Bioremediation of heavy metals using newly isolated native algae from Balanagar effluent channel, Hyderabad

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Research Article

Keywords: Bioremediation, Algae, Heavy metals, Wastewater, Molecular identification

Posted Date: May 6th, 2025

DOI: https://doi.org/10.21203/rs.3.rs-6481309/v1

Additional Declarations: No competing interests reported.

Abstract

Natural algal samples were collected and tested for their efficiency to degrade the heavy metals like Lead, Arsenic, Chromium and Mercury. Overall, 10 algal samples were collected and tested for their degradation efficiency.

The present study assessed the ability of the local algal stains to degrade the heavy metals. The following parameters were chosen for this research: Mercury, Cadmium, Chromium, Manganese, Iron, Copper, Zinc, Nickel and Lead. Among the ten isolates, one had to encourage degrading ability and apply genotypic approaches to the strain involved using the 16S rRNA gene sequence. We discovered the algal strain *Pandorina morum YSCVR24* through NCBI BLAST analysis. The isolation grew most efficiently at pH 7.0, 30°C, and a 24-hour incubation period. Measurements of degradation potential showed that lead and arsenic had the highest removable rates (80 and 85%), whereas mercury and chromium had the lowest (62 and 65%). In addition to showing a reduction of 60–80% of all heavy metals evaluated, *Pandorina morum YSCVR24* may be able to degrade heavy metals using plasmids, which may be used to modify plasmids to allow them to collect heavy metals.

1. INTRODUCTION

Heavy metal contamination is a major problem in the environment due to the rise of industrialization, urbanization, and agricultural runoff. These dangerous metals, which include lead (Pb), mercury (Hg), cadmium (Cd), zinc (Zn), copper (Cu), and arsenic (As), pose a significant threat to aquatic ecosystems and human health. The accumulation of these toxins in water sources has a domino effect, harming aquatic life and eventually reaching human populations through the food chain. Chemical precipitation and filtration are two common ways to clean up heavy metal pollution. However, they can be expensive, use a lot of energy, and create extra waste, so we need other, more environmentally friendly ways to do it.

Heavy metals are highly toxic and can have carcinogenic, teratogenic, and mutagenic effects, in addition to their tendency to accumulate in organisms [1–2] Microbes such as microalgae, fungi, yeast, and bacteria are increasingly recognized for their potential to remediate heavy metal contamination. These microorganisms have demonstrated impressive effectiveness in removing environmental contaminants [3–4]. Their rapid growth and ease of manipulation make them valuable tools in bioremediation [5]. It is essential to further explore and utilize microbial bioremediation techniques to enhance sustainability in environmental management [6–7]. Various bioremediation strategies, such as bioaccumulation, myco remediation, bio-reduction, bacterial remediation, phytoremediation and biosorption, are not only cost-effective but also environmentally responsible approaches for managing contamination [8]. Among these, biosorption is considered the most economical and environmentally sustainable technique for eliminating heavy metal ions from contaminated water [9].

Bioremediation, especially when employing algae, is a viable, economical, and ecologically benign method for eliminating heavy metals from polluted water bodies. Because algae have a large surface area and can change how they use energy, they can absorb heavy metals, store them, and get rid of them

in various ways, such as biosorption, bioaccumulation, and biotransformation [10]. The use of native algal species for bioremediation is particularly advantageous as they are adapted to local environmental conditions and can potentially offer more effective removal rates compared to non-native species [11]. The bioaccumulation of heavy metals and metalloids from sources such as air and water lead to their gradual infiltration into plants, animals, and humans, ultimately advancing through the food chain [12].

The Balanagar effluent channel in Hyderabad, an area known for industrial discharges, is a decisive site for assessing the potential of native algae in remediating heavy metal contamination. The effluent channel receives a significant amount of wastewater from nearby industries, which may contain elevated concentrations of various heavy metals. Isolating native algae from this area offers an opportunity to discover strains that are specifically adapted to these contaminated conditions. These native algae may possess inherent mechanisms for tolerating and removing heavy metals, making them ideal candidates for bioremediation applications in this region.

Recent research has demonstrated how natural algae can effectively remove heavy metals from a variety of environments worldwide. For example, *Chlorella vulgaris* has been shown to efficiently remove metals like cadmium and copper from wastewater [13]. While *Scenedesmus obliquus* has demonstrated effective biosorption of lead and zinc [14]. Furthermore, recent advancements in algae-based bioremediation have underscored the importance of selecting locally adapted strains to ensure the sustainability and efficiency of the process [15].

This research aims to explore the potential of newly isolated native algal strains from the Balanagar effluent channel in Hyderabad for bioremediation of heavy metals. By evaluating their capacity to absorb and accumulate heavy metals, this research could contribute to the development of sustainable, locally based solutions for managing industrial pollution in the region.

2. Materials and Methods

2.1 Study area

The effluents channel originates near Jeedimetla industrial area and passes through Balanagar after which it merges with Kukatpally nala at Fathenagar and finally enters Hussain Sagar Lake. It receives various industrial waste at the first phase of its flow. Later it receives domestic waste from various localities along its stretch and serves as the main feeding channel to Lake Hussain Sagar. Three sampling locations were carefully chosen beside the course of the channel. Station-I located at Balanagar, where it receives industrial effluents. Station-II at Fathenagar, which receives industrial effluent and domestic sewage. Station-III is located at Begumpet flyover bridge. Algal samples labelled AS1to AS10 consisted of a mix of wastewater collected from the sampling site.

2.2 Sample Preparation: Filter the collected wastewater to remove large debris and particles. This can be done using a mesh or sieve with an appropriate pore size to retain microalgae while allowing larger particles to pass through [16].

2.3 Isolation of algae from wastewater samples

2.3.1 Enrichment Cultures:

Inoculate filtered wastewater samples into growth media such as Bold Basal Medium (BBM) to promote the growth of algae. Incubate under controlled light and temperature conditions to encourage algal proliferation.

2.4 Enumeration of algal samples:

The wastewater samples were collected in 50 ml volumes and subsequently filtered using gauze. In that order, the samples have been diluted to 10^{-1}, 10^{-2}, 10^{-3}, 10^{-4}, 10^{-5}, 10^{-6}, and 10^{-7}. Using the spread plate approach, samples with varying dilution concentrations were equally coated on nutrient-rich media in a sterile operating table. They were then incubated for five to seven days at 32°C [17]. The APHA 24th edition (2023) [18] gave a precise formula for figuring out colony-forming units (CFU), which are a way to count the number of bacteria in an area. The serial dilution colonies were separated and streaked one at a time onto nutrient-rich media for two days at 37°C [19]. Until pure colonies were achieved, the colonies were re-streaked.

The isolation of algae from wastewater involves collecting samples, enriching cultures, and using DNA sequencing for identification and cultivation in controlled environments. The isolated algae can be tested for nutrient removal efficiency and utilized for various applications, making this method a sustainable approach to wastewater treatment.

2.5 Nutrient removal efficiency of isolated algal samples

pH, TDS, nitrates, phosphates, and COD were key parameters used to measure how well algal isolates removed pollutants. The parameters were assessed prior to and following the inoculation of prospective algal isolates. The capabilities of bacteria in reducing pollutants were evaluated. The removal percentage of pollutants was assessed in comparison to the control group, which did not include algal inoculation [12].

2.6 Heavy metal analysis

The quantities of particular heavy metals, such as lead, manganese, zinc, iron, and copper, were evaluated using an Atomic Absorption Spectrophotometer (AAS) (Shimadzu AA6600). Atomic Absorption Spectroscopy (AAS) is a method utilized to measure the concentration of chemical components in samples by evaluating the radiation received by the targeted element [20].

2.7 Determination of minimum inhibitory concentration (MIC):

To assess MIC, heavy metal resistant selected isolates were grown on heavy metal incorporated media against respective heavy metal; was identified by gently inclining the concentration of the heavy metals

(Lead, Manganese, Zinc, Iron and Copper) on nutrient agar plates until the isolates failed to give colonies on the petri plate. The starting concentration of the heavy metals was 50 mg/kg and the culture growing on the final concentration was transferred to the higher concentration each time by streaking on the agar plate. When the isolates failed to grow on petri plate, MIC was assessed according to standard protocol of ASTM 22 edition [14].

The culture grows at a given concentration were subsequently transferred to the next concentration. Based on the evaluation, MIC was determined at 30^0C for 6 days. The strains showing higher MIC values for heavy metals were selected for further studies like molecular identification.

2.8 The biological molecular identification of best performed algal strain.

The genetic identification of the isolated algae exhibiting the most significant degradation rate of organisms was conducted using 16S rDNA analysis. The DNA was extracted utilizing the Ezup Column Bacteria Genomic DNA Purification Kit (Sangon Biotech, China). The 16S rDNA of bacteria was amplified using universal primers 27F (50-AGAGTTTGATCCTGGCTCAG-30) and 1492R (50-TACGGC TACCTTGTTACGACTT-30), which had been used by other researchers with similar goals before [21]. PCR was done with 25 µL of Prime master mix, 20 µL of sterile water, and 1 µL of pooled DNA as the template. Primers 27F and 1492R were also used. The total reaction volume was 100 µL. The PCR protocol commenced with 4 minutes at 94°C, followed by 32 cycles consisting of 30 seconds at 94°C, 30 seconds at 60°C, and 2 minutes at 72°C, concluding with a final expansion step of 10 minutes at 72°C.

2.9 Optimization of growth parameters

2.9.1 pH, Temperature, and incubation period optimization

To ascertain the optimal pH for the growth of isolates, 0.4% (v/v) of fresh culture was infused into 10 ml of nutrient agar media modified to pH 5–10 and incubated at 30°C with shaking at 180 rpm for 24 hours. Following incubation, the optical density was assessed at 600 nm using a UV-visible spectrophotometer (Thermo Scientific). All experiments were conducted in triplicate [16].

A 0.4% (v/v) fresh culture was added to 10 ml of nutritional agar media, and the mixture was incubated at 20, 25, 30, 35, and 40^oC to maximize the growth of the isolates. A UV-vis spectrophotometer was used to assess absorbance at 600 nm following a 24-hour incubation period. Three duplicates of each experiment were conducted.

To ensure the best possible growth for the isolates, 10ml of nutrient agar medium were inoculated with 0.4% (v/v) of fresh culture and incubated at 30 degrees Celsius. Utilizing a UV-vis spectrophotometer, absorbance was recorded at 600 nm during the incubation periods of 6, 12, 24, 36, 48, and 72 hours. Three duplicates of each experiment were conducted.

3. Results and Discussion

Wastewater samples have been collected from the effluent channel balanager, Hyderabad, Telangana. The wastewater samples, labelled AS1 to AS10. The average values of heavy metals are given in Table 1. It is distinct from the table that station-I recorded very high concentrations of metals whereas station-III recorded comparatively low concentrations of metals. Iron was recorded in very high concentration at all the stations.

Lead is a serious cumulative poison. It ranged between 1.9 and 7.5 mg/L at stations- 1, 1.5 to 5.2 mg/L and 1.5 to 8.0 mg/L at stations-II and III respectively. Manganese in surface waters present both in interruption in the quadrivalent state and trivalent state in a moderately static soluble compound. At station-I it differed widely between 16.52 and 28.0 mg/L. At station-II it was noted in between 2.19–14.59 mg/L, thus showing a 116% decrease from station-I. At station-III it was observed 2.7 to 8.7 mg/L. showing a 44% decrease in its concentration from station-II.

Zinc is an important micronutrient for growth and metabolism of algae, Zinc varied between 7.08–21.8 mg/l at station-1 and 1.0-15.9 mg/L at station-II showing 116% decrease from station- I. At station-III, it was recorded between 0.7 and 10.4 mg/l showing a 65% decrease in its concentration from station-II. Maximum values were recorded during summer. Iron was the range of 9623–20547 mg/L and at station-II it fluctuated between 16.4 and 383.0 mg/L., showing 100000% decreases from station-I. At station-I copper fluctuated between 0.63 and 6.8 mg/L. At station-II in the range of 0.5-5.0 mg/L, showing a decrease of 102% from station-I. At station-III it was recorded in the range of 0.08–2.28 mg/L, showing 41% decrease from station-II. High values were recorded during summer.

Table 1
Heavy metal concentration of the sampling sites.

Metals	Station-I	Station-II	Station-III
Lead	5.57	3.4	4.5
Manganese	25.13	9.2	7.16
Zinc	17.01	9.1	5.9
Iron	1395	149.2	108.24
Copper	4.96	2.48	2.0
pH	5.8	7.2	7.8
Nitrates	58	62	45
Phosphates	2.8	2.32	3.46
Hardness	809	512	556
Organic matter	28.3	29.32	22.82
All values expressed in the units of mg/L, except pH			

10-ml of algal samples were taken, and underwent dilutions to yield respective concentrations of 10^{-1}, 10^{-2}, 10^{-3}, 10^{-4}, 10^{-5}, 10^{-6}, and 10^{-7}. Using the spread plate method on a clean operating table, nutritional agar was spread out over samples with varying concentrations of dilution and left to sit at 30°C for 24 hours. In order to get pure colonies, the colonies were re-streaked. We isolated 10 different algal strains for our study: AS1, AS2, AS3, AS4, AS5, AS6, AS7, AS8, AS9, and AS10. These strains were then cultured and grown on nutrient agar (NA) medium for extensive growth.

The range of 50 to 1900 mg/L was considered for each heavy metal's minimum inhibitory concentration (MIC). The MIC values of algal strains obtained from AS1 to AS10 varied in concentration, ranging from 2.32 to 149mg/L. Findings revealed that the isolate 7 showed resistance to every one of the ten heavy metals (Table 2).

Algal strain Isolate 7, the best-performing strain from the balanagar channel wastewater sample, underwent 16S rRNA identification tests. NCBI received the isolates' assembled RNA sequences. MACROGEN (Seoul, Korea) amplified ten samples of the sample's 16S rRNA sequences using universal 16S rRNA primers. The isolate 7 revealed identification with *Pandorina morum YSCVR24* (Fig. 1) based on the relatedness of closely related algal species *(*Fig. 2).

Table 2
MIC values of heavy metals verses Isolated algal strains.

Minimum Inhibitory Concentration values of heavy metals verses Isolated algal strains.											
S. No.	Heavy metals	AS1	AS2	AS3	AS4	AS5	AS6	AS7	AS8	AS9	AS10
1	Zinc	1.12	NA	1.38	1.4	1.84	1.55	3.25	NA	1.34	1.26
2	Manganese	1.25	NA	1.65	NA	2.25	1.25	4.88	1.46	1.82	1.86
3	Iron	2.23	2.5	2.2	2.54	4.58	NA	6.66	2.5	2.34	2.28
4	Copper	3.5	3.2	NA	3.2	4.82	3.45	6.57	NA	3.24	NA
5	Chromium	NA	NA	1.66	NA	1.55	NA	2.98	NA	NA	NA
6	Lead	NA	NA	0.06	0.02	0.84	NA	1.55	0.04	NA	NA
7	Arsenic	NA	NA	0.02	NA	0.22	0.02	0.34	NA	NA	NA

Heavy metal concentrations in the wastewater dropped as a result of algae breaking them down into simpler molecules, according to the present decomposition study. The best conditions for the growth of isolated algal strain are a culture pH of 7 and a 24-hour incubation time at 30^{o}C. Biodegradation capacity of wastewater sample was increased in the isolate 7 (*Pandorina morum YSCVR24*) with 60 to 80%. as can be seen in Fig. 3. This research opens up new avenues for the potential discovery of enzymes with real-world commercial uses in wastewater, and it also increases the likelihood of isolating algae strains with industrial significance.

3.1 Phylogenetic tree construction:

The phylogenetic tree shows the evolutionary relationships of *Pandorina morum* strain YSCVR24 with other related species (Fig. 3). The *Pandorina morum* strain YSCVR24 is closely related to *Pandorina morum* strain KMMCC 1257, Both belong to the *Pandorina* genus, indicating they share a recent common ancestor. The tree includes several *Eudorina elegans* isolates (hyx1507e14, hyx1507e13, hyx1507e17, hyx1505e8). *Eudorina* is another colonial volvocine algae, with typically 32 or 64 cells in a colony. The presence of *Eudorina compacta* (2018-1205-E-14) suggests that *Eudorina* species form a monophyletic group (sharing a common ancestor) within Volvocaceae. Since *Pandorina* and *Eudorina* appear closely related, it is possible that *Eudorina* evolved from a *Pandorina*-like ancestor by increasing the number of cells per colony. The percentages (e.g., 38%, 37%, 28%) likely represent bootstrap support values, which indicate the reliability of certain branching points. Lower values suggest weaker confidence in those specific branches.

4. Conclusion

The ability of natural algae samples to break down heavy metals like lead, arsenic, chromium, and mercury was examined. Ten different algal samples were isolated for their degrading efficiency. The current invention examined the potential of local algal stains to decompose mercury, cadmium, chromium, manganese, iron, copper, zinc, nickel, and lead. One of the ten isolates had to accelerate degradation. Using 16S rRNA gene sequence genotyping and NCBI BLAST, the best performed isolate found as Pandorina morum YSCVR24. The isolate grew best at pH 7.0, 30°C, and 24 hours. Lead and arsenic had the highest degradation potential (80 and 85%), while mercury and chromium had the lowest (62 and 65%). Using plasmids, Pandorina morum YSCVR24 may degrade and accumulate heavy metals. It also reduced 60–80% of all heavy metals tested. These results have broadened the potential for identifying commercially essential types of algae in wastewater. These isolated strains could serve as a crucial resource for identifying enzymes that have practical uses in various industries.

Statements and Declarations

Author contribution: Y. Seeta was responsible for study conception and design, material preparation, data collection and analysis, writing and original draft preparation.

T. Raghavendra contributed to the study conception and design, editing, reviewing and scientific discussion, and supervised the production of the final version.

Funding Declaration: The authors declare that no funds, grants, or other support were received during the preparation of this manuscript.

Data availability Declaration: No datasets were generated or analyzed during the current study.

Ethics approval and consent to participate: Not applicable.

Consent for publication: Not applicable.

Competing interests: The authors have no relevant financial or non-financial interests to disclose.

Clinical trial number: not applicable.

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Figures

Pandorina morum strain YSCVR24 small subunit ribosomal RNA gene, partial sequence; internal transcribed spacer 1 and 5.8S ribosomal RNA gene, complete sequence; and internal transcribed spacer 2, partial sequence

GenBank: PQ110233.1
FASTA Graphics

Go to:

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REFERENCE   1  (bases 1 to 923)
  AUTHORS   Seeta Reddy,Y., Raghavendra,T. and Manikya Reddy,P.
  TITLE     Heavy metal degradation by using new isolate Pandorina morum
            YSCVR24
  JOURNAL   Unpublished
REFERENCE   2  (bases 1 to 923)
  AUTHORS   Seeta Reddy,Y., Raghavendra,T. and Manikya Reddy,P.
  TITLE     Direct Submission
  JOURNAL   Submitted (30-JUL-2024) Environmental Science, Humanities and
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Figure 1

16S RNA sequence molecular identification of *Pandorina morum YSCVR24* of the algal isolate.

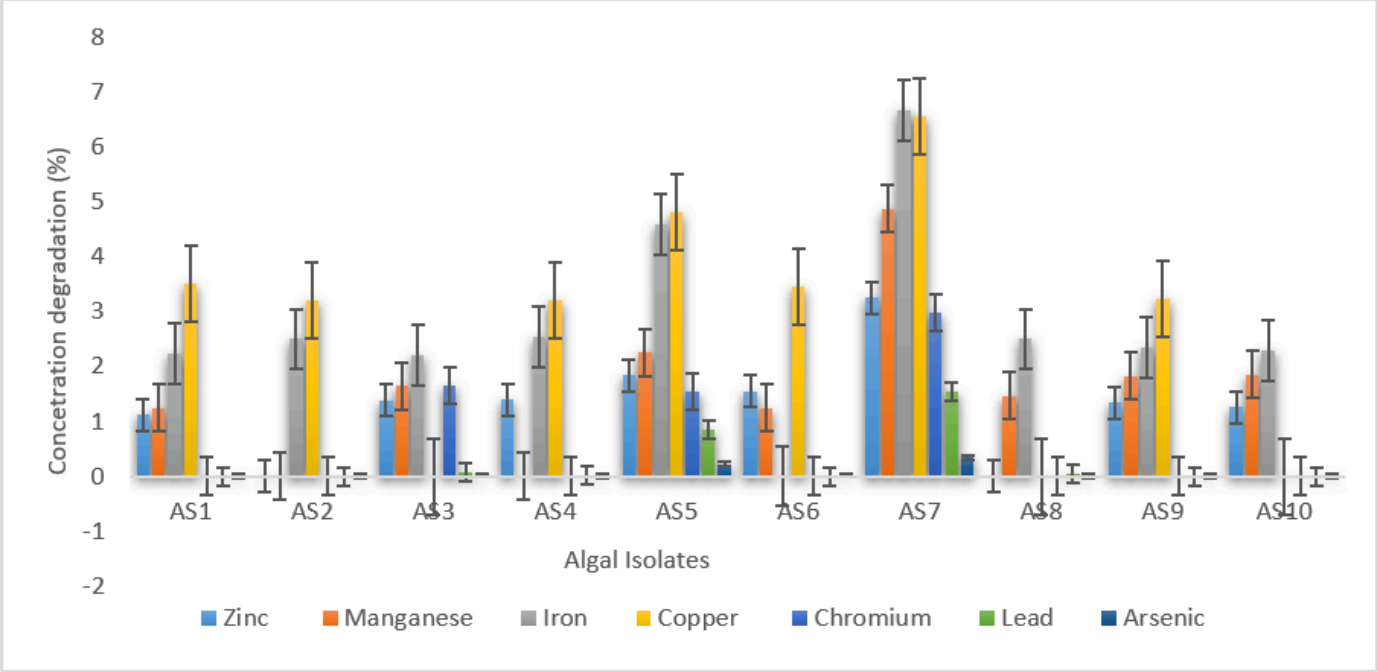

Figure 2

Heavy metal degradation of algal isolates.

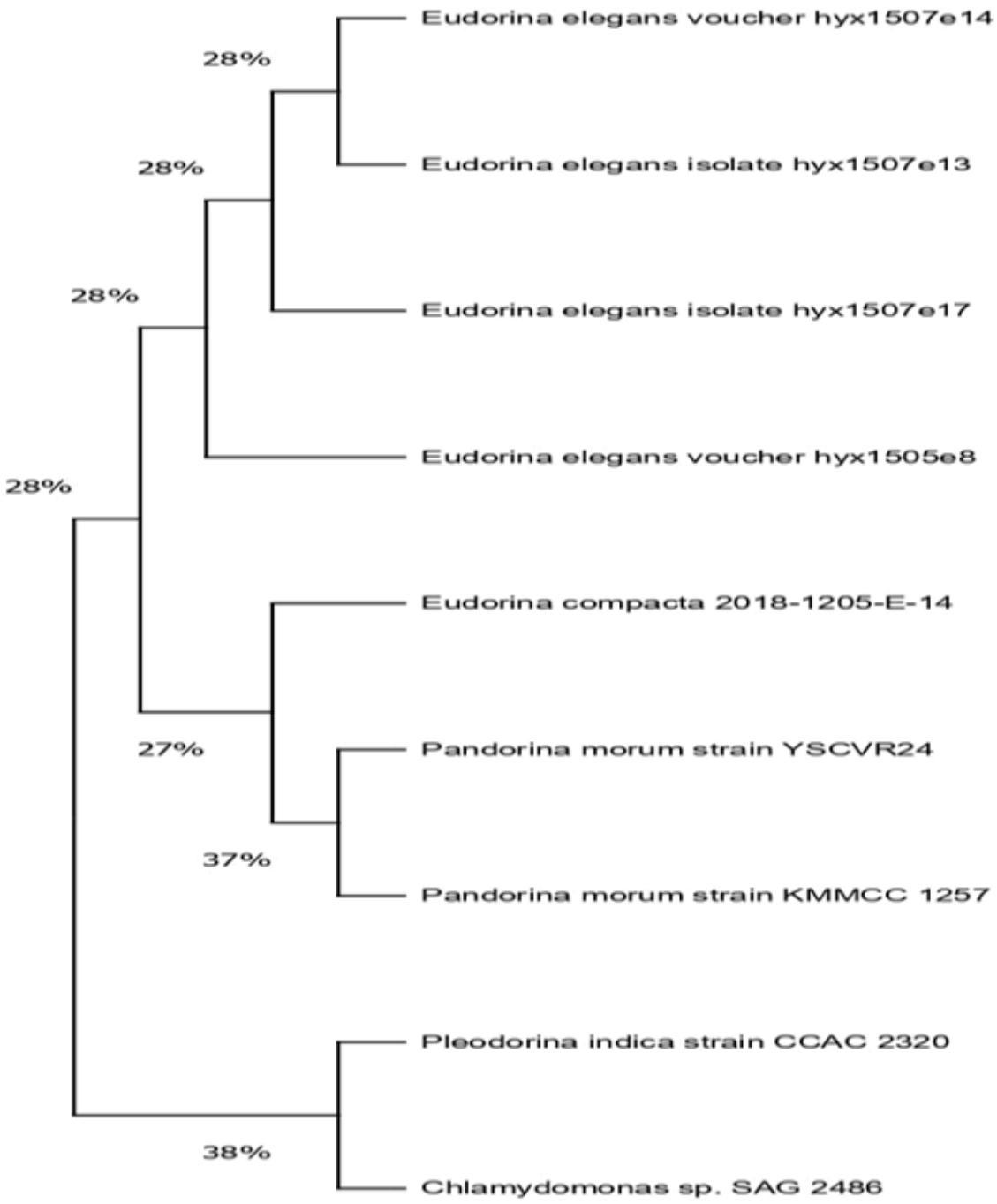

Figure 3

Phylogenetic tree of algal isolate 7 (*Pandorina morum YSCVR24*).