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Bioprospecting Biodegradative Enzymes and Related Genes in Bacteria Isolated from Paint-Contaminated Environment

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Biodegradation, Enzymes, Paints, Phosphatase (*pho*D), Dehydrogenase (*adh*), β-glucosidase (*bgl*)

ABSTRACT

Bacteria offer substantial biodegradative potential to cope with environmental pollutants because of having strong genetic machinery (genes) and enzymes that finally get the job done. This study relates with the fermentative and molecular screening of the three most important biodegradative enzymes and their target genes, i.e. phosphatase (*pho*D), dehydrogenase (*adh*) and β-glucosidase (*bgl*) for bioremediation of paints. Enzymatic screening of 22 bacterial isolates revealed strong activity for dehydrogenase in 5(22%) isolates, phosphatase activity in 20(87%) isolates, while only 2(9%) isolates showed strong β-glucosidase activity. The molecular screening for enzyme-specific genes revealed that 16 isolates harbor *pho*D gene, 20 isolates carry *bgl* gene while *adh* gene was amplified in 13 isolates. Conclusively, based on enzymatic activities and enzyme-specific genes screening results 13 isolates (5 G+ve and 8 G –ve) possess strong potential for environmental applications like bioremediation.

INTRODUCTION

Environmental pollution has been recognized as one of the major modern world problems that directly or indirectly affect biodiversity. Paint effluents have great concern for environmental pollution worldwide, due to their prevalent applications that cause expulsion and accidental leakages into the environment (Phulpoto *et al*., 2016). Paint contains hazardous pollutants like toluene, benzene, volatile organic compounds (VOCs), metals, phenol, gases, polyaromatic hydrocarbon (PAH), tributyline. VOCs may cause short as well as long-term environmental impacts and may lead to respiratory, allergic and immunogenic faults in human beings. Paint contains a high level of lead or mercury and their ingestion may lead to serious health related problems like nerve and kidney damage. Paint also contains tributyline which is highly toxic to biodiversity (Ishfaq *et al*., 2015). Paint exposure to different chemical and physical phenomena, such as humidity and high temperature, facilitates microbial attack (Phulpoto *et al*., 2016). The paint biodegradation serves as a principle model for understanding the mechanism of bacterial benzene ring metabolism (Joutey *et al*., 2013). Different bacterial genera such as *Arthrobacter, Gracilibacillus, Bacillus, Paenibacillus, Virgibacillus, Salibacillus, Pseudomonas, Nocardia, Aeromicrobium, Micrococcus, Sphingomonas, Thiobacillus, Alcaligenes, Shewanella, Gallionella, Escherichia,* and *Flavobacterium* are reported for degradation of xenobiotic pollutants (La Rosa *et al*., 2008; Obidi *et al*., 2009; Ravikumar *et al*., 2012; Ishfaq *et al*., 2015; Phulpoto *et al*., 2016; Ghosh *et al*., 2022).

Hazardous environmental pollutants are safely removed by biological and chemical methods. One of the cheapest and safest solutions is bioremediation. Bioremediation is processing to return the surrounding contaminants to its original condition by using microbes or their enzymes (Maitlo *et al*., 2024). The use of microbial enzymes for the removal of environmental and industrial

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pollutants has attracted increasing attention worldwide. The enzymes such as phosphatases, esterase, lipase, peptidases, glycosidase, peroxidases, tyrosinase, reductase, catalase, superoxide dismutase and laccases have been evaluated for the removal of toxic compounds from industrial wastewater and the degradation of recalcitrant xenobiotics (Budolla *et al*., 2014).

Three major pollutant-degrading enzymes: dehydrogenase, phosphatase and β-glucosidase are used as biological markers to monitor biodegradation. β-glucosidases are linked to the carbon cycle and hydrolyze β-glucosyl residues, thereby releasing β-D-glucose in the final reaction step (Maitlo *et al*., 2024). Phosphatases are enzymes involved in the phosphorous cycle and catalyze the hydrolysis of oxygen phosphate (O-P) bonds, thereby yielding free phosphate, whereas the dehydrogenase enzyme is involved in the total metabolic activity of live microorganisms (Margesin *et al*., 2003; Rosado *et al*., 2013). These three enzymes are collectively applied and monitored in the degradation of pollutants like paint, wastewater, pesticides and hydrocarbon contaminated soils (Antunes *et al*., 2011; Balestri *et al*., 2013; Rosado *et al*., 2013).

Biological parameters like respiration or structure of degrading communities do not reflect the biodegradation phenomenon. Recently, functional gene detection for pollutant degradation is being considered as a direct, reliable and straightforward method to evaluate the degradation of pollutants (Hendrickx *et al*., 2006; Nõlvak *et al*., 2012). Specific catabolic genes are located on plasmids of microbes, which enable the microbes to tolerate the xenobiotic pollutants like paint pollutants and utilize these pollutants as an energy source. The catabolic genes such as *alk*B (*alk*B1 and *alk*B2), *alk*M, *xyl*E, *ndo*B, *nid*A, *Cdo*, *tbm*D, *tmo*A, *xyl*M, *xyl*A, *nah*A, *hdq*A, *hdq*B, *hap*C, *hap*D, *hap*E and *sfp* gene are detected in the degradation of such pollutants (Kim *et al*., 2000; Malkawi *et al*., 2009; Hesham *et al*., 2014). Pollutant-degrading genes *adh, bgl* and *pho*D expressing dehydrogenase, β-glucosidases and phosphatase, have been reported in bacteria and are involved in the degradation pathway of many recalcitrant xenobiotic pollutants (Antunes *et al*., 2011; Balestri *et al*., 2013; Rosado *et al*., 2013).

So far, to the best of our knowledge, these enzymes were evaluated either on application basis (bioremediation studies) or molecular basis (enzyme characterization studies). No one has monitored and evaluated such enzymes at both application and molecular levels, simultaneously. Additionally, there isn't any gene expression study so far that reported the catabolic potential of all the three enzymes, i.e. dehydrogenase, phosphatase and β-glucosidase in a single bioremediation system, although intensive work has been done on the application of individual enzymes (Wu *et al*., 2018; Tozakidis *et al*., 2016; Ragot *et al*., 2015; Chaperon and Sauvé, 2007; Margesin *et al*., 2003). Moreover, none of those studies have yet reported their comparative appraisal of the interplay between genes and applications.

Contrarily, this study includes expression of three biodegradative enzymes, for molecular characterization and application, in a single bioremediation system containing complex recalcitrant pollutants like paints. Our study mainly focused on the role of such enzymes in the remediation of paint. Furthermore, for the first time, comparative performance analysis was done for bacterial isolates containing single genes or single enzyme vs. multiple-genes or multiple-enzymes in a single bioremediation process. Thus, the present study was designed to explore the enzyme analysis and functional gene localization in the indigenous paint-degrading bacterial isolates collected from the Postgraduate Research Laboratory, Institute of Microbiology, Shah Abdul University, Khairpur.

MATERIALS AND METHODS

The present study was subdivided into two parts, i.e. (1) Enzyme screening and (2) detection of the gene encoding biodegradative enzymes. Enzyme screening was carried out at the Postgraduate Research Laboratory (PGRL), Institute of Microbiology, Shah Abdul Latif University, Khairpur, and gene detection was carried out at Molecular Lab, Pir Syed Abdul Qadir Shah Jillani, Institute of Medical Sciences, Gambat, Pakistan.

Enzyme screening

Microorganisms and culture conditions

Twenty-two paint-degrading indigenous bacterial isolates (Gram-positive and negative) were collected from the culture repository of PGRL. All the paint-degrading bacterial (PDB) isolates were screened for production of three different biodegradative enzymes viz. phosphatase, dehydrogenase and β-glucosidase and molecular detection of their respective genes. The bacterial isolates were cultivated as individual pure isolates as well as in the form of consortia in minerals salts medium (MSM) containing oil-based paint as the sole carbon source to screen their enzymatic activities according to Phulpoto *et al*. (2016).

Twenty-two PDB were identified on the basis of 16S RNA (*Pseudomonas aeruginosa, Bacillus subtilis*) and biochemical characteristics (*Pseudomonas aeruginosa, Pseudomonas putida, Pseudomonas fluorescens, Bacillus subtilis, Bacillus licheniformis, Bacillus* spp.).

Sample processing for enzyme activity

Freshly grown bacterial cells (1.5×10^8 CFU/ml), were

inoculated into the 250 ml conical flasks containing 100 ml media (MSM+Paint) and incubated at 37 ºC at 160 rpm for 5 days (Phulpoto *et al*., 2016). On the 5th day of incubation, 3 ml sample was transferred from each flask to sterilized tubes and these tubes were kept at 4°C at PGRL. Enzymes analysis was carried out from these tubes according to the method of enzyme analysis from previous reports by using an ELISA plate reader.

Detection of dehydrogenase

The activity of the dehydrogenase enzyme was determined according to Rosado *et al*. (2013). CFS samples (0.1ml) were incubated at 40 ºC for 60 min, in the dark along with 1M of Tris-HCl buffer pH 7.5 and 0.2 ml of 0.5% 2-(p-iodophenyl)-3-(p-nitrophenyl)-5 phenyl tetrazolium chloride (INT). Then 0.1 ml of ethanol/ DMF (1:1), was added and immediately centrifuged at 10,000 rpm for 15mins. Then about 200 µl from each sample was transferred to the microplate. The amount of iodonitrotetrazolium formazan (INTF) released was determined in the sample at 490 nm by using ELISA plate reader.

Detection of phosphatase

The activity of the phosphatase enzyme was determined according to Rosado *et al*. (2013). Briefly, the samples (CSF) of treated paints (0.1 ml) were incubated for 60 min, with universal buffer (UB), pH 5.0 ± 0.2 and 0.2 ml of 115mM *p*-nitrophenyl phosphate (p-PNP). Then 0.1 ml of 0.5M NaOH was added and immediately centrifuged at 10,000 rpm for 15 min. Then about 200 µl from each sample was transferred to the microplate. The *p*-nitrophenol amount generated from p-PNP was determined in the sample at 405 nm by using ELISA plate reader.

Detection of β -glucosidase

The activity of the β-glucosidase enzyme was determined according to Rosado *et al*. (2013) technique. The samples (CSF) of treated paint (0.1 ml) were incubated for 60 min, with universal buffer (UB), pH 6.0±0.2 and 0.2ml of 2mM *p*-nitrophenyl β–D-glucoside. Then 0.1 ml of 0.5M NaOH was added and immediately centrifuged at 10.000 rpm for 15 min. Then about 200 µl from each sample was transferred to the microplate. The *p*-nitrophenol amount generated from *p*-nitrophenyl β–D-glucoside substrate was determined in the sample at 405 nm by using ELISA plate reader.

Amplification of biodegradative gene using PCR

The bacterial isolates were grown in nutrient broth for 48 h, OD was observed at 600 nm using UV-Vis Spectrophotometer. 1 ml of broth containing culture (OD=1 approximately, at 600 nm) from each tube was transferred to 1.5 ml Eppendorf tube. All the Eppendorf tubes containing culture were centrifuged at 8000 rpm for two minutes and the supernatant was discarded. Then the DNA extraction process was carried by the standard protocol of Molecular DNA Extraction Kit (Biobasic, USA). Later, the extracted DNA was quantified using the standard protocol of the QUBIT 3.0 fluorometer. After quantification DNA was stored at -20 °C, until further processing.

DNA from all 22 PDB isolates was processed with specifically designed primers. Firstly, gene sequences were retrieved from GenBank@NCBI. Retrieved sequences were subjected to PickPrimer tool @NCBI, and primer specificity was analyzed by Primer blast tool (NCBI). The primers used in this study are listed in the Table I. Total 50 ul PCR mixture tube was made for all samples along with control by adding 25 µl of Master Mix, 2 µl from Forward Primer, 2 µl from Reverse Primer, 5 µl from DNA sample, and 16 µl of PCR water. The control contained all the above components except the DNA sample. The reaction mixture was then kept in a thermocycler machine for amplification (PCR ADVANCE GeneMate Series, TBG Biotechnology, China). Total 30 cycles were run by, the initial denaturation at 96±2 °C for 45 sec, Annealing temperature 59±2°C for 45 sec., Extension at 72±2 °C for 1 min. Amplified products were subjected to gel electrophoresis. Consequently, 2% Agarose gel was made by mixing with TBE buffer 0.5X. About 10 µl sample from each PCR tube was put into the whole in gel along with negative control and IKB DNA ladder. The gel was run at 220 v for 30 min using horizontal gel-electrophoresis. Then the gel was examined under UV Transilluminator.

Table I. List of primers used for amplification of target genes.

Gene	Primer sequences 5′→ 3′	Reference
*pho*D	Fw: TGCTGCGCCTAACTTCTCAA Rv: GCCGGTACAGCTGCATATCT	This study
	Fw: GGTCACTCAGATCCGTGCAA Rv: TGATCTGGACACGTCTTGCC	
Bgl	Fw: ACGCTCTATCACTGGGACCT Rv: TCCGAATTCATGTGCGGGAA	
*bgl*S	Fw: TCCTTGCCGTCCACGTAATC Rv: AGCACGACTACCTGCTCAAC	
*adh*B	Fw: CCAGCTGGAAAGCCAATGTG Rv: TACCATCCATCCCAACAGCG	
*adh*P	Fw: GGTATTGGCGTGGTTCAGGA Rv: CCCACCACTTCGATCCCATC	

Note. Fw, Forward; Rv, Reverse

RESULTS

Paint degrading bacterial (PDB) isolates

Twenty two paint-degrading bacteria were identified on the basis of 16S rRNA (*Pseudomonas aeruginosa, Bacillus subtilis*) and biochemical characteristics (*Pseudomonas aeruginosa, Pseudomonas putida Pseudomonas fluorescens, Bacillus subtilis, Bacillus licheniformis, Bacillus* sp.).

These PDB isolates were screened for biodegradative enzymes *viz*. phosphatase, dehydrogenase and β-glucosidase. According to absorbance, 10 isolates (06 Gram-positive and 04 Gram-negative) *viz*. PDB1, PDB2, PDB4, PDB9, PDB10, PDB11, PDB15, PDB16, PDB17 and PDB22 showed maximum growth as shown in Figure 1.

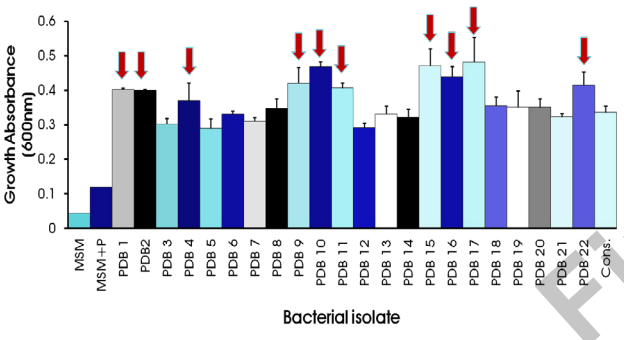


Fig. 1. Spectrophotometric absorbance (600 nm) of the samples used for Enzyme Analysis showing bacterial growth status on 5th day of incubation.
PDB1, PDB3, PDB5, PDB7, PDB8, PDB12= *Pseudomonas aeruginosa*; PDB2, PDB4, PDB6 and PDB14, PDB16-21= *Bacillus subtilis*; PDB9, PDB13= *Pseudomonas putida*; PDB10, PDB11= *Pseudomonas fluorescens*; PDB15= *Bacillus licheniformis*; PDB22= *Bacillus* spp.

PDB expressing dehydrogenase, phosphatase and β-glucosidase activities

In case of dehydrogenase 5(22%) isolates were considered as having a strong activity (absorbance ≥0.5), 9(43%) isolates were considered as having a moderate activity (≥0.3 and < 0.5), 7(30%) isolates were considered as having a low activity (≥0.2 to 0.3) while 2 isolates along with PC and NC didn't show any activity (<0.2) (Fig. 2). By comparing bacterial growth and enzyme activity samples 7 isolates showed a direct correlation of growth and enzyme activity. However, PDB4 was strongly active in enzyme production but not in growth. 04 isolates showed significant growth but low enzyme activity.

In the case of phosphatase, 19(87%) isolates were considered as having a strong activity (absorbance ≥3.5), 3(13%) isolates were considered as having a moderate activity (≥2 and < 3.5), while none of the isolates showed low or no activity (Fig. 3). All the isolates except PDB10 showed a positive relationship. Only PDB 10 showed significant growth but low enzymatic activity.

In case of β-glucosidase only 2((9%) isolates were considered as having strong activity (absorbance ≥0.05), 8(35%) isolates were considered as having moderate activity (≥0.025 and < 0.05), while 12(56%) isolates were considered as having low activity (≥0.015 and < 0.025) (Fig. 4). None of isolate showed no activity. In comparison of enzyme activity and growth only two isolates PDB1 and PDB 14 showed positive relation. However most of isolates showed significant growth but low enzyme activity.

Molecular amplification of target genes

Pair of Primers for gene encoding enzyme dehydrogenase, *adh*P successfully amplified target genes in 13 isolates with the size of 665 bp, while pair of primer for *adh*B successfully amplified target gene in 09 isolates with the size of 485bp. Pair of Primers for target gene encoding phosphatase, *pho*D A successfully amplified gene of interest in 16 bacterial isolates with the size of 794 bp, While Pair of Primers for gene *pho*D B did not amplify any gene. Pair of Primers for gene encoding β-glucosidase**,** *bgl* successfully amplified target gene in 05 isolates, having size about 629 bp. While Primer *bgl*S successfully amplified the target gene in 20 isolates with size 604. However, there was no amplification (band) in NC of all primers used.

Comparative enzymatic and genetic profiling

According to comparative enzymatic profile, 13 isolates (PDB1, PDB2, PDB5, PDB6-8, PDB11, PDB14, PDB16, PDB18-20 and PDB22) were found significant strain during enzymatic screening. On the other hand, genetic profiling indicated different bacterial strains in which more than one target genes were amplified using designed primers. Comparative enzymatic (Fig 5A) and genetic (Fig 5B) profiling of the bacteria to decipher the most important bacterial isolates. By comparing genetic profile and enzymatic screening, 13 isolates (5 G+ve and 8 G –ve) (PDB2, PDB4, PDB5, PDB7-9, PDB12-16 and PDB20), were found to be potential isolates in xenobiotic bioremediation.

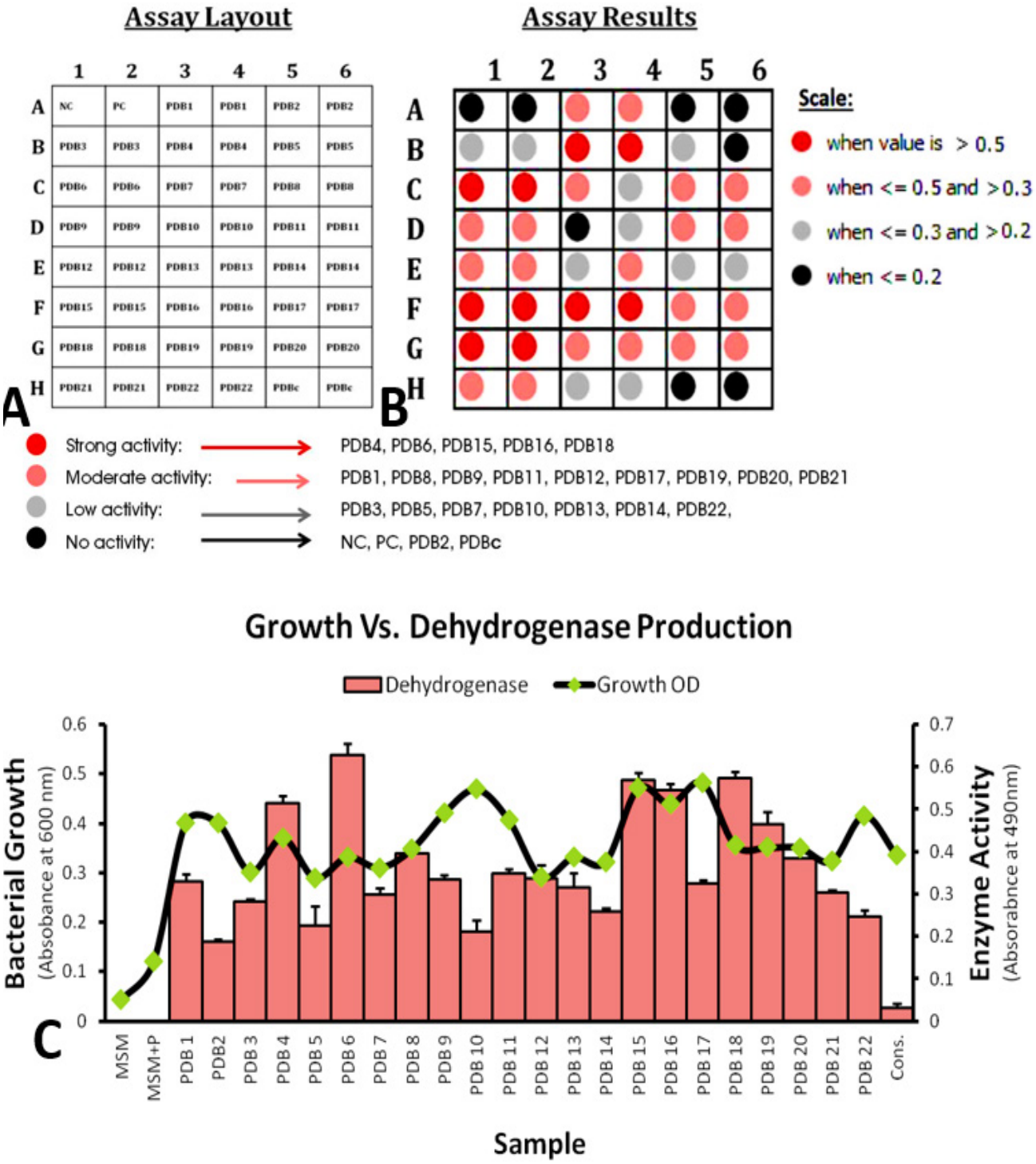
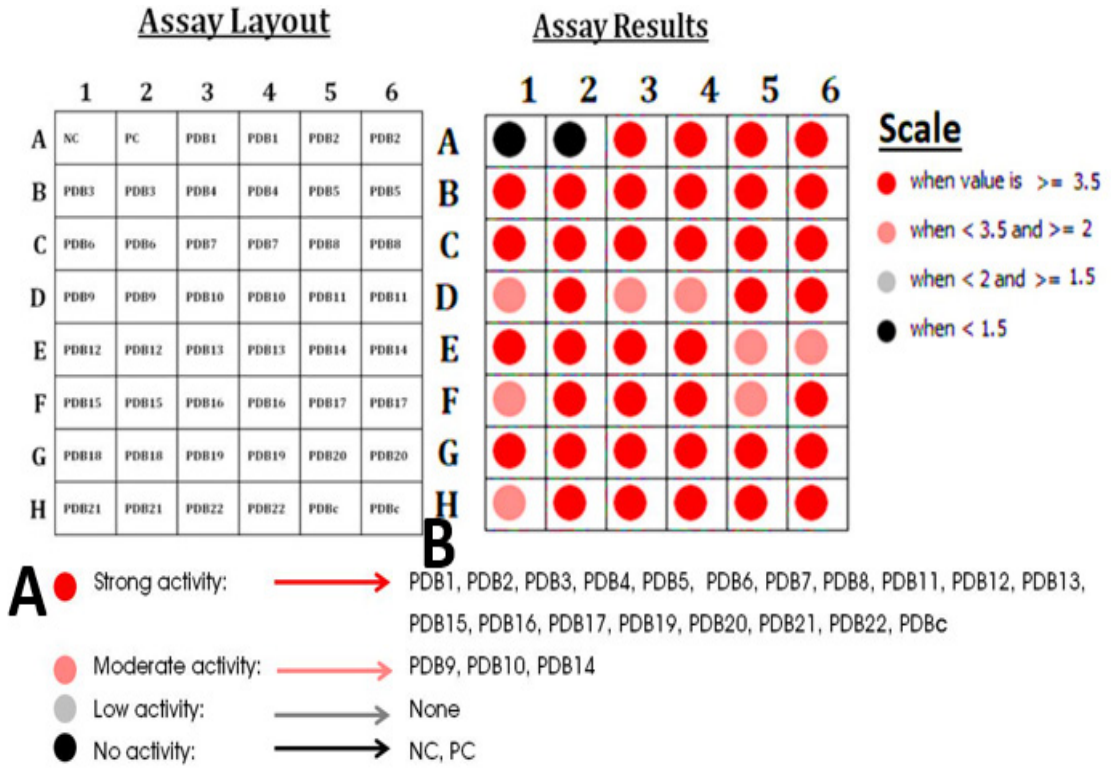


Fig. 2. The enzymatic activity of dehydrogenase was checked at 490 nm using ELISA plate reader. A shows assay layout. All the samples were run in duplicate along with positive control (PC) and negative control (NC). B shows results of enzyme activity. C shows comparative bacterial growth enzyme activity



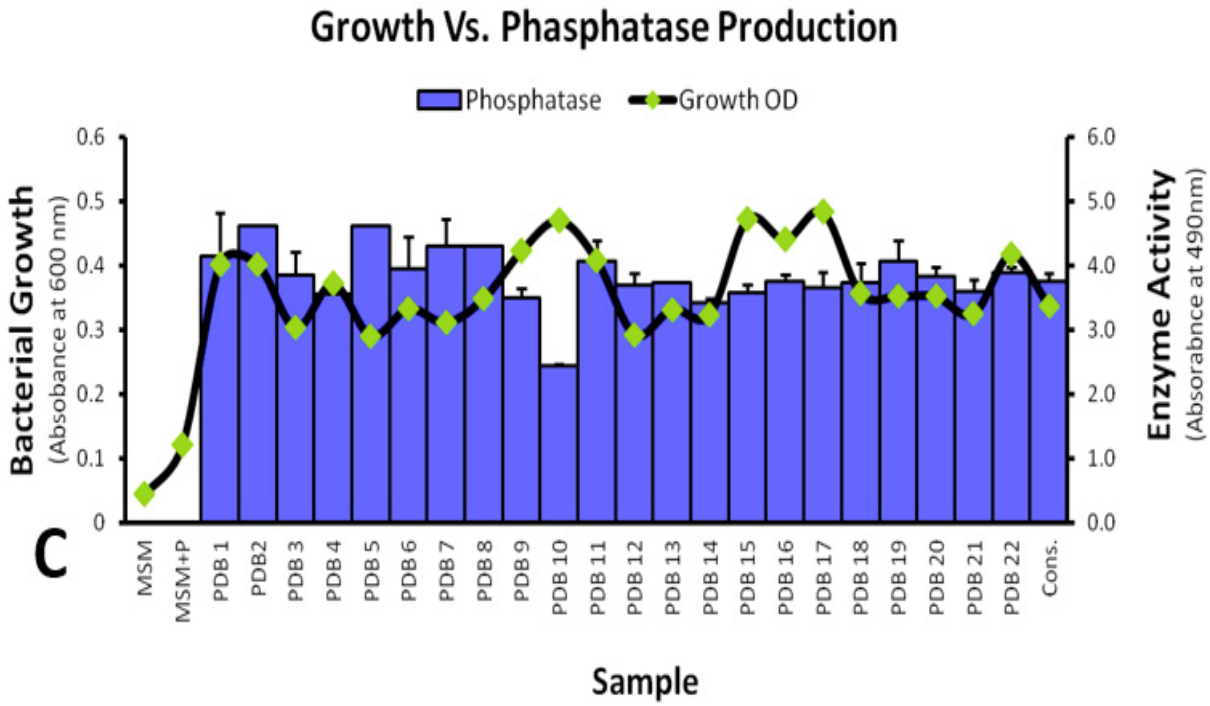


Fig. 3. The enzymatic activity of phosphatase was checked at 405 nm using ELISA plate reader. A is shows assay layout. All the samples were run in duplicate along with PC and NC. B shows results of enzyme activity. C show enzyme production vs. growth relationship.

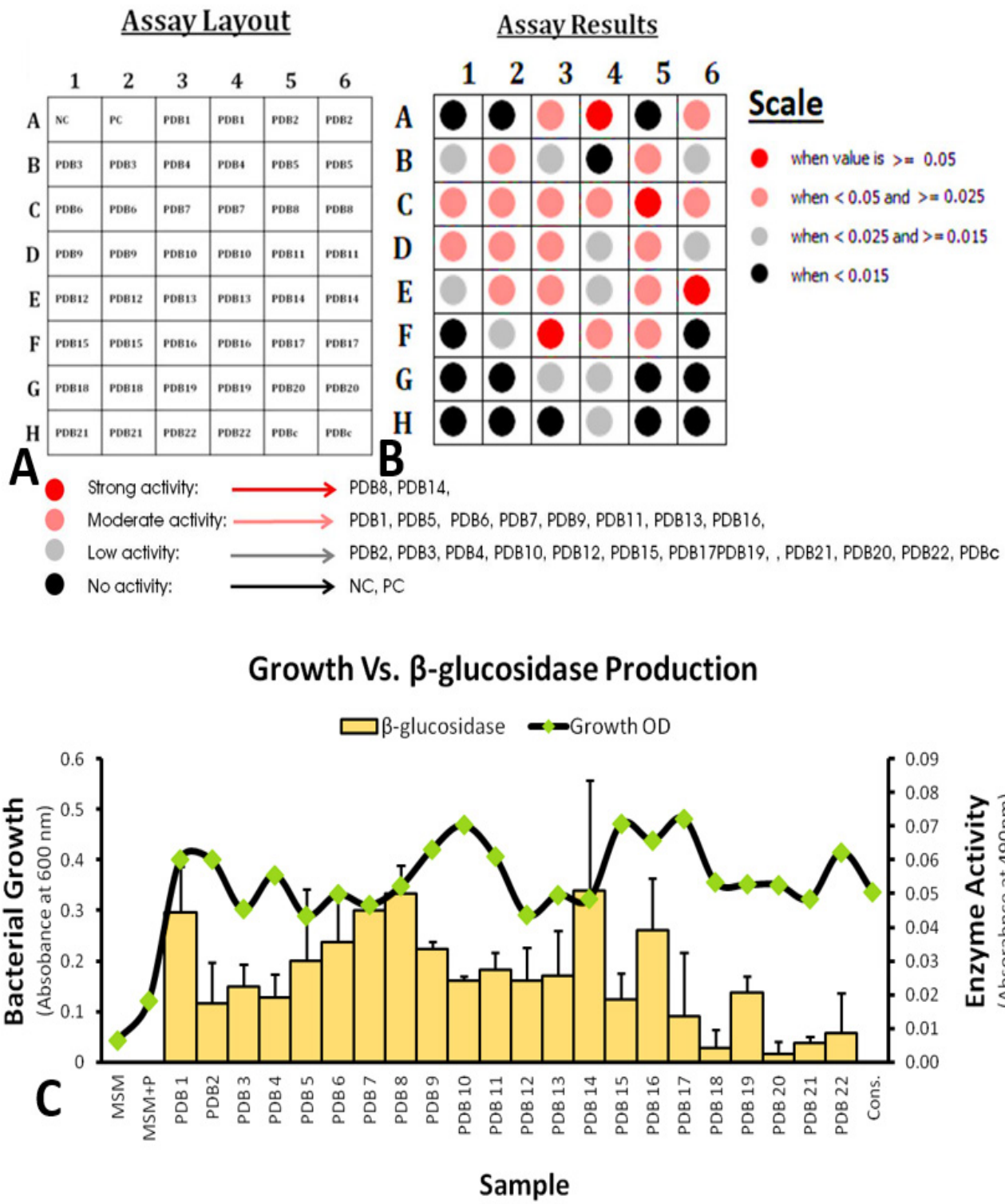


Fig. 4. Enzymatic activity of β-glucosidase was checked at 405 nm using ELISA plate reader, A is showing assay layout. All the samples were run in duplicate along with PC and NC while B is showing results of enzyme activity. C shows enzyme production vs. growth relationship.

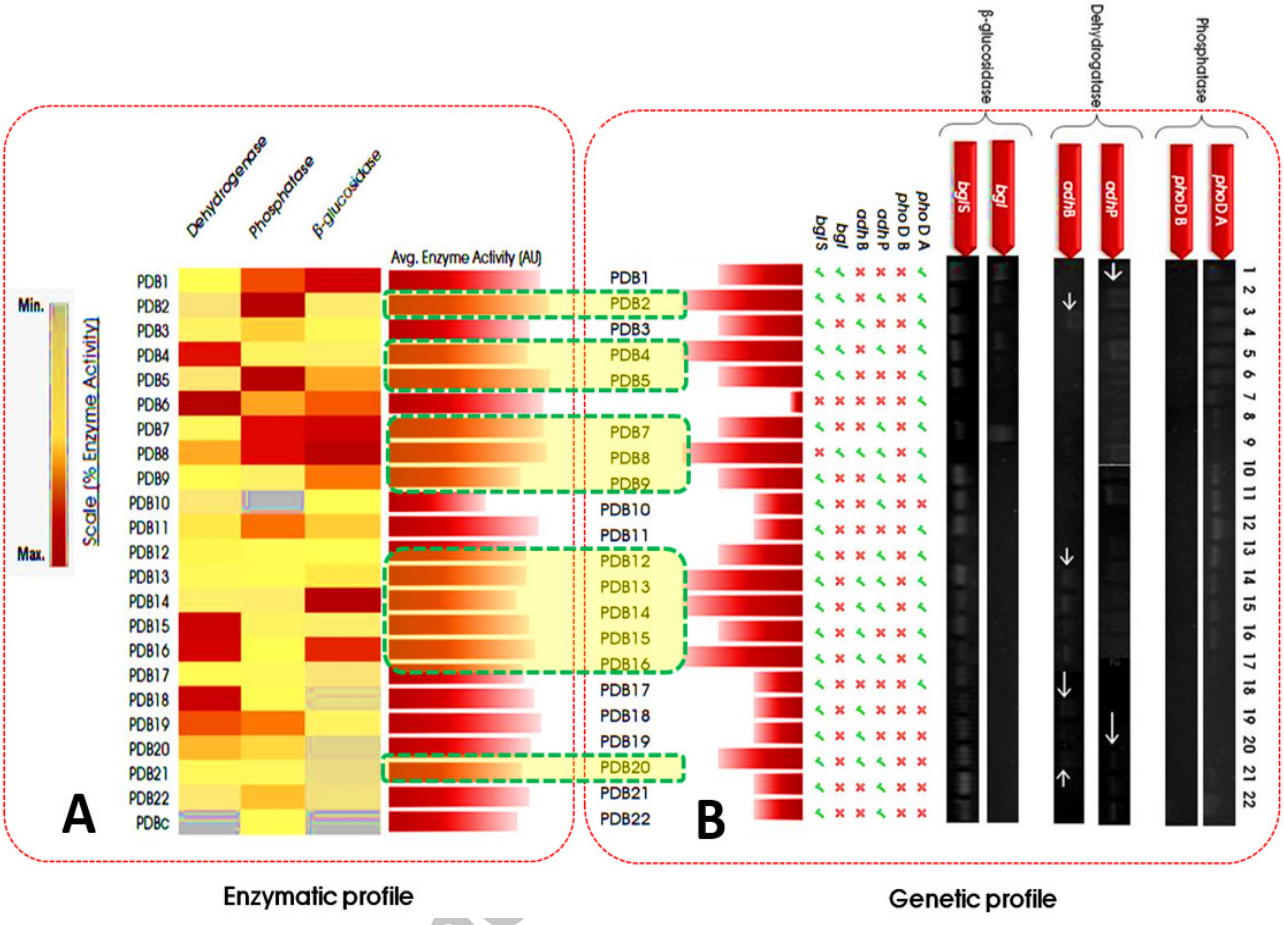


Fig. 5. Comparative enzymatic (A) and genetic (B) profiling of the bacteria to decipher the most important bacterial isolates.

DISCUSSION

The environment is mainly polluted with anthropogenic contaminants including paints or paint-related products, pesticides, dyes, excretion of untreated industrial effluents, smoke, oil spills, etc. Among these pollutants, paint is a complex pollutant containing a wide range of hazardous pollutants (Phulpoto *et al*., 2016; Okafor and Orji, 2022). Luckily these hazardous pollutants are being degraded naturally by bacteria, fungi, and algae, etc. Biological remediation, especially microbial remediation, is an efficient, cost-effective, less invasive and eco-friendly approach than physical and chemical methods of remediation (Ruggaber and Talley, 2006). The applications of microbial extracellular enzymes are proved efficient means of advanced bioremediation for a wide variety of pollutants including paints, polychlorinated biphenyls (PCBs), dyes, wastewater, phenols, PAHs, BTEX and VOCs, etc. Microbial enzymes such as manganese-peroxidases, lignin-peroxidases and laccases play a potential role in the bioremediation of such xenobiotic compounds. Likewise, paints appear to be easily degraded by cellulase, carboxylesterase and glucosidases (Watanabe, 2001; Ruggaber and Talley, 2006). Therefore, in this study, 22 pollutant-degrading isolates belong to broad genera of environmental isolates genus *Bacillus* and genus *Pseudomonas* were selected for the screening of three major pollutant-degrading enzymes: dehydrogenase, phosphatase and β –glucosidase.

Many microorganisms utilize low molecular-weight sugars as energy sources during metabolism (Donderski *et al*., 1998), which is mainly achieved using β-glucosidase enzyme (Li *et al*., 2025). Dehydrogenase, on the other hand, is a key requirement for aerobic bacterial respiration; thus, dehydrogenase activity is used to measure overall microbial activity (Rosado *et al*., 2013). Phosphatase synthesis increases when bacteria are subjected to phosphorous limiting conditions. Hence it is supposed

that bacterial extracellular phosphatases release inorganic phosphates from organic phosphate compounds in the environment (Hinjosa *et al*., 2008).

Previous studies have revealed that these three enzymes are being monitored, evaluated and applied in different bioremediations from various microbial groups like fungi, bacteria and yeasts (Antunes *et al*., 2011; Balestri *et al*., 2013; Rosado *et al*., 2013). These enzymes are considered good indicators of biological activity. Rosado *et al*. (2013) screened these enzymes from mural paintings from major fungal strains: *Cladosporium* sp., *Rhodotorula* sp., *Penicillium s*p., *Aspergillus* sp. Antunes *et al*. (2011) applied these three enzymes in soil pollutants remediation. However, Balestri *et al*. (2013) applied these enzymes in water and wastewaters quality/contamination studies, so the current study was intended to screen enzymatic potential against paint pollutants.

The results as described in Figures 2-4 reveal that the pollutant-degrading enzymes, β-glucosidase, phosphatase and dehydrogenase are found active in tested bacterial strains. The current study directly correlates with Rosado *et al*. (2013) who also found them active in mural paintings. The difference is only that Rosado *et al*. (2013) tested these enzymes from various fungal strains, however, in our study, these enzymes were screened from bacterial strains. Wei *et al*. (2018) have also reported dehydrogenase from *Pseudomonas* and *Sphingomonas* in the degradation of benzoic compounds. Scientists have described that it takes an active part in different pollutant degrading pathways: Polyvinyl alcohol degradation, alkane, SDS, dichlropropene, cellulose, aliphatic and aromatic hydrocarbons (Van *et al*., 2007; Dvořák *et al*., 2017; Ivshina *et al*., 2017; Wu *et al*., 2018; Wei *et al*., 2018; Panasia and Phailipp, 2018). Chaperon and Sauvé (200) reported dehydrogenase having a key role in organic matter degradation from bacteria, fungi and actinomycetes, whereas Taguchi *et al*. (2001) reported it in the degradation of polychlorinated biphenyls using bacterial strains.

Our findings pertaining to enzyme phosphatase also correlate with Bradford (1976) and Rosado *et al*. (2013). Bradford (1976) reported phosphatase from *Desulfovibrio desulfuricans* in the degradation of nitrocellulose paints while Rosado *et al*. (2013) reported from different fungal strains in the degradation of mural paintings.

It is noteworthy to mention here that phosphatase was found to be more active in bioremediation experiments as compared with β-glucosidase, and dehydrogenase (Figs. 2-4). 21 samples out of 23 samples showed strong activity of phosphatase. The activity of phosphatase and β-glucosidase is correlated to the transformation of pollutants that provide various components to microbes for their growth and development. The activity of the dehydrogenase enzyme reflects total oxidative activity hence showing the presence of living microbes. Dehydrogenase can be used as an indicator of the presence of metabolically active cells (Rosado *et al*., 2013). Moreover, it was verified in the present study that the tested bacterial strains have all the three active enzyme complexes, i.e. dehydrogenases, phosphatases and β–glucosidases, therefore, these enzymes could be recommended as bio-markers to evaluate the metabolic activity of bacterial strains involved in the degradation of recalcitrant pollutants like paints or paint-related products.

Functional gene detection related to the metabolic pathway of pollutants degradation is a direct straightforward method to monitor the biodegradation of pollutants (Nõvak *et al*., 2012). Specific genes on plasmid are maintained in microbial population only by few cells which need minimal energy and remain ready to tolerate the pressure of pollutants (Llosa and de la Cruz 2005). Thus the kinetics of specific catabolic genes in a polluted environment can provide a standpoint to understand the bioremediation process.

These catabolic genes are detected with the help of specific primers that target the only gene of interest. For this one must use primer already reported in literature against that specific gene or by designing the new primer using bioinformatics tools (Table I).

In this study, the genes for pollutant-degrading dehydrogenase (*adh*) were successfully screening from 13 isolates belonging to the genus *Pseudomonas* and *Bacillus*. Van Beilen and Funhoff (2007) reported 12 *adh* genes for alkane degradation. Our study correlates with Wu *et al*. (2018), who reported 9 genes coding for dehydrogenase from *Pseudomonas aeruginosa* involved in hydrocarbon degradation pathways. Wei *et al*. (2018) reported *adh* gene in *S. rhizophila, Stenotrophomonas,* and *Xanthomonas* for polyvinyl alcohol degradation. Panasia and Philipp (2018) reported *adh* from *Pseudomonas aeruginosa* for alkane- and SDS degradation. However, Dvořák *et al*. (2017) reported dehydrogenase (*adhB*) genes from *Zymomonas mobilis* for dichloropropene degradation and Ivshina *et al*. (2017) reported it from *Rhodococcus* for aliphatic and aromatic hydrocarbons degradation.

Gene for the pollutant-degrading enzyme, β-glucosidase (*bgl*) was successfully amplified in 20 selected strains belonging to the genus *Pseudomonas* and *Bacillus*. Similarly, Tozakidis *et al*. (2016) detected genes coding for endoglucanase, β-glucosidase, for cellulose degradation from *P. putida*. Van Rensburg *et al*. (1998) screened *bgl* from yeast for cellulose degradation pathway.

Metagenomics datasets reveal that *pho*D gene is predominant in 20 bacterial phyla. *pho*D is considered ubiquitous in the ecosystem. Terrestrial ecosystem

metagenomes show the highest occurrence of *pho*D. Bacterial *pho* (phosphatase) is categorized into three main families, *pho*A, *pho*D and *pho*X. The most prevalent *pho*D genes identified are affiliated with the following orders: Actinomycetales, Bacillales, Gloeobacteriales Rhizobiales, and Pseudomonadales (Ragot *et al*., 2015). Kageyama *et al*. (2011), detected *pho*D genes from other orders, including, Burkholderiales, Caulobacterales, Deinococcales, lanctomycetales and Xanthomonadales. Frankenberger *et al*. (1983) and Myer *et al*. (2016) detected *pho*D from *B. subtilis*. Similarly in our study gene *pho*D was amplified in 13 isolates belonging to *Bacillus* and *Pseudomonas* species.

CONCLUSION

The current study was designed to screen enzymes involved in the bioremediation of xenobiotic pollutants. The paint was used as a model pollutant. Work was divided into two broad categories physiological screening by which pollutant-degrading enzymes: Phosphatase, dehydrogenase and were screened and advance molecular techniques to detect catabolic genes. Expression of catabolic genes was observed in a single bioremediation system. A comparative study was also done between enzymatic and gene profiling to find out the significance. From this study, it is evident that microbes have genetic machinery that is responsible for catabolic pathways of pollutants. The current study was conducted on bacterial species belonging to the genus *Pseudomonas* and *Bacillus*. The current approach can be applied for other numerous bacterial genera, fungi and algae as well. The present approach was straight forward, reliable, simple, time saving and cost-effective for xenobiotic bioremediation of recalcitrant pollutants.

DECLARATIONS

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Generative AI and AI-assisted technology statement

The authors declare that no generative AI and AI assisted technology was used in the creation of this manuscript.

Statement of conflict of interest

The authors have declared no conflict of interest.

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