

REVIEW ARTICLE

BIODEGRADATION OF ENDOCRINE DISRUPTOR BISPHENOL A BY INDIGENOUS MICROBIAL CONSORTIUM OF WASTE WATER: A CASE STUDY

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Manuscript Info

Abstract

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*Keywords:-*Endocrine Disruptor Chemical, Bisphenol A, Biodegradation, Microbial Consortium Bisphenol A(BPA) or 2,2-bis-(4-hydroxyphenyl propane) has beenthe most havoc-wrecking polycarbonate pollutant released majorly from plasticandresin manufacturing industriesintothemunicipalwastes.As per USEPA, if it contaminates open water bodies and enters our food chain, it can act as an endocrine disruptor to aquatic creatures and a potential carcinogen to humans. Hence, removal of this compound is necessary from the environment. Conventional chemical/physical/mechanical mitigation processes further add to accumulation of toxic reagents in the environment. Hence, many researchers reported use of a few bacterial strains for biodegradation of BPA in sustainable, environmentally friendly pathways. Strains of Sphingomonas sp. MV1, Sphingomonasbisphenolicum A01, Sphingobium sp.BiD 32, Citrobacter freundii and Pseudomonas sp. have been reported to degrade 99.87% - 100% BPA within 72-110 hours at rates 1.61-2.2 µg/L/h by using enzyme coenzymes in tandem pathways. Laccase and oxidase enzymes with coenzymes NADH, NAD+, NADPH and NADP+ perform zero/first order oxidative degeneration reactions of BPA. Reported HPLC, GC-MS analysis showed formation of end products oxalic acid, 1,2,4-trimethylbenzene and 2.9-dimethyldecane which proved to be non-toxic by algal toxicity testing. This information can further help future researchers to genetically engineer the established strains for faster, cost-effective mitigation of BPAin a green-technological mechanism.

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Introduction:-

The most prevalent environmental contaminants are polycyclic aromatic hydrocarbons, or PAHs. Numerous individuals worldwide are exposed to these substances, which are widely dispersed throughout a varietyof habitats, including water supplies. A man-made PAH with two phenol rings, bisphenol A (BPA; 2,2-bis(4-hydroxyphenyl) propane;CASregistryno. 80-05-7) isusedextensivelyinthe manufacturingofsyntheticpolymers, especiallyepoxy resins and polycarbonate plastics (Atacag et al., 2015). This substance has been identified as one of the endocrine-disrupting compounds (EDCs) that can impact humans and other organisms' reproduction because of its androgenicor oestrogenic action, despite its significant industrial uses (Wang et al., 2017). Additionally, a number of studies have demonstrated that BPA has mutagenic, carcinogenic, immunotoxic, and embryotoxic effects that pose a major risktobothhumanandenvironmentalhealth(Alexanderetal.,1988).Thesedays,theincreasedmanufacturingof

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BPA is a result of the extensive global demand for plastic products. Thus, during production, significant levels of BPA may be discharged into the environment, particularly in industrial and municipal wastewaters.

Transportation or consumption procedures. It is therefore imperative to provide an effective, environmentally acceptable method for removing it from exposed natural habitats. Based on our earlier research and the findings of other investigations, bioremediation employing bacteria that break down BPA has been shown to be an efficient method of getting rid of this substance. Numerous BPA-degrading bacteria have been identified thus far from soil, sediment, water, and petrochemicalwastes, and the majorityofthemcontain BPA-biodegradation routes. Lobosand colleagues(19) andSpivackand colleagues(30) werethe first to giveanexplanationofthetwo main and secondary mechanisms that *Sphingomonas sp.* strain MV1 uses to biodegrade BPA. The primary mechanism that has been suggested generates 4-hydroxyacetophenone and 4-hydroxybenzoicacid.

As metabolic intermediary compounds, a novel indigenous *Pseudomonas pseudoalcaligenes* bioremediates a salty petrochemical wastewater containing bisphenolA, while the minor one yields 2,2-bis(4-hydroxyphenyl)-1-propanol and 2,3-bis(4-hydroxyphenyl)-1,2-propanediol as primary metabolites. Later, in addition to confirming these pathways in other bacterial strains, distinct metabolic pathways were also identified in particular bacterial strains, suchas*Pseudomonasaeruginosa* PAb1 isolatedfromthermalpaper industryeffluent byVijayalakshmiet al. in2018 and *Bacillus pumilus* strains BP-2CK, BP-21DK, and BP-22DK isolated from kimchi by Yamanaka et al. in 2007. BPA can be broken down by bacteria that break down sphingosine, including *Bacillus, Ochrobactrum, andPicocystis sp*.However, microbes have a hard time breaking down BPA due to its benzene ring structure. Thus, additionalcarbonor nitrogensources, like yeast orglucose, aretypicallyrequired for screening individualspeciesof bacteria to enhance their BPA breakdown. Nevertheless, a suitable strainofbacterialresources for the breakdown of BPA is still absent because this process will make degradation more complex and incur additional operating and maintenance expenses. BPA was thus the sole carbon source in this experiment to identify microorganisms capable of effectively breaking down BPA and investigate how they degraded in water.

Through enzymatic processes controlled by functioning genes, microorganisms can frequently break down contaminants. Microorganisms will use the metabolic regulatory mechanisms to withstand BPA stress during the microbial breakdown of BPA.

Theyaredeteriorating it. Researcherscanbetter graspthekeyelements inthedegradation process by identifying the mechanism of BPA breakdown in microbial cells and screening the most significant genes engaged in this process. Though more research and analysis are required, some progress has been made in understanding the molecular mechanism underlying the biodegradation of BPA. Some studies have looked at how specific elements, such as temperature, a solution's acidity, the condition of the bacteria, or bacterial metabolites, affect degradation.

Despite extensive research on the BPA degradation pathways, little is known about the essential genes andmetabolic processes involved in BPA degradation. Consequently, the analysis of a microbial genome is a crucial phase that can be applied to further our understanding of the genome, particularly with regard to the defense and degradation mechanisms of bacteria associated with BPA. This will encourage the development of accurate bioremediation techniques for contaminated areas.

Since BPA is one of the most prevalent endocrine disruptors in the environment, it is essential to screen for bacteria that break down BPA and investigate their genome.

In order to ascertain strain P1's degradation capability under the impact of various environmental variables, qPCR was used to confirm the expression of genes encoded by enzymes involved in BPA degradation. The defense mechanism of bacteria against harmful contaminants was examined and explained based on functional annotation. The goal of this research is to provide a theoretical framework for microbial remediation of BPA-polluted settings, enhancetheBPAdegradationandresistance mechanismofstrains, and further enrichthe bankofbacteriathat break down BPA.

Several BPA-degrading bacteria have been reported, but most ofthem are only able to degrade low amounts of high BPA concentrations within several days in lab conditions and thereby cannot be used for practical removal of this compound. Hence, in the present study, isolation and characterization of a novel indigenous BPA-degrading bacteriumforeliminationofBPAfromsaltywastewaterwasconsidered.PotentialofisolatedbacteriumforBPA

removal from petrochemical wastewater was investigated on lab scale. The possible metabolic pathway for BPA biodegradationbyisolatedbacteriumwas also proposedby the identificationofthe metabolic intermediarycompounds using high-performance liquid chromatography (HPLC) and gas chromatography mass spectrometry (GC/MS) analysis.

Case Study:

Vijavalakshmi et al. in 2018established Escherichia coli (DH5a) as a strain for gene cloning (Novagen, Germany). BPA, 4-hydroxybenzaldehyde (4-HBAL), 4-hydroxybenzoic acid (4-HBA) and 4-hydroxyacetophenone (4-HAP) with a purity of 99% were purchased from Alfa Aesar (Spain). All chemicals, enzymes, plasmids and kits were purchased from specific manufacturers. Solvents for HPLC were of HPLC grade. They used the following mediaand growth Conditions: Basal salt medium (BSM, containing 1.0 g K2HPO4, 1.0 g (NH₄)₂SO₄, 0.2 gMgSO4·7H₂O, 0.01 g FeCl₃, 0.05 g NaCl and 0.05 g CaCl₂ per liter, pH 7) and Luria-Bertani (LB) medium (10 g Peptone, 10 g Na Cl, 5 g Bacto Yeast extract per litre, pH 7) were used for isolation and cultivation of BPA and phenol-utilizingbacteria. BPA was added to BSM and LB medium through 2methods. In some experiments, BPA as a sole carbon source was added to the above-mentioned media before autoclaving at an initial concentration of 300 mg/L of LB-BPA, unless other concentrations stated. In the other experiments, BPA solution (1 g/L) was prepared by dissolving 100 mg of BPA in 5 mL of pure ethanol(99%) and adding distilled water up to 100 mL, and resulting BPA solution was added to BSM (BSMBE); at initial concentrations of 300 mg/L and 1% (v/v) for BPA and ethanol, respectively. Growth and BPA-degradation activity of the selected bacterial isolates in the original petrochemical wastewater were also confirmed in 250 mL Erlenmeyer flasks containing Khuzestan petrochemical wastewater (PWW) and supplemented with concentrated solutions of BSM (PWW-BSM) and 200 mg/L BPA. After incubation of cultures with rotaryshaking in the dark (200 rpmat 30°C), growth was monitored based on absorbance at 600 nm (OD600) spectrophotometrically (Beckman, USA) during different cultivation times. Media having 300 mg/L BPA and 1.5% (w/v) pure agar were used for colony purifying and growth of the individual isolates of the bacterial consortium (from 2 to 4 days of incubation at 30°C). For isolation of BPA-resistant bacteria and its use in degradation of the same, the authors performed primary screening. In primary screening experiments, 1 mL of each petrochemical wastewater sample was inoculated directly into 50 mL of BSMBE (100) containing 20 g/L NaCl. Theresultant cultureswere incubatedat 200rpmand30°C for 7days. When the turbidity appeared, 1mLofthe grown culture media was transferred in a stepwise manner into 50 mL of fresh BSMB, containing 40 g/L NaCl for secondary screening. Finally, the grown bacterial cells in BSMB containing 40 g/L NaCl were cultivated on BSMB agar plates. The morphologically distinct bacterial isolates were purified on the BSMB plates by the repeated streakplate methodandstoredin30% (v/v) glyceroland1% (v/v) tryptonesolutionat -70°C.Certaineffectsoftemperature and pH were also checked: 5mL of LB medium was inoculated with a colony of selected isolates from the agar plate. The culture was incubated at 37 °C and 200 rpm until OD600 of 0.6. Thereafter, the resultant pre-culture was inoculated into 50 mL of BSMB containing 40 g/L NaCl at a final OD600 of 0.2. Cultures were incubated under shaking (200 rpm) at 25, 30, 35 and 40 °C for 48 h. At 12 h intervals, the OD600 of each culture was determined spectrophotometrically (Beckman, USA). The effect of pH on the growth rates of the selected isolate wasdetermined by cultivation of pre-culture in BSMB containing 40 g/L NaCl media with pH 5, 6, 6.5, 7, 7.5, 8 and 9 under the same above-mentioned conditions. During the growth of the cultures, OD600 was determinedperiodically as described previously. The authors also checked the Chemical Oxygen Demand of degrading bacteria: The cells from log-phase culture (18 h at 37 °C) of the selected isolate in LB medium were harvested by centrifugation (5000 rpm, 20 min). The pellet was re-suspended in BSM medium and washed twice. Then, an appropriate amount of the resulting suspension was inoculated in 50 mL BSMB containing 40 g/L NaCl to obtain an initial absorbance of 0.2at 600nm. The culturewasgrownin anincubator shaker at 30°Cand 200rpmfor 48 h. Samples werecollected from the culture at time points of 6, 12, 18, 24, 30, 36, 42 and 48 h and centrifuged at 13000 rpm for 5 min. The supernatant was used for the estimation of COD, whose values indicated the mean value of the two independent determinations repeated each time in duplicate. Determination of BPA Degradation: The selected isolate was pre- cultured in LB medium and grown aerobically under shaking (200 rpm) for 18 h at 37 °C. The cells were centrifugally separated (5000 rpm, 20 min) and washed twice with 5 mL of fresh culture medium. The cells inoculatedintoBSMBcontaining50mg/LPWW(petrochemicalwastewater),PWW-BSMmediaatanOD600of 0.2 and incubated under shaking (200 rpm) at 30 °C for 48 h. After incubation, samples(1 mL) were collected from the cultures at certaintime points and centrifuged at 13,000 rpm for 5 min. The resultant supernatants were filtrated through a 0.2µ membrane filter (Millipore, USA). The amount of phenol and BPA in the filtrates was determined by a high-performance liquidchromatography(HPLC) and reverse phase C18 column(4.6×250 mm, 5 mm Zorbax RX-C18). The samples were eluted with a linear gradient (10-90% acetonitrile-water) at 1 mL/min for 40 min. The injectionvolumewas25µL, and the absorbance was monitored at 280nm. Identification of metabolites from BPA

decompositionwithgaschromatography-massspectrometry(GC/MS) andHPLCwasalso carriedout bytheauthors as follows. Three compounds of 4-HAP, 4-HBAL, and 4-HBA acid were previously reported as BPA degradation metabolites of Sphingomonas sp. strain MV1, Pseudomonas alkylphenolica, and other bacterialstrains in the KEGG database. For identification of metabolic intermediary compounds of the BPA-biodegradation pathway in the selected isolate, thestandardsolutions of the threeabove mentioned compounds (200 mg/L) and BPA (300 mg/L) were prepared in the BSM medium and analyzed by HPLC. The metabolites derived from the biodegradation of BPA by the selected isolate were identified based on the comparison and matching of the peak retention time belonged to known (standard) and unknown compounds. Process of BPA Removal: Pre-culture for fermentation was prepared by inoculating 200 mL LB medium with a single colony of selected isolate. Flasks were incubated for 18 h at 37°C aerobically under shakingat 200rpm. Batchfermentationwascarriedoutina2L fermenter with a1.2Lworking volume(Biolog3000; New Brunswick Scientific Co., New Jersey, USA). The fermenter was equipped with a built-in controller for pH, temperature, agitation, dissolved oxygen (DO), and peristaltic pumps for base and acid additions. Pre-culture was centrifuged (5000 rpm, 20 min) and collected cells were washed twice with fresh BSM medium. The washed cells were inoculated into PWW, PWW-BSM at 5% (v/v) in separate batch tests. After inoculation, the temperature and pH of fermenters were automatically maintained at 35°C and 7, respectively. The DO was maintained automatically at 10% by controlling the agitation speed up to 500 rpm. Sampling was carried out at certain time points. Phenol and BPA concentrations of samples were determined using HPLC analysis at the same condition mentioned.

Outcome Of Case-Study:

Eachcollectedsamplewas inoculated into liquid BSMBE (100) containing20g/LNaClandcultivatedat 30°C for 7 days. Considerable turbidity (OD600 = 0.6 - 1.1) was observed in 4 samples during 1 to 3 days of incubation. For obtaining bacteria with higher BPA-degrading activity in salty conditions, four grown samples were cultured in BSMB containing 200 and 300 mg/L BPA as the sole carbon source and 40 g/L NaCl using a stepwise enrichment manner. Onlyone BSMB culture showed turbidity after 24 h; suggesting the existence of BPA-degrading and NaCl- tolerant bacteria in that sample. Colony purification was performed by spreading the grown liquid culture on BSMB agar plate. All colonies on the solid medium were derived from one bacterial strain on the basis of the colony morphology. Consequently, one isolate was selected for further experiments and designated as YKJ isolate. TheYKJ isolate was a Gram-negative, catalase and oxidase-positive bacilliform bacterium. Colonies of this isolate onLB agar plates were milk-white (1-2 mm size), non-transparent, circular with convex appearance, and smooth margin. Antibioticresistance evaluation on LB agar plates containing different antibiotics and the antibiogram test, showed sensitivityofYKJ isolate to rifampicin (100 µg/L), kanamycin (50 μ g/L), and tetracycline (25 μ g/L) and its resistance to ampicillin (50 μ g/L) and chloramphenicol (34 μ g/L) as previously realized genetically. Growth condition determination: The growth parameters, including suitable temperature and pH, were determined for the YKJ isolate in the presence of BPA as the sole carbon and energy source in salty conditions. The results showed that the isolate was able to grow in liquid BSMB containing 40 g/L NaCl at 25, 30, 35 and 40 °C. In addition, measuring OD600 of collected samples within 12, 24, 36 and 48 h of cultivation demonstrated the higher growth rate of YKJ isolate at 30°C compared to other temperatures. Therefore, a temperature of 30°C was applied as one of the growth parameters in further experiments (Fig. 1).



Figure1:-EffectoftemperatureongrowthofBPA-degradingbacteria atOD600.

Subsequently, the growthrate(OD600)of the YKJ isolatewas evaluated inliquid BSMB containing40 g/LNaClat a pH of5-9 and a temperature of30°C. OD600 ofcultures after 12, 24, 36 and 48 h of incubation showed that the YKJ isolate wasabletogrowat alltestedpHsexcept 5. However, thebest growthofthis isolatewasat a pHof6.5, 7and7.5. The growth pattern in different pHs indicated that the growth of the YKJ isolate was not limited to a specific pH (Fig. 2). Moreover, the potential of the YKJ isolate for growth in high concentrations ofNaCl (up to 40 g/L) as well as different pHs (6-9) and temperatures (25-40 °C) can presumably illustrate the ability of the isolate to grow in the conditions existing in the petrochemical wastewater.



Figure2:-EffectofpHongrowthofBPA-degradingbacteria asobservedatOD600.

Chemicaloxygendemandofdegrading bacteria:

BPA-degradation activity of the YKJ isolate was determined by measuring the COD of the isolate culture in BSMB containing40g/L NaClwithin6, 12, 18, 24, 30, 36, 42and48 hofcultivation. Accordingto theresults, theCODof the above-mentioned culture reduced from the initial value of655.2 mg/L to 109.2 mg/L (about 83% decrease) after 36 hoursand remained almost constant up to 48 hours(Fig. 3). Also, growth monitoring of the culture at the same time points showed close correlation between growth rate of YKJ isolate and COD reduction in BSMB containing 40 g L–1 NaCl (Fig. 3). Reducing COD of BSMB culture and increasing the growth of YKJ isolate could indicate the ability of this isolate to utilize BPA as the sole carbon and energy sources.

PLAUSIBLEMECHANISM OF BPADEGRADATION BY ISOLATES

BPA-degradation activity of *P. pseudoalcaligenes* strain YKJ was also confirmed in BSMB containing 40 g/L NaCl by HPLC analysis. According to the chromatogram of the HPLC, the retention time of BPA was 23.772 min (Fig.4). The calibrationcurve equation for detectionoffhe BPA concentrationwas as follows: peak area = 21.12 CBPA - 9.742 ($R^2 = 0.999$), where CBPA was the BPA concentration (within the range of 1 - 300.0 mg/L). The results demonstrated that BPA at 300 mg/L was reduced to 243.7, 97.57, 11.14 and 0 mg/L by P. pseudoalcaligenes strain YKJ within 12, 18, 24, and 36 h, respectively. This strain was able to degrade high levels (288.86 mg/L) of BPA within 24 h and utilize 100% (300 mg/L) of BPA without detectable new peaks in HPLC analysis within 48 h. Therefore, *P. pseudoalcaligenes* strain YKJ can utilize BPA as its sole carbon source to produce CO₂, H₂O and all cell components.



Time (h)

Figure3:-Inter-relationoftime, COD, biomassgrowthofBPA-degradingbacteria.



 $Figure 4: \mbox{-} Plausible mechanism of BPA degradation by BPA-degrading isolates.$

Subsequently, the growth and phenol, BPA-degradation activity of *P. pseudoalcaligenes* strain YKJ were evaluated in PWW and PWW-BSM which contained 100 mg/L phenol and 300 mg/L BPA on the basis of HPLC analysis. Growth increased to OD600 of 0.597 ± 0.009 in PWW and OD600 of 0.570 ± 0.005 in PWW-BSM until 24 h and thereafter until 48 h, it did not increase. BPA was decreased to an undetectable level by HPLC in both cultures within 24 h. Thus, the similarity of the results in both cultures showed that the BSM mineral salts could not stimulatethe growth and phenol, and BPA-degradation activity of *P. pseudoalcaligenes* strain YKJ in PWW. In addition, thisstrain was able to grow up to OD600 of 0.5 and degrade300 mg/L BPA (100%) in PWW and PWW-BSM within 24 h which was higher than that in the BSMB containing 300 mg/L BPA within 48 h. Therefore, it was probable that other organic compounds existing in the petrochemical wastewater could stimulate the growth and degradation activity of *P. pseudoalcaligenes* strain YKJ. In addition, other living microorganisms existing in petrochemical wastewater might synergistically enhance the growth and BPA degradation activity of this strain. **Comparative Study OfBpa-Degradation Capacity Among Bpa-Degrading Strains Asper Reorted Literature** Asobserved in the table below (Table 1), we can see the comparison of BPA-degradation capacity of the strain discussed in the present case study vis-à-vis other reported literature.

Microorganism	BPA	NaCl	BPA removal	Time(h)	Reported
	concentration mg/L	concentration g/L	(%)		Literature
Р.	300	40	96.28	24	Present case
<i>pseudoalcaligeness</i> train YKJ					study
Pseudomonas putidastrainYC-AE1	200	0	100	20	Eltoukhyet al. (2020)
Sphingobiumsp. YC- JY1	100	0	100	12	Jiaetal. (2020)
Sphingobiumsp. YC- JY1	100	6–10	0(inhibited)	10	Jiaetal.(2020)
Pseudomonas sp. strain KU1	1000	0	78	288	Kamarajet al.(2014)
Pseudomonassp. strain KU2	1000	0	81	288	Kamarajet al. (2014)
Bacillussp.strainKU3	1000	0	74	288	Kamarajet al.(2014)
Enterobacter gergoviaestrainBYK-7	200	0	11.55	8	Badiefaret al. (2015)
Bacilluspumilusstrains BP-2CK	25	10	100	48	Yamanakaet al.(2007)
Bacilluspumilusstrains BP-21DK	25	10	100	48	Yamanakaet al. (2007)
Bacilluspumilusstrains BP-22DK	50	10	100	120	Yamanakaet al.(2007)

Table1:-ComparisonofBPA-degradationcapacityofvariousstrainsas reported.

It is also worth noting that biodegradation of PAHs as BPA depends on environmental factors such as temperature, pH, and salinity. These parameters have important effects on the growth of bacteria and catabolic activity of the enzymes involved in the BPA-biodegradation process. The P. pseudoalcaligenes YKJ can grow in a temperature range of 25 - 40°C with an optimum growth temperature of 30 °C. Increasing the temperature improves solubility of the BPA and thus significantly increases the bioavailability of BPA molecules. In return, higher temperature reduces the metabolic activity of mesophilic aerobic microorganisms, which is also seen as reduced growth of this bacterium in BSMB containing 40 g/L NaCl. Optimum growth of the YKJ strain at 30°C can be due to the optimum temperature for activityofthe enzymes involved in the BPA-biodegradationpathwaythat is lower than 40°C. pH of the medium also affects microbial activity, including enzymatic activity, solubility, and accessibility of nutrients. P. pseudoalcaligenes YKJ grows at pH 6–9 with the best growth in the pH 6.5 - 7.5. The growth pattern indicates that the BPA-biodegradation activity of this strain is not limited to a specific temperature and pH. These results again suggest that antibiotic resistance genes are in bacterial plasmids which can be horizontally transferred between environmental bacteria. Therefore, the strains with the least resistance to antibiotics should be considered for potential applications to minimize environmental risks. P. pseudoalcaligenes YKJ is resistant to chloramphenicol and slightlyto ampicillin(whichcanberemoved through a geneticengineeringapproach) but nottoother antibiotics. These characteristics may be suited for the bioremediation purpose.

Conclusion:-

The isolation and identification of a novel BPA-degrading *P. pseudoalcaligenes* strain YKJ. It was able to degrade BPA as the only source of carbon and energy in the basal salt medium containing a high concentration of NaCl more rapidly than the other reported bacteria. BPA biodegradation pathways by this strain were proposed based on the analysis of themetabolites. Our results showed that strain YKJ was applicable for treatment of salty petrochemical

wastewater containing highconcentrationsofphenoland BPA. A strainofPseudomonas sp. P1, which is capable of efficiently breaking downBPA, wasacquired for this investigation. Whenthe temperature was30 °C, the pH was7, the BPAconcentration was 30 mg/L, and 3 mL ofinoculationwasused, the maximum breakdownrate was96.89%. There are 5636 protein-encoding genes in strain P1's genome. All of the critical genes for BPA biodegradation in strainP1were screenedusing comparativegenomicanalysis, including138 functionalgenesthat maybeengaged in BPA degradation and 72 functional genes involved in the mechanism of BPA stress. Under BPA induction, seven genes were expressed, including laccase, ferredoxin, cytochrome P450, and ferredoxin reductase complex. Strong environmental adaptability is exhibited by strain P1, which can withstand temperatures between 25 and 40 °C, pH values between 5 and 8, and BPA concentrations between 15 and 100 mg/L. During the BPA degradation process, six intermediates were identified, including 4-vinylphenol, which was discovered for the first time. The biodegradation of various BPA-contaminated environments due to its abundance of functional genes and strong environmentaladaptability.

Conflict Of Interest:

Theauthorsdeclarenoconflictofinterest with anyone.

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Reference:-

- 1. Atacag EH. Biodegradation and detoxification of BPA: involving laccase and a mediator. CLEAN Soil Air Water. 2015; 43:932–9. doi:10.1002/clen.201400628
- Wang Q, Chen M, Shan G, Chen P, Cui S, Yi S, et al. Bioaccumulation and biomagnification of emerging bisphenol analogues in aquatic organisms from Taihu Lake, China. Sci Total Environ. 2017; 598:814–20. 10.1016/j.scitotenv.2017.04.167
- 3. Alexander HC, Dill DC, Smith LW, Guiney PD, Dorn P. Bisphenol A: acute aquatic toxicity. Environ Toxicol Chem. 1988; 7:19–26. https://doi.org/10.1002/etc.5620070104
- 4. Seachrist DD, Bonk KW, Ho SM, Prins GS, Soto AM, Keri RA. A review of the carcinogenic potential of bisphenol a. ReprodToxicol. 2016; 59:167–82. https://doi.org/10.1016/j.reprotox.2015.09.006.
- Welshons WV, Thayer KA, Judy BM, Taylor JA, Curran EM, Vom Saal FS. Large effects from small exposures. I. Mechanisms for endocrine-disrupting chemicals withestrogenicactivity. EnvironHealthPerspect. 2003; 111:994– 1006. https://doi.org/10.1289/ehp.5494
- 6. Vom Saal FS, Hughes C. An extensive new literature concerning low-dose effects of bisphenol a shows theneed for a new risk assessment. Environ Health Perspect. 2005; 113:926–33. https://doi.org/10.1289/ehp.7713
- Vandenberg LN, Colborn T, Hayes TB, Heindel JJ, Jacobs DR Jr, Lee D-H, et al. Hormones and endocrinedisrupting chemicals: low-dose effects and nonmonotonic dose responses. Endocr Rev. 2012; 33:378–455. 10.1210/er.2011-1050
- 8. Kang JH, Kondo F. Bisphenol a in the surface water and freshwater snail collected fromrivers around a secure landfill. Bull Environ ContamToxicol. 2006; 76:113–8. https://doi.org/10.1007/s00128-005-0896-4
- Ye X, Pierik FH, Angerer J, Meltzer HM, Jaddoe VWV, Tiemeier H, et al. Levels of metabolites of organophosphate pesticides, phthalates, and bisphenol a in pooled urine specimens from pregnant women participating in the Norwegian mother and child cohort study (MoBa). Int J Hyg Environ Health. 2009; 212:481–91. https://doi.org/10.1016/j.ijheh.2009.03.004
- 10. Larsson K, Lindh CH, Jönsson BAG, Giovanoulis G, Bibi M, Bottai M, et al. Phthalates, non-phthalate plasticizers and bisphenols in Swedish preschool dust in relation to children's exposure. Environ Int. 2017; 102:114–24. https://doi.org/10.1016/j.envint.2017.02.006
- Zhou NA, Kjeldal H, Gough HL, Nielsen JL. Identification of putative genes involved in bisphenol a degradation using differential protein abundance analysis of Sphingobium sp. BiD32. Environ Sci Technol. 2015; 49:12232–41. https://doi.org/10.1021/acs.est.5b02987
- 12. Chouhan S, Yadav SK, Prakash J, Singh SP. Effect of Bisphenol a on human health and its degradation by microorganisms: a review. Ann Microbiol. 2014; 64:13–21.

- 13. Yamada H, Furuta I, Kato EH, Kataoka S, Usuki Y, Kobashi G, et al. Maternal serum and amniotic fluid bisphenol a concentration in the early second trimester. ReprodToxicol. 2002; 16:735–9. 10.1007/s13213-013-0649-2
- Zhang Y, Tao S, YuanC, Liu Y, Wang Z. Non-monotonic dose-response effect ofbisphenola onrare minnow Gobiocypris rarus ovarian development. Chemosphere. 2016; 144:304–11. https://doi.org/10.1016/j.chemosphere.2015.08.079
- Chen J, Xiao Y, Gai Z, Li R, Zhu Z, Bai C, et al. Reproductive toxicity of low-level bisphenola exposures in a twogeneration zebrafish assay: evidence of male-specific effects. AquatToxicol. 2015; 169:204–14. https://doi.org/10.1016/j.aquatox.2015.10.020
- 16. Yamamoto T, Yasuhara A, Shiraishi H, Nakasugi O. Bisphenol a in hazardous waste landfill leachates. Chemosphere. 2001; 42:415-8. https://doi.org/10.1016/s0045-6535(00)00079-5
- 17. Lee H-B, Peart TE. Bisphenol a contamination in Canadian municipal and industrial wastewater and sludge samples. Water Qual Res J. 2000; 35:283–98. http://dx.doi.org/10.2166/wqrj.2000.018
- Kleywegt S, Pileggi V, Yang P, Hao C, Zhao X, Rocks C, et al. Pharmaceuticals, hormones and bisphenola in untreated source and finished drinking water in Ontario, Canada—occurrence and treatment efficiency. SciTotal Environ. 2011; 409:1481–8. https://doi.org/10.1016/j.scitotenv.2011.01.010
- Bolz U, Hagenmaier H, Körner W. Phenolic xenoestrogens in surface water, sediments, and sewage sludgefrom Baden-Württemberg, south-West Germany. Environ Pollut. 2001; 115:291–301. https://doi.org/10.1016/s0269-7491(01)00100-2
- Kolpin DW, Furlong ET, Meyer MT, Thurman EM, Zaugg SD, Barber LB, et al. Pharmaceuticals, hormones, and other organic wastewater contaminants in US streams, 1999–2000: A national reconnaissance. Environ Sci Technol. 2002; 36:1202–11. https://doi.org/10.1021/es011055j
- 21. Staples CA, Dome PB, Klecka GM, Oblock ST, Harris LR. A review of the environmental fate, effects, and exposures of bisphenol a. Chemosphere. 1998; 36:2149–73. https://doi.org/10.1016/s0045-6535(97)10133-3
- Yamauchi K, Ishihara A, Fukazawa H, Terao Y. Competitive interactions of chlorinated phenol compoundswith 3, 3', 5-triiodothyronine binding to transthyretin: detection of possible thyroid-disrupting chemicals in environmental waste water. Toxicol Appl Pharmacol. 2003; 187:110–7. https://doi.org/10.1016/s0041-008x(02)00045-5
- Wang R, Diao P, Chen Q, Wu H, Xu N, Duan S. Identification of novel pathways for biodegradation of bisphenola bythe greenalga Desmodesmus sp. WR1, combined with mechanistic analysis at the transcriptome level. Chem Eng J. 2017; 321:424–31. https://doi.org/10.1016/j.cej.2017.03.121.
- 24. Kang J-H, Katayama Y, Kondo F. Biodegradation or metabolism of bisphenol a: from microorganisms to mammals. Toxicology. 2006; 217:81–90. https://doi.org/10.1016/j.tox.2005.10.001
- 25. Zhang C, Zeng G, Yuan L, Yu J, Li J, Huang G, et al. Aerobic degradation of bisphenol a by Achromobacterxylosoxidans strain B-16 isolated from compost leachate of municipal solid waste. Chemosphere. 2007; 68:181–90. https://doi.org/10.1016/j.chemosphere.2006.12.012
- 26. Kang JH, Kondo F. Bisphenol a degradation by bacteria isolated from river water. Arch Environ ContamToxicol. 2002; 43:265–9. https://doi.org/10.1007/s00244-002-1209-0
- Suyamud B, Inthorn D, Panyapinyopol B. Biodegradation of Bisphenol a by a newly isolated Bacillus megateriumstrainISO-2fromapolycarbonateindustrialwastewater;2018. https://link.springer.com/article/10.1007/s11270-018-3983-y
- 28. Oshiman KI, Tsutsumi Y, Nishida T, Matsumura Y. Isolation and characterization of a novel bacterium, Sphingomonasbisphenolicum strain AO1, that degrades bisphenol a. Biodegradation. 2007; 18:247–55. https://doi.org/10.1007/s10532-006-9059-5
- 29. Xu C-P, Kim S-W, Hwang H-J, Choi J-W, Yun J-W. Optimization of submerged culture conditions formycelial growth and exo-biopolymer production by Paecilomyces tenuipes C240. Process Biochem. 2003; 38:1025–30. https://doi.org/10.1007/s11274-004-5841-x
- Khambhaty Y, Mody K, Jha B, Gohel V. Statistical optimization of medium components for κ-carrageenase production by Pseudomonas elongata. EnzymMicrob Technol. 2007; 40:813–22. https://doi.org/10.1007/BF02931084
- Kumar P, Satyanarayana T. Optimization of culture variables for improving glucoamylase production by alginateentrapped Thermomucorindicae-seudaticae using statistical methods. Bioresour Technol. 2007; 98:1252–9. https://doi.org/10.1016/j.biortech.2006.05.019
- 32. Eio EJ, Kawai M, Tsuchiya K, Yamamoto S, Toda T. Biodegradation of bisphenola by bacterial consortia. Int BiodeteriorBiodegrad. 2014; 96:166–73. https://doi.org/10.1016/j.ibiod.2014.09.011.
 - 33. Ren L, Jia Y, Ruth N, Shi Y, Wang J, Qiao C, et al. Biotransformations of bisphenols mediated by a novel Arthrobacter sp. strain YC-RL1. Appl MicrobiolBiotechnol. 2016; 100:1967–76. 10.1007/s00253-015-7076-1
 - 34. Karthikeyan RS, Rakshit SK, Baradarajan A. Optimization of batch fermentation conditions for dextran production. Bioprocess Eng. 1996; 15:247–51. 10.1007/BF02391585
 - 35. Engqvist MKM. Correlating enzyme annotations with a large set of microbial growth temperatures reveals

metabolic adaptations to growth at diverse temperatures. BMC Microbiol. 2018; 18:1-14. https://doi.org/10.1186/s12866-018-1320-7

- Ulrich AC, Guigard SE, Foght JM, Semple KM, Pooley K, Armstrong JE, et al. Effect of salt on aerobic biodegradation of petroleum hydrocarbons in contaminated groundwater. Biodegradation. 2009; 20:27–38. https://doi.org/10.1007/s10532-008-9196-0
- De Carvalho CC, De Fonseca MM. Degradation of hydrocarbons and alcohols at different temperatures and salinities by Rhodococcuserythropolis DCL14. FEMS Microbiol Ecol. 2005; 51:389–99. https://doi.org/10.1016/j.femsec.2004.09.010
- 38. Lee SG, Yoon BD, Park YH, Oh HM. Isolation of a novel pentachlorophenol-degrading bacterium, Pseudomonas sp. Bu34. J Appl Microbiol. 1998; 85:1–8. https://doi.org/10.1046/j.1365-2672.1998.00456.x
- 39. Kumar A, Kumar S, Kumar S. Biodegradation kinetics of phenol and catechol using Pseudomonas putida MTCC 1194. Biochem Eng J. 2005; 22:151–9. https://doi.org/10.1016/j.bej.2004.09.006
- Peng X, Qu X, Luo W, Jia X. Co-metabolic degradation of tetrabromobisphenol a by novel strains of Pseudomonas sp. and Streptococcus sp. Bioresour Technol. 2014; 169:271–6. https://doi.org/10.1016/j.biortech.2014.07.002
- 41. Yamada T, Takahama Y, Yamada Y. Biodegradation of 2, 4, 6-tribromophenol by Ochrobactrum sp. strain TB01. BiosciBiotechnolBiochem. 2008; 72:1264–71. https://doi.org/10.1271/bbb.70755
- Yu K, Yi S, Li B, Guo F, Peng X, Wang Z, et al. An integrated meta-omics approach reveals substrates involved insynergistic interactionsina bisphenola(BPA)-degrading microbialcommunity. Microbiome.2019; 7:1–13. https://doi.org/10.1186/s40168-019-0634-5
- 43. Das R, Li G, Mai B, An T. Spore cells from BPA degrading bacteria Bacillus sp. GZB displaying high laccase activityandstabilityforBPAdegradation.SciTotalEnviron.2018;640–641:798–806. https://doi.org/10.1016/j.scitotenv.2018.05.379.
- 44. Wang J, Ren L, Jia Y, Ruth N, Shi Y, Qiao C, et al. Degradation characteristics and metabolic pathway of 4nitrophenol by a halotolerant bacterium Arthrobacter sp. CN2. Toxicol Environ Chem. 2016; 98:226–40. https://doi.org/10.18006/2022.10(4).743.766
- Nahurira R, Ren L, Song J, Jia Y, Wang J, Fan S, et al. Degradation of Di(2-Ethylhexyl) phthalate by a novel Gordonia alkanivorans strain YC-RL2. Curr Microbiol. 2017; 74:309–19. https://doi.org/10.1007/s00284-016-1159-9
- RenL, Jia Y, RuthN, Qiao C, WangJ, Zhao B, et al. Biodegradationofphthalic acidestersbya newlyisolated Mycobacterium sp. YC-RL4 and the bioprocess with environmental samples. Environ Sci Pollut Res. 2016; 23:16609–19. https://doi.org/10.1007/s11356-016-6829-4.
- 47. Tamura K, Peterson D, Peterson N, Stecher G, Nei M, Kumar S. MEGA5: molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. Mol Biol Evol. 2011; 28:2731–9. https://doi.org/10.1093/molbev/msr121
- 48. Sarkar S, Zafar Z, Sourav P, Das B, Biswas D, Panda R J. Strategies for Removing Endocrine Disrupting Chemicals (EDCs) from Wastewater. Curr. Pharm. Biotechnol. 2024; 10.2174/0113892010317251240826051110
- Aslam, Z., Alam, P., Islam, R., Khan, A. H., Samaraweera, H., Hussain, A., Zargar, T. I. Recent developments in moving bed biofilm reactor (MBBR) for the treatment of phenolic wastewater-A review. Jn. Tai. Engg.,2024; https://doi.org/10.1016/j.jtice.2024.105517
- Macedo, S., Teixeira, E., Gaspar, T. B., Boaventura, P., Soares, M. A., Miranda-Alves, L., Soares, P.Endocrinedisrupting chemicals and endocrine neoplasia: A forty-year systematic review. Env. Res., 218, 2023. https://doi.org/10.1016/j.envres.2022.114869.