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Bisphenol A degradation by *leclercia adecarboxylata* and synergistic effects bacterial consortia

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ABSTRACT

This study investigated the potential of bacterial isolates from plastic waste disposal and wastewater treatment sites in Bilaspur, Chhattisgarh, for the biodegradation of Bisphenol A (BPA). The findings revealed that BPA concentration significantly influenced bacterial survival and growth. A total of 44 bacterial isolates were identified, of which 12 showed growth at the highest BPA concentration (160 ppm), highlighting their resilience and adaptability. Among these, isolate S2-4 (S2GGV-4) demonstrated the highest BPA degradation efficiency (75%) when grown individually. Co-culturing experiments further enhanced BPA degradation, with the consortium of S2-4 achieving 90.16% degradation, representing a synergistic improvement in efficiency. These results emphasize the potential of using microbial consortia for efficient BPA bioremediation, as metabolic complementarity among isolates enhances degradation performance. Molecular characterization of the most efficient isolate, S2-4 (S2GGV-4), identified it as Leclerciaadecarboxylata. Phylogenetic analysis confirmed its taxonomic placement within the genus Leclercia and highlighted its evolutionary relationship with other strains of L. adecarboxylata. The identification of this strain is particularly significant, as it may possess unique metabolic pathways enabling BPA degradation, which could be further exploited for environmental and industrial applications. Overall, this study underscores the potential of microbial isolates, particularly Leclerciaadecarboxylata S2-4 (S2GGV-4), in BPA bioremediation. The results provide a foundation for future research focused on optimizing environmental conditions and exploring the metabolic pathways involved in BPA degradation, as well as scaling up the use of microbial consortia for sustainable remediation of BPA-contaminated environments.

Key Word: Bisphenol A (BPA) degradation, Bioremediation, *Leclerciaadecarboxylata*, Bacterial consortia, Environmental contamination

INTRODUCTION

Bisphenol A (BPA) is an industrial chemical widely used in the production of polycarbonate plastics and epoxy resins, which are commonly found in a variety of consumer products such as food containers, water bottles, and thermal paper (Vijayalakshmi *et al.* 2018). During the polymerization process, BPA is synthesized from phenol and acetone through a condensation reaction catalyzed by either an acid or an alkali, which explains the "A" in its name. It is internationally recognized as a harmful chemical (Yi *et al.* 2025; Razia *et al.* 2024; Han *et al.* 2023). Despite its versatility and economic significance, BPA is a recognized environmental pollutant and endocrine-disrupting compound (EDC) with potential adverse effects on human health and wildlife. Once released into the environment, BPA is highly resistant to natural degradation processes, resulting in its accumulation in soil, water bodies, and even the food chain. This persistence, coupled with its toxicity, has made BPA a critical environmental concern, necessitating the development of effective strategies for its removal (Husain and Qayyum, 2013; Mishra *et al.* 2015). One promising approach to mitigate BPA pollution is microbial biodegradation, a process that utilizes microorganisms to break down complex organic pollutants into less harmful or non-toxic compounds (Cheng and Wang, 2024). Among the diverse microbial species capable of degrading pollutants, *Leclerciaadecarboxylata* has emerged as a notable candidate for BPA degradation. This bacterium, known for its metabolic adaptability, has demonstrated the ability to utilize BPA as a carbon source, effectively transforming it into environmentally benign byproducts. However, despite the potential of *L. adecarboxylata*, its specific mechanisms for BPA degradation remain underexplored, and the environmental conditions that optimize its activity are not well understood (Swargiary, MD., 2022).

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The persistence of BPA in the environment poses significant risks to ecosystems and human health, necessitating efficient and sustainable methods for its removal (Tarafdar *et al.* 2022; Abu Hasan *et al.,* 2023). While microbial biodegradation offers a promising solution, the effectiveness of this approach depends on identifying and optimizing microbial strains capable of efficiently degrading BPA. Although *Leclerciaadecarboxylata* has shown potential for this purpose, there is limited knowledge about the pathways and enzymatic mechanisms it employs to degrade BPA. Additionally, there is a lack of comprehensive studies that evaluate the environmental factors influencing its degradation efficiency, limiting the practical application of this bacterium in real-world scenarios(Hemavarshini *et al.* 2024). Keeping this we have design this study to investigate the biodegradation of Bisphenol A (BPA) by *Leclerciaadecarboxylata*

MATERIALS AND METHOD

Sample collection

To investigate Bisphenol A (BPA) degradation potential in bacteria, soil and water samples were collected from six different sites in and around Bilaspur, Chhattisgarh. These sites were selected based on their proximity to plastic waste disposal areas and wastewater treatment facilities, where environmental exposure to BPA and other pollutants is significant. The collected samples were utilized to isolate bacterial strains capable of degrading BPA.

Microbial Isolation

Microbial isolation for Bisphenol A (BPA) degradation was performed using the serial dilution method followed by enrichment in Basal Salt Medium (BSM) supplemented with BPA as the sole carbon source. Soil and water samples collected from various plastic waste disposal sites and wastewater treatment plants were diluted, plated on Nutrient Agar, and incubated at 37°C overnight to obtain initial colonies. Enrichment involved incubating 5 grams of each sample in 100 mL of sterile BSM containing 100 mg/L BPA at 30°C and 180 RPM for 7 days. The culture was sequentially transferred to fresh BSM with increasing BPA concentrations (up to 500 mg/L) over four cycles. Final enriched cultures were spread on BPA-containing agar plates and incubated for 5 days at 30°C. Colonies exhibiting growth were isolated and purified by repeated streaking. Morphological characterization revealed colonies of various colors (cream, white, yellow, pink, and brown), shapes (round or uneven), and textures (rough or smooth). An abiotic control confirmed that BPA degradation was solely due to microbial activity. This method successfully enriched and isolated bacterial strains capable of BPA degradation, providing potential candidates for bioremediation (Wu *et al.* 2024; Matsumura *et al.,* 2009).

Primary screening for BPA removing microbes

Primary screening of BPA-degrading microbes was carried out using BSM and nutrient agar with BPA as the sole carbon source. Sterilized plates were prepared with BSM containing varying BPA concentrations (10 ppm, 20 ppm, 40 ppm, and 160 ppm). The microbial isolates were inoculated onto these plates and incubated under controlled conditions: temperature of 37°C, pH 7, and duration of 72 hours (Dai *et al.,* 2022).

Determination of biodegradation of BPA using UV-Vis

To assess the level of Bisphenol A (BPA) degradation, the sulphanilic acid method was employed, utilizing a diazo-coupling reaction between BPA and sulphanilic acid. In the first step, sulphanilic acid reacts with acidic sodium nitrite at low temperature (0–5°C) to form a diazonium salt. This intermediate compound then couples with BPA in an alkaline medium, resulting in the formation of a yellow azo-dye with a maximum absorbance at 439 nm, which can be quantified using a UV-Visible spectrophotometer. Freshly prepared stock and working solutions were stored in amber bottles to prevent degradation. For the reaction, 1 mL of sulphanilic acid (120 µg/mL) was mixed with 1 mL of cold acidic sodium nitrite solution (0.1 M HCl and 140 µg/mL $NaNO_2$, mixed in a 1:1 ratio), and the mixture was left to stand on ice for 5–10 minutes. Subsequently, 1 mL of varying BPA concentrations (0.5–3 ppm) was added and

shaken for 5 minutes. To complete the coupling reaction, 1 mL of 0.1 M NaOH (pH 13) was added, and the mixture was allowed to stand for 10 minutes before measuring the absorbance at 439 nm. A solution without BPA was used as the blank. The percentage of BPA degradation was calculated using the formula:

$$\%\text{Biodeterioration} = \frac{\text{InitialConcentration} - \text{FinalConcentration}}{\text{InitialConcentration}} \times 100$$

This method provides a reliable approach to quantify BPA degradation by monitoring absorbance changes, ensuring accurate estimation of biodegradation efficiency (Mengting *et al.* 2020).

Molecular identification of bisphenol A degradation bacteria

Molecular identification of two high BPA-degrading bacterial isolates, S2-4 (S2GGV-4) and S4-1 (S4BV-1), was performed through 16S rRNA gene analysis. Genomic DNA was extracted from bacterial cultures grown on nutrient agar for 2 days at 28°C using a spin column kit (HiMedia, India). DNA purity was assessed using a UV spectrophotometer. The service was outsourced from National Collection of Industrial Microorganisms (NCIM), CSIR-NCL, Pune, India. The 16S rRNA gene (~1284 bp) was amplified via polymerase chain reaction (PCR) using specific primers (907RC_704F) and a Biorad Mini thermal cycler. The PCR reaction involved 40 cycles of denaturation at 94°C for 1 minute, annealing at 48°C for 1 minute, and extension at 72°C for 2 minutes, followed by a final elongation step at 72°C for 5 minutes. The PCR products were electrophoresed on a 1.5% agarose gel and visualized using ethidium bromide staining. Purified amplicons were sequenced using the Sanger method (ABI 3500xL genetic analyzer) (Clarridge, 2004; Darby *et al.,* 2005).

The sequencing data were processed using CHROMASLITE software, and sequence similarity searches were performed with the BLASTN algorithm against the NCBI nucleotide database to identify closely related species through (https://blast.ncbi.nlm.nih.gov/) (Altschul *et al.,* 1990). The top five to ten hits were recorded for each isolate. Multiple sequence alignment was conducted using ClustalW, and evolutionary distances were estimated using the MEGA11 software. Phylogenetic analysis was performed using the Tajima-Nei method, and a phylogenetic tree was constructed with 1000 bootstrap replicates to ensure accuracy. The analysis provided insights into the taxonomic identity and evolutionary relationships of the isolates, confirming their novelty and potential application in BPA biodegradation (Myers *et al.,* 1988).

Statistical analysis

In this study, results were expressed as means of triplicate values with standard deviation (±SD). Statistical analysis was performed by one-way single factor ANOVA was calculated with the help of the SPSS version 16.0 software. The P values obtained less than the level of significance which is expressed as $P< 0.05$.

RESULTS & DISCUSSION

Selection of bacterial isolates for BPA degradation

Soil and water samples from six plastic waste disposal and wastewater treatment sites in Bilaspur, Chhattisgarh were collected and cultured. Based on colony morphology, 44 bacterial isolates were identified, labeled, and stored for further study. These isolates were tested for their ability to degrade Bisphenol A (BPA) by growing them on Basal Salt Medium (BSM) with BPA concentrations of 10 ppm, 20 ppm, 40 ppm, 80 ppm, and 160 ppm. The results showed that bacterial survival decreased with increasing BPA concentration. At 10 ppm, 32 isolates survived, while only 12 isolates grew at 160 ppm, indicating that higher BPA levels inhibited bacterial growth (Razia *et al.,* 2024).

Table 1: isolation and selection of bacteria for BPA Degradation

BPA Concentration (ppm)	10 ppm	20 ppm	40 ppm	80 ppm	160 ppm
No. of Bacteria (After 24h)	32	25	22	15	12
		Bacterial Isolates			
S1	S1-1, S1-2, S1-3, S1-5, S1-6 (5 isolates)	S1-1, S1-2, S1-3, S1-6 (4 isolates)	S1-2, S1-3, S1-6 (3 isolates)	-	-
S2	S2-1, S2-2, S2-3, S2-4, S2-6, S2-7, S2-8, S2-10 (8 isolates)	S2-1, S2-2, S2-3, S2-4, S2-7, S2-8, S2-10 (7 isolates)	S2-1, S2-2, S2-3, S2-4, S2-7, S2-8, S2-10 (7 isolates)	S2-1, S2-2, S2-3, S2-4, S2-7, S2-8 (6 isolates)	S2-1, S2-2, S2-4, S2-7, S2-8 (5 isolates)
S3	S3-1, S3-2, S3-3, S3-4, S3-6 (5 isolates)	S3-1, S3-2, S3-3, S3-4 (4 isolates)	S3-1, S3-2, S3-3, S3-4 (4 isolates)	S3-1, S3-2, S3-3, S3-4 (4 isolates)	S3-1, S3-4 (2 isolates)
S4	S4-1, S4-2, S4-3, S4-4, S4-5, S4-6, S4-7 (7 isolates)	S4-1, S4-2, S4-3, S4-4, S4-5, S4-6, S4-7 (7 isolates)	S4-1, S4-2, S4-3, S4-5, S4-6, S4-7 (6 isolates)	S4-1, S4-2, S4-3, S4-5, S4-6 (5 isolates)	S4-1, S4-2, S4-3, S4-5, S4-6 (5 isolates)
S5	S5-1, S5-2, S5-3, S5-5 (4 isolates)	-	-	-	-
S6	S6-2, S6-7, S6-9 (3 isolates)	S6-2, S6-7, S6-9 (3 isolates)	S6-2, S6-9 (2 isolates)	-	-

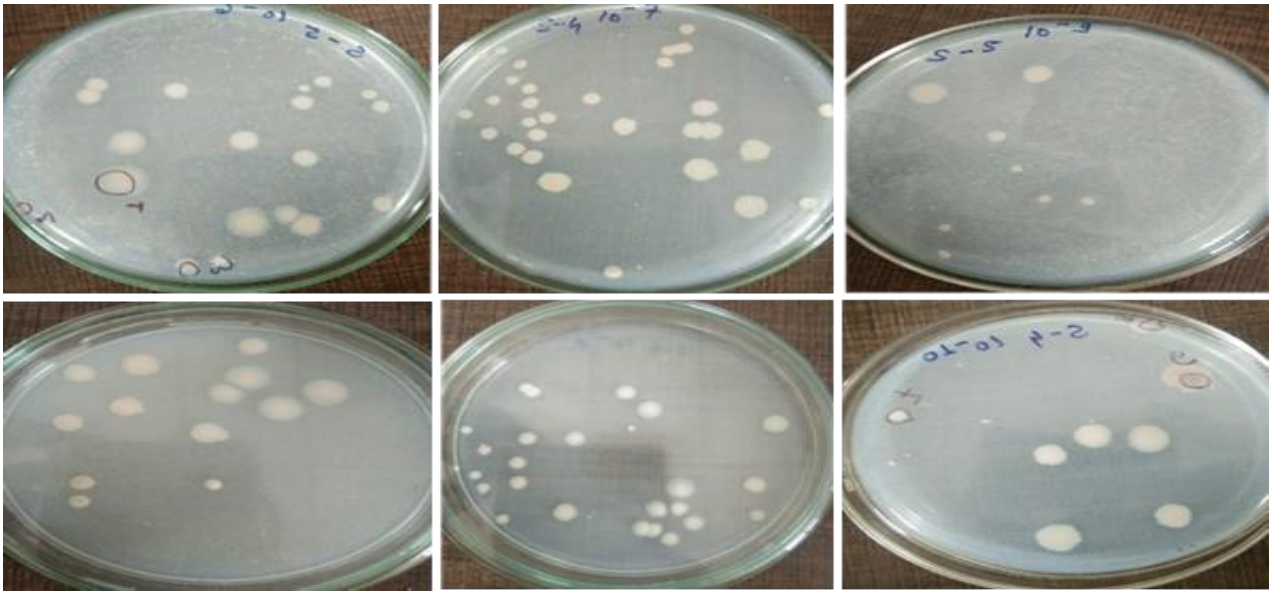

Figure 1: Screening of soil and water samples collected from waste material recycling hub

Growth of selected bacterial isolates on BPA

The growth of the bacterial isolates on BPA-supplemented BSM was concentration-dependent, with a decline in the number of viable isolates as BPA concentration increased. While 32 isolates grew on 10 ppm BPA, only 12 showed growth at 160 ppm. The ability to grow at higher BPA concentrations highlights these isolates' potential for BPA degradation. In shake flask cultures, the 12 selected isolates exhibited varied growth patterns using BPA as the sole carbon source. At 160 ppm BPA, isolates S2-4 and S4-1 achieved maximum growth, with optical densities (OD600) of 0.94 and 0.99, respectively, after 24 hours (Darby and Hine, 2014). Both isolates exhibited minimal lag phases, indicating rapid adaptation to BPA. S4-3 also showed promising growth but experienced a decline after 18 hours. The remaining isolates displayed similar growth trends, with most failing to surpass an OD 600 of 0.8 within 24 hours (Figure- 2). These results suggest that isolates S2-4 and S4-1 are particularly suited for BPA degradation studies due to their resilience and rapid growth in high BPA concentrations. The ability of S4-1 to maintain strong growth at 160 ppm BPA underscores its potential application in bioremediation (Kamaraj *et al.* 2014).

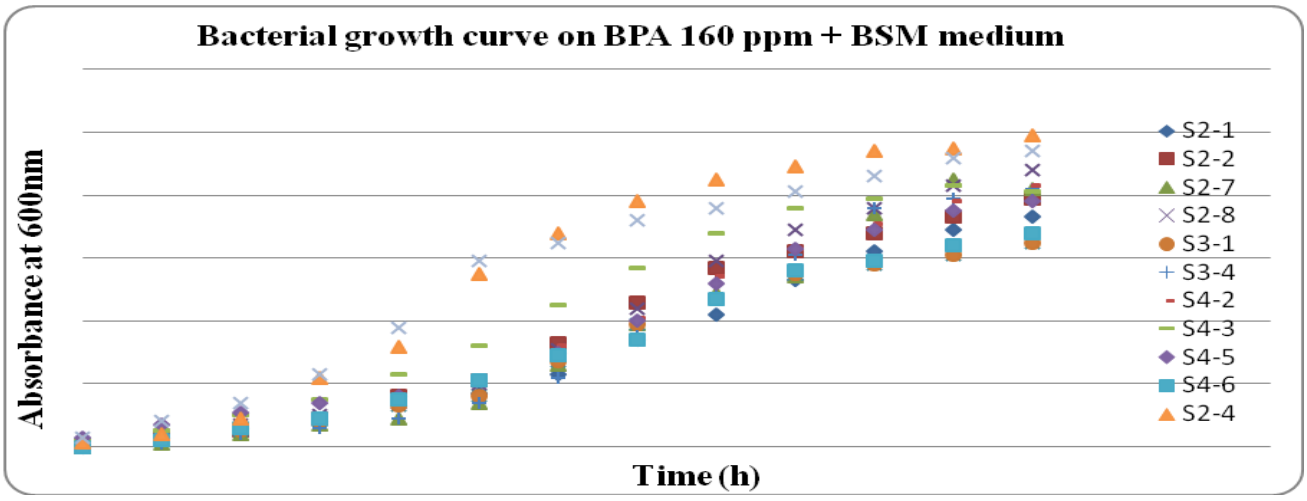

Figure 2: Growth profile of different bacterial isolates cultivated on 160 ppm BPA supplemented BSM media over a period of 24 hr, at 37 °C (P< 0.05.)

BPA degradation level by selected bacterial isolates

In order to address the ability of these isolates towards BPA degradation, the culture sample was taken and assessed for the residual BPA in each flask. To establish a relationship between the absorbance and the Bisphenol A content for spectrophotometric measurement, a calibration curve is required.

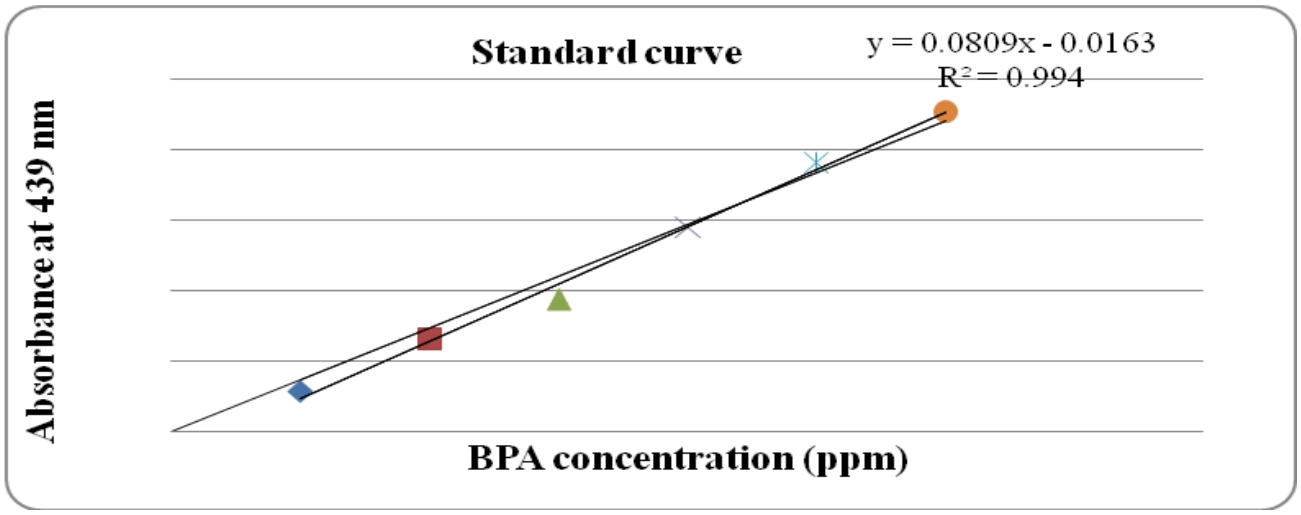

Figure 3: Standard curve for the determination of bisphenol A

The ability of selected bacterial isolates to degrade Bisphenol A (BPA) was assessed, and significant variation in degradation efficiencies was observed among the isolates. Based on the calibration curve used for spectrophotometric measurements, the residual BPA levels in the culture samples were determined. Out of the 12 isolates tested, S2-4 (S2GGV-4) exhibited superior BPA degradation capabilities, achieving >75% degradation after 4 days of incubation. Five isolates (S2-4, S2-7, S2-8, and S2-2) showed ≥50% degradation efficiency, indicating their potential for BPA bioremediation and were selected for further studies. Notably, isolate S2-4 (S2GGV-4) demonstrated the highest individual degradation efficiency of 75%, highlighting its strong metabolic activity towards BPA as a sole carbon source (Razia *et al.,* 2024).

Table 2: Bis phenol A degradation by 12 selected bacterial isolates on BPA as sole carbon source after 4 days incubation

Bacterial Isolates	Degradation of Bis phenol A (%)
S2-1	24.83
S2-2	26.93
S2-4(S2GGV-4)	75.00
S2-7	51.29
S2-8	52.17

To enhance the degradation efficiency, co-culturing experiments were conducted, forming bacterial consortia by combining two isolates at a time. A total of 45 consortia were tested, and the results revealed a significant improvement in BPA degradation due to the synergistic interactions between the isolates (Sarma *et al.,* 2019). The bacterial consortium of S2-4 (S2GGV-4) achieved the highest BPA degradation efficiency of 90.16%, representing a 1.2-fold increase compared to the individual degradation abilities of the isolates. This result underscores the importance of cooperative metabolic activity in biodegradation, as different bacterial species may utilize complementary enzymatic pathways to break down BPA more effectively. The consortium containing S2-4 (S2GGV-4) consistently showed high degradation efficiencies across multiple combinations, further confirming its key role in BPA metabolism (Yu *et al.* 2019).

Interestingly, out of 45 combinations, 22 consortia achieved BPA degradation rates exceeding 20%, indicating that co-culturing enhanced degradation efficiency in nearly half of the tested combinations. The variability in degradation rates among consortia suggests that the metabolic compatibility and enzymatic diversity of paired isolates play a critical role in determining overall efficacy. The results highlight the potential of using microbial consortia for efficient BPA bioremediation, as co-culturing enhances both degradation capacity and ecological adaptability.

Table 3: Bisphenol A degradation by different bacterial consortia

S.No.	Bacterial Consortia	Bis phenol A Degradation (%)	S.No	Bacterial Consortia	Bis phenol A Degradation (%)
1	S2-1 & S2-2	11.77	23	S2-4 & S2-8	23.95
2	S2-1 & S2-4	16.93	24	S2-4 & S3-1	26.2
3	S2-1 & S2-7	15.4	25	S2-4 & S3-4	21.61
4	S2-1 & S2-8	1.5	26	S2-4 & S4-1	90.16
5	S2-1 & S3-1	4.3	27	S2-4 & S4-2	35
6	S2-1 & S3-4	12.9	28	S2-4 & S4-3	28.14
7	S2-1 & S4-1	21.61	29	S2-4 & S4-5	14.27
8	S2-1 & S4-2	4.3	30	S2-4 & S4-6	29.75
9	S2-1 & S4-3	7.6	31	S2-7 & S2-8	17.25
10	S2-1 & S4-5	11.85	34	S2-7 & S4-1	30.24
11	S2-1 & S4-6	5.87	35	S2-7 & S4-2	17.66
12	S2-2 & S2-4	12.9	36	S2-7 & S4-3	22.25
13	S2-2 & S2-7	26.37	37	S2-7 & S4-5	12.24
14	S2-2 & S2-8	17.04	38	S2-7 & S4-6	13.95
15	S2-2 & S3-1	6.46	39	S2-8 & S3-1	14.11
16	S2-2 & S3-4	22.49	40	S2-8 & S3-4	10.88
17	S2-2 & S4-1	19.27	41	S2-8 & S4-1	13.22
18	S2-2 & S4-2	15.4	42	S2-8 & S4-2	22.58
19	S2-2 & S4-2	30.88	43	S2-8 & S4-3	16.2
20	S2-2 & S4-5	24.03	44	S2-8 & S4-5	23.38
21	S2-2 & S4-6	26.62	45	S2-8 & S4-6	20.4
22	S2-4 & S2-7	27.41			

The cooperative interaction between different bacterial strains in a consortium can lead to more efficient pollutant breakdown compared to individual strains, as metabolic complementarity and the sharing of enzymatic pathways contribute to the overall success of the degradation process. This synergy not only improves the efficiency of bioremediation but also allows for the adaptation of bacterial communities to a wider range of environmental

conditions, enhancing the ecological relevance of this approach. The ability of consortia to degrade complex pollutants like BPA makes them a promising tool for addressing major environmental concerns, such as plastic waste contamination and industrial pollution. This study underscores the significance of microbial consortia in offering a sustainable and effective solution for the bioremediation of hazardous contaminants, marking a significant step forward in environmental biotechnology (Magodia *et al.* 2024).

Future studies should focus on characterizing the metabolic pathways involved in BPA degradation and optimizing environmental conditions to maximize the performance of promising consortia, particularly those involving S2-4 (S2GGV-4). This study demonstrates the feasibility of employing bacterial consortia for the effective and sustainable remediation of BPA-contaminated environments (Hemavarshini *et al.,* 2024).

4.5. Molecular characterization of BPA degrading bacteria

Bacterial isolates S2-4(S2GGV-4) were found to perform well towards the BPA degradation and thus were evaluated for taxonomic relation using NCBI database search. The 16S rDNA sequencing was carried out and the data was processes as described earlier. The final processed sequence was checked using BLAST search tool, NCBI. Strain S2-4(S2GGV-4) was identified as *Leclerciaadecarboxylata* and (Table S4). The nucleotide sequences for 16S rDNA gene of both the isolates have been submitted to NCBI **(**National Centre for Biotechnology Information, USA) with an Accession number of PQ416792 (Table S6). Phylogenetic analysis done using MEGA11 further revealed that the two isolates belong to the genus *Leclercia*, respectively.

Figure 4: Phylogenetic analysis of S2GGV-4 16S rDNA sequence

The phylogenetic analysis of *Leclerciaadecarboxylata S2GGV-4*, based on 16S rRNA gene sequences, demonstrated its close evolutionary relationship with other strains of *Leclerciaadecarboxylata*, such as *ATCC 23216* and *CIP 82.92*. These strains clustered together in a distinct clade supported by high bootstrap values, confirming the taxonomic placement of S2GGV-4 within the *Leclercia* genus. The robustness of the bootstrap values provides strong statistical support for the inferred evolutionary relationship. Furthermore, the separation of *L. adecarboxylata* from other genera, such as *Enterobacter*, highlights its

genetic divergence, affirming its distinct identity. The phylogenetic results suggest that *Leclerciaadecarboxylata S2GGV-4* shares significant evolutionary similarities with previously characterized strains, reinforcing the reliability of the 16S rRNA gene as a molecular marker for species identification (Naum *et al.* 2008).

The identification of *L. adecarboxylata S2GGV-4* is significant due to its potential ecological and biomedical implications. Members of the species are known to inhabit diverse environments and occasionally act as opportunistic pathogens. Strain S2GGV-4 may possess unique genetic or metabolic traits that could contribute to understanding its ecological roles or applications in biotechnology. Additionally, the presence of closely related strains in the clade suggests conserved evolutionary characteristics, which could be further explored through comparative genomic studies. The robust clustering also indicates that strain S2GGV-4 may share functional or phenotypic traits with other *Leclerciaadecarboxylata* strains, opening possibilities for its application in fields such as bioremediation or as a source of bioactive compounds

Future research could focus on the practical application of L. adecarboxylata S2GGV-4 in bioremediation, particularly in field conditions where pollutants like Bisphenol A (BPA) are present. Further exploration of bacterial consortia and their synergistic effects could optimize biodegradation efficiency, with an emphasis on understanding metabolic complementarity among different strains. Additionally, genetic and metabolic pathway studies could uncover novel enzymes or mechanisms involved in pollutant degradation, offering insights into the strain's environmental adaptability and resistance. Given the close relationship with pathogenic species, further investigation into its biomedical implications, including its potential as an opportunistic pathogen or a source of bioactive compounds, would be valuable. These directions would provide a deeper understanding of the strain's broader ecological, biotechnological, and biomedical potential.

CONCLUSION

This study investigated the potential of bacterial isolates from plastic waste disposal and wastewater treatment sites in Bilaspur, Chhattisgarh, for the biodegradation of Bisphenol A (BPA). The findings revealed that BPA concentration significantly influenced bacterial survival and growth. A total of 44 bacterial isolates were identified, of which 12 showed growth at the highest BPA concentration (160 ppm), highlighting their resilience and adaptability. Among these, isolate S2-4 (S2GGV-4) demonstrated the highest BPA degradation efficiency (75%) when grown individually. Co-culturing experiments further enhanced BPA degradation, with the consortium of S2-4 and S4-1 achieving 90.16% degradation, representing a synergistic improvement in efficiency. These results emphasize the potential of using microbial consortia for efficient BPA bioremediation, as metabolic complementarity among isolates enhances degradation performance. Molecular characterization of the most efficient isolate, S2-4 (S2GGV-4), identified it as *Leclerciaadecarboxylata*. Phylogenetic analysis confirmed its taxonomic placement within the genus *Leclercia* and highlighted its evolutionary relationship with other strains of *L. adecarboxylata*. The identification of this strain is particularly significant, as it may possess unique metabolic pathways enabling BPA degradation, which could be further exploited for environmental and industrial applications.

Overall, this study underscores the potential of microbial isolates, particularly *Leclerciaadecarboxylata* S2-4 (S2GGV-4), in BPA bioremediation. The results provide a foundation for future research focused on optimizing environmental conditions and exploring the metabolic pathways involved in BPA degradation, as well as scaling up the use of microbial consortia for sustainable remediation of BPA-contaminated environments.

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