diversity of carbon source utilization in Shewanella is
limited, the metabolic machinery for the catalysis of the other carbo-
hydrates could not be fully elucidated in S. algae 2NE11. It has already
been confirmed that several strains of Shewanella can improve the
decolorization efficiency when grown under additives such as carbo-
hydrates [10]. S. algae 2NE11 seems to have the complete genomic
machinery necessary to metabolize lactate and N-acetylglucosamine,
which has been related to the results obtained by physiological tests.
Previous studies indicate that they are promoters of the decolorization
efficiently, for which we highlight that these two sugars could be used to
optimize the decolorization process on a larger scale with strain 2NE11
[10, 55, 58].

5. Conclusion

We present the complete genome sequence and physiological profile
of S. algae 2NE11, a bacteria dye-degrading isolated from an industrial
effluent in Peru. It can tolerate up to 10% NaCl, and it can decolorize azo
and anthraquinone dyes. The molecular decolorization mechanisms
involved include the catalytic activity of azoreductases, dyp-
peroxidases, oxidoreductases, and the Mtr respiratory pathway. Like-
wise, we identified several genes related to metal resistance and car-
bohydrate metabolism, which would enhance their potential
applicability in textile effluents bioremediation. This explorative view
of strain 2NE11 reveals interesting genomic features, however future
research is required to show functionality.

Authors contributions

Wendy Lizarraga & Carlo Mormontoy & Ruth García & Pablo Ram-
irez consigned and designed the experiment. Wendy Lizarraga
performed and participated in all experiments. Carlo Mormontoy
performed the phylogenetic tree and participated in the analysis of
the data. Mario Taira & Hedersson Calla & Maria Castañeda performed part
of the physiological tests. Claudia Marin sampled and isolated the strain
under study. Michel Abanto participated in the assembly of the genome.
All authors participated in the final revision of the manuscript.

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[45] H. Suzuki, Remarkable diversification of bacterial azoreductases: primary sequences, structures, substrates, physiological roles, and biotechnological applications, Appl. Microbiol. Biotechnol. 103 (2019) 3965–3978, https://doi.org/10.1007/s00253-019-09775-2.

[46] M.L. Swift, GraphPad prism, data analysis, and scientific graphing, J. Chem. Inf. Comput. Sci. 37 (1997) 411–412, https://doi.org/10.1021/ci960402b.

[47] T. Tatusova, M. Micucio, A. Badretdin, V.chetverin, E.P. Nawrocki, L. Zaslavsky, A. Lomsadze, K.D. Pruitt, M. Borodovsky, J. Ostell, NCBI prokaryotic genome annotation pipeline, Nucleic Acids Res 44 (2016) 6614–6624, https://doi.org/10.1093/nar/gkw669.

[48] A.C.M. Toes, M.H. Daleke, J.G. Kuenen, G. Muyzer, Expression of copA and cusA in Shewanella during copper stress, Microbiology 154 (2008) 2709–2718, https://doi.org/10.1099/mic.0.2008/016857-9.

[49] K.J. Tsai, K.P. Yoon, A.R. Lynn, ATP-dependent cadmium transport by the cadA cadmium resistance determinant in everted membrane vesicles of Bacillus subtilis, J. Bacteriol. 174 (1992) 116–121, https://doi.org/10.1128/jb.174.1.116-121.1992.

[50] K. Venkateswaran, M.E. Dollhopf, R. Aller, E. Stachebrandt, K.H. Nealon, Shewanella amazonensis sp. nov., a novel metal-reducing facultative anaerobe from Amazonian shelf muds, Int. J. Syst. Bacteriol. 48 (1998) 965–972.

[51] R.R. Wick, L.M. Judd, C.L. Gorrie, K.E. Holt, Unicycler: Resolving bacterial genome assemblies from short and long sequencing reads, PLoS Comput. Biol. 13 (2017), https://doi.org/10.1371/journal.pcbi.1005595.

[52] A.J. Worlock, R.L. Smith, ZntB is a novel Zn2+ transporter in Salmonella enterica serovar Typhimurium, J. Bacteriol. 184 (2002) 4369–4373, https://doi.org/10.1128/JB.184.14.4369-4373.2002.

[53] X. Xiao, C.C. Xu, Y.M. Wu, J.P. Cai, W.W. Li, D.L. Du, H.Q. Yu, Biodecolorization of Naphthol Green B dye by Shewanella oneidensis MR-1 under anaerobic conditions, Bioreour. Technol. 110 (2012) 86–90, https://doi.org/10.1016/j.biortech.2012.01.099.

[54] M. Xu, J. Guo, Y. Cen, X. Zhong, W. Cao, G. Sun, Shewanella decolorationis sp. nov., a dye-decolorizing bacterium isolated from activated sludge of a waste-water treatment plant, Int. J. Syst. Evol. Microbiol. 55 (2005) 363–368, https://doi.org/10.1099/ijs.0.63157-0.

[55] M. Xu, J. Guo, G. Sun, Biodegradation of textile azo dye by Shewanella decolorationis S12 under microaerophilic conditions, Appl. Microbiol. Biotechnol. 76 (2007) 719–726, https://doi.org/10.1007/s00253-007-1032-7.

[56] M. Xu, J. Guo, G. Zeng, X. Zhong, G. Sun, Decolorization of anthraquinone dye by Shewanella decolorationis S12, Appl. Microbiol. Biotechnol. 71 (2006) 246–251, https://doi.org/10.1007/s00253-005-0144-1.

[57] K. Yousfi, S. Bekal, V. Usongo, A. Touati, Current trends of human infections and antibiotic resistance of the genus Shewanella, Eur. J. Clin. Microbiol. Infect. Dis. 36 (2017) 1353–1362, https://doi.org/10.1007/s10096-017-2962-3.

[58] Q. Zhang, X. Xie, Y. Liu, X. Zheng, Y. Wang, J. Cong, C. Yu, N. Liu, Z. He, J. Liu, W. Sand, Sugar sources as Co-substrates promoting the degradation of refractory dye: A comparative study, Ecotoxicol. Environ. Saf. 184 (2019), 109613, https://doi.org/10.1016/j.ecoenv.2019.109613.

[59] C. Zhong, M. Han, S. Yu, P. Yang, H. Li, K. Ning, Pan-genome analyses of 24 Shewanella strains re-emphasize the diversification of their functions yet evolutionary dynamics of metal-reducing pathway, Biotechnol. Biofuels 11 (2018) 1–13, https://doi.org/10.1186/s13068-018-1201-1.

[60] W. Zhou, W. Zimmermann, Decolorization of industrial effluents containing reactive dyes by actinomycetes, FEMS Microbiol. Lett. 107 (1993) 157–161, https://doi.org/10.1111/j.1574-6941.1993.tb05003.x.