

Studies on Screening of Paint Degrading Microorganisms Isolated from Wall Scrapings

Yamuna G.¹, Anooj O.², Roopika K.³, Sabitha Rani⁴ & Rashmi D.^{5*}

¹⁻⁵Assistant Professor, Department of Biotechnology & Microbiology, Padmashree Institute of Management and Sciences, Bangalore-560060, Karnataka, India. Email id: rashmi.pcr@gmail.com*



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ABSTRACT

The present study was an effort to obtain paint degrading bacterial isolate from wall scrapings. The study included that microorganisms such as bacteria, not only cause discoloration of paint surfaces but also, they can directly cause degradation of the materials through their metabolic activities. The Halophilic microorganisms are well known for their paint degrading activity. As evidenced from the literature survey, there is a great diversity of bacteria and fungi that are capable of growing on paint coating. The presence of various polymer compounds used in paint manufacturing makes it resistant to degradation and continue to be a potential hazard to the environment as well as humans. Use of nonabrasive and environmentally safe methods, to reduce the impact of microbial activities can further reduce the damage as well as help in bioremediation of paint contaminated water, soil and environments to clean up.

Keywords: Bioremediation, Contamination, Halophilic microbes, Polymers.

Introduction

Paint is one of the oldest synthetic substances known to mankind with a history stretching back into pre historic times (Karigar *et.al*, 2017). These are uniformly dispersed mixtures which have viscosity ranging from a thin liquid to a semisolid plate (Nwachukwu *et.al*, 2015). Paint is eventually a mixture of: Binder, solvent, pigment, thinner, drier, extender, additives, (Fichera *et.al*, 2015, Monico *et.al*, 2016, Fraga *et.al*, 2018, Hayashi *et.al*, 2019 & Hewlett *et.al*, 2019).

The modern household paints fall into two broad categories, i.e.,

- (a) 'orthodox', the oil-based or solvent-based paints, thinned with mineral turpentine or other organic solvents, and
- (b) 'emulsion paints', the water-based vinyl or acrylic paints that may be thinned with water (Kanhar *et.al*, 2016).

Degradation of paints

Factors that influence deterioration of paints and paint products include the anaerobic environment in the paint, the organic nature of the paint's components, the microbial quality of the packaging materials and the hygiene level of the manufacturing plant processing units.

The consequence of their microbial deterioration such as foul smell, viscosity loss, discoloration and visible surface growth has serious economic, implication on the paint industry (Altenburg *et.al*, 2017).

Health hazards

Volatile organic compounds (VOC's) present in paint may lead to respiratory, allergic or immunogenic defects in humans (Mendell *et.al*, 2013). Frequently, paints contain a high level of mercury or lead and their ingestion may lead to serious health problems such as nerve and kidney damage. In addition, other metal such as chromium and cadmium are also reported that they provide many health risks. The exposure of VOCs has been related to organic

solvents syndrome (Spurgeon *et.al*, 2006). The world health organisation (WHO) has reported a 20%-40% increased risk of certain types of cancer (particularly lung cancer).

Bioremediation of paints

Bioremediation involves in indigenous microbial populations with or without nutrient supplementation, or it can involve inoculation of exogenous organisms into the site to transfer or degrade toxic compounds to harmless or less toxic substances. Mohsen Zadeh *et.al*, (2012) have reported biological removal (bioremediation) treatment technology has turned out to be a favourable alternative because it produces no toxic end products and is cost effective. These processes typically rely on the use of aerobic indigenous microorganisms at site or enrichment with naturally occurring microorganisms to degrade the contaminants. Safia Ahmed *et.al*, (2018) have discussed bioremediation is nowadays a much widely acceptable technique due to its eco-friendly approach, although plenty of pollutant remedial technologies. Boopathy *et.al*, (2015) and Zakie *et.al*, (2016) have discussed the use of relatively cost effectively, non-invasive, and are attractive alternative for either pollutant treatment by successes during the present technology rely on appropriate microbes in appropriate habitat with supporting ecological conditions to detoxify the environmental paint pollutants in an open ecosystem.

Microbial degradation

Rosado *et.al*, (2013) stated that environmentalists have recently reported variety of microorganisms isolated from the paints having capability of biodegradation and bioremediation of paints and paint related wastes. Soudi and Kolahchi (2011) studied that the environment is contaminated with key pollutants could be an ideal source of the microbes utilizing or degrading them. The study conducted by Wojturska *et.al*, (2017) concerned the susceptibility of polyurethane coatings to enzymatic degradation. The authors studied the growth of bacteria in a culture medium using polyurethane coating as the sole source of carbon. They used the following bacteria; *Pseudomonas*, *Corynebacterium*, *Bacillus* and *Brevibacterium*. The entire microorganism in the study showed an ability to grow using polyurethane coating as a sole source of carbon.

Microorganisms involved in degradation

Gaylarde *et.al*, (2016) stated that major groups of microorganisms involved in paints deterioration are bacteria and fungi, which can grow on applied paint film and solvent and wanted based coatings. Jakubowski *et.al*, (2015) reported most commonly isolated bacterial and species in paints include *Bacillus*, *Pseudomonas*, *Enterobacter*, *Proteus*, *Escherichia*, *Micrococcus*, *Serratia* and *Aeromonas*. A wide range of anaerobic bacteria including *Bacteriodes*, *Clostridium*, *Desulphovibrio* and *Bifidobacterium* have also been isolated in paints (Opperman *et.al*, 2018). *Rhizopus arrhinus*, *Aspergillus niger*, *Penicillium citrinum*, *Alternaria altanata* fungi associated with the deterioration of paints (Grant *et.al*, 2017). The bacterial degradation of paints is faster than fungi to mineralize the paints through combined metabolic mode of aerobic anaerobic reaction in form of consortia (Chand *et.al*, 2016).

Enzymatic biodegradation

Cifferi *et.al*, (2016) have discussed due to the lack of water solubility and large size of polymer molecules the microorganism are unable to transport the polymeric materials directly into the cells, where most biochemical

processes take place, so they are forced to produce certain enzymes which could penetrate the polymer and degrade them into smaller transportable molecules. Enzymes play an important role in metabolism and detoxification. The enzymes may be extracellular or intracellular and their mode of action will be different. Enzymatic degradation occurs by catalytic process. The enzymes can be denatured quickly by change in temperature or solvent or pH. Some enzymes require coenzymes. Coenzymes act as a donor or acceptor for a specific group. Hydroxylases and oxygenase are the main enzymes used.

Bacterial degradation of paint

Ashwini *et.al.*, (2018) reported on basis of their study on bacterial degradation of paint that microorganisms such as bacteria, not only cause discoloration of paint surfaces but also, they can directly cause degradation of the materials through their metabolic activities. The bacterial isolates also vary with the type of paint used. The microbes isolated in the study, has used paint as the sole source of carbon for its growth and metabolic activity and contributed towards the degradation of the paint materials.

The presence of acrylic compound in the paint samples inhibits growth of many organisms which made the isolation process tedious. Reports on fungal isolation from paint are quite common than bacteria. The following bacteria showed the ability to grow by utilizing paint: *Bacillus*, *Brevibacterium*, *Pseudomonas*, *Lactobacillus sp.*

Materials and Methods

Sample collection

Paint flakes were collected from 20 different places. The sample was collected in different containers which were properly sealed. The sealed individual containers are labelled neatly and moved to laboratory for further analysis.

Isolation of bacteria from wall scrapings

Mineral salt medium was used to isolate the bacteria from paint samples. Mineral salt medium was prepared, where paint acted as the sole source of carbon. Media was sterilised for 20 minutes at 121°C at 15lbs. The flask was allowed to reach the room temperature. The pH is checked and adjusted to 7.2 using 1N NaOH or 1N HCL. 1gm of paint flakes were added into the medium aseptically and incubated at room temperature for 7 days.

Screening of bacterial isolates for their paint degrading potentiality

Screening of bacterial isolates on mineral salt agar media amended with oil emulsion

Mineral salt agar medium was prepared, sterilized at 121°C for 20 minutes at 15 lbs and plated in sterilized petri plates and left for solidification. After the media got solidified 0.1ml of sample was transferred to mineral salt agar medium and spread plating was carried out. The inoculated plates were incubated at 37°C for 24 hours.

Screening of bacterial isolates on LB agar media amended with oil emulsion

LB agar was prepared, sterilized at 121°C for 20 minutes at 15 lbs and plated in pre-sterilized petri plates and left for solidification. After the media got solidified 0.1ml of sample was transferred to mineral salt agar medium and spread plating was carried out. The inoculated plates were incubated at 37°C for 24 hours.

Biochemical studies on different bacterial isolates

- IMViC, Oxidase test, Catalase test, Urease production, Starch hydrolysis
- Growth at different incubation temperatures (10°C, 15°C, 25°C and 45°C)
- Growth at different pH (2, 5, 7 and 9)

Screening of paint degrading potentiality of bacterial secondary metabolites

The paint degrading potentiality of the secondary metabolites was screened by observing the leaching of the paint from the pre coated metal strips treated with the crude bacterial extract having desirable concentration. Thin metal strips were coated with oil emulsion with the approximate thickness of 0.001mm and allowed to dry. The dried metal stripes were immersed in sterile distilled water containing crude bacterial extracts at the concentration of 1mg/ml. The whole setup was incubated at room temperature for 48 hours and observed for leaching of the paint. Coated metal strip in distilled water served as negative control, coated metal strip in 1N HCL was positive control.

Molecular identification of biologically active bacterial isolates

For the molecular identification of bio active bacterial isolates exhibiting exhibiting paint degrading capacity, DNA extraction, amplification was done using forward (27F-50-AGAGTTTGATCCTGGCTCAG-30) and reverse (1492R-50ACGGCTACCTTGTTACGC TT-30) primers (Genie, Bangalore, India), according to the protocol followed by Aman and Rai, (2016). Final PCR amplicon was eluted in 1.5% agarose gel and purified using GenElute gel elution kit (Sigma Aldrich, USA). Sequencing of DNA was accomplished and blast analysis was done. Similarities of Nucleotides were determined by NCBI (National Centre for Biotechnology Information databases). The identified isolate sequence was deposited and allotted with accession number.

Phylogenetic analysis of bacterial isolates

16S rDNA sequences obtained from bioactive bacterial isolates were homologically aligned with reference sequences from NCBI database with the aid of multiple sequence alignment program of MEGA 4.0 (Tamura, *et.al*, 2007). Unrooted phylogenetic tree was constructed using neighbour-joining and was maintained by 1000 boot straps using the MEGA-5 software.

Result and Discussion

Isolation and characterization of bacteria from wall scrapings

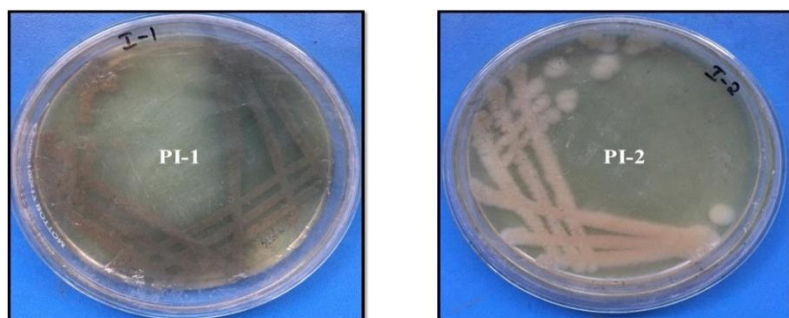


Fig.1. Bacterial isolates of wall scrapings on LB agar

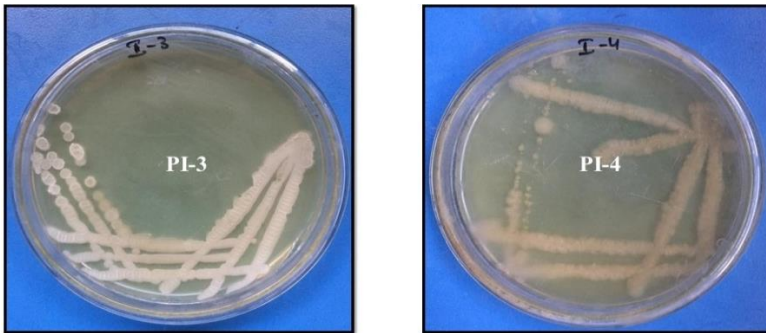


Fig.2. Bacterial isolates of wall scrapings on LB agar

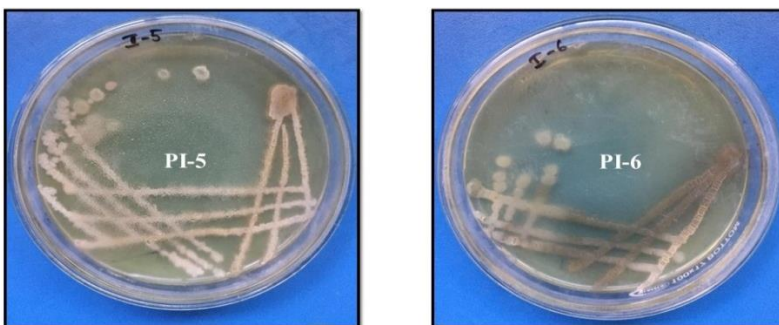


Fig.3. Bacterial isolates of wall scrapings on LB agar

Growth was observed on LB agar plates. Among six isolates PI-1, PI-5 and PI-6 was found to be gram negative rods. PI-2, PI-3 and PI-4 was found to be gram positive rods (Figs. 1,2 and 3).

Biochemical studies on different bacterial isolates

Identification of isolated culture was done by conduction of microbiological and biochemical test as referred in Bergey 's manual Whitman *et.al*, (2012). The results of various test & tabulated.

The organism showed:

1. All organisms showed indole negative.
2. 66.66% positive and 33.33% negative for methyl red test.
3. All organism showed negative for voges-proskauer test.
4. All organisms showed positive for citrate utilization test.
5. 66.66% positive and 33.33% negative for urease test.
6. All organisms showed positive for catalase test.
7. 50% positive and 50% negative for oxidase test.
8. 66.66% positive and 33.33% negative for starch hydrolysis.

Physiological characterization

Table 1. Growth of isolates at different pH & temperatures

Samples	pH			
	2	5	7	9
Control	-	-	-	+
P.I-1	-	+	+	+
P.I-2	-	-	+	+
P.I-3	-	-	+	+
P.I-4	-	+	+	+
P.I-5	-	+	+	+
P.I-6	-	+	+	+

Samples	Temperature			
	4°C	15°C	27°C	37°C
Control	-	-	+	-
P.I-1	-	-	+	+
P.I-2	-	+	+	+
P.I-3	-	-	+	+
P.I-4	-	-	+	+
P.I-5	-	+	+	+
P.I-6	-	-	+	+

Key: (+) = positive and (-) = negative

None of the isolates were able to grow at pH2 and two isolates were not able to grow at pH 5. At pH 7 and 9 all organism showed positive growth (Table 1).

All organisms showed negative growth at 4°C and four isolates were not able to grow at 15°C. All the organisms showed positive growth at 27°C and 37°C (Table 2).

Paint degrading potentiality of bioactive bacterial isolates on precoated metal strips

Secondary metabolite extraction

By the addition of ethyl acetate for supernatant obtained, secondary metabolite present in bacteria are dissolved and precipitation was seen (Fig.4).

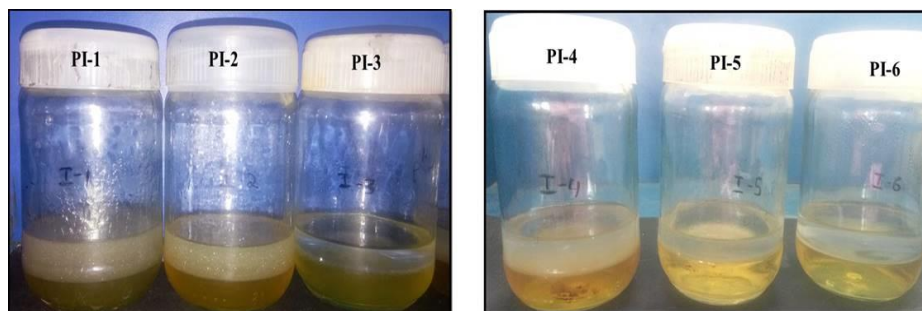


Fig.4. Secondary metabolites extraction

Leaching of paint was observed on the metal strip that was coated with paint. Graph was plotted using the optical density taken (Fig.5).

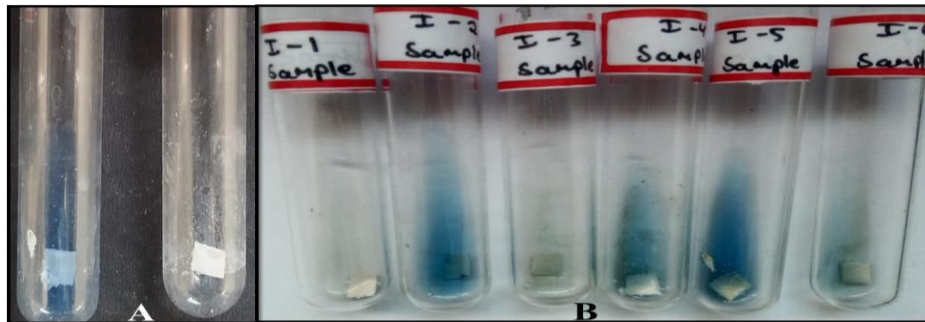
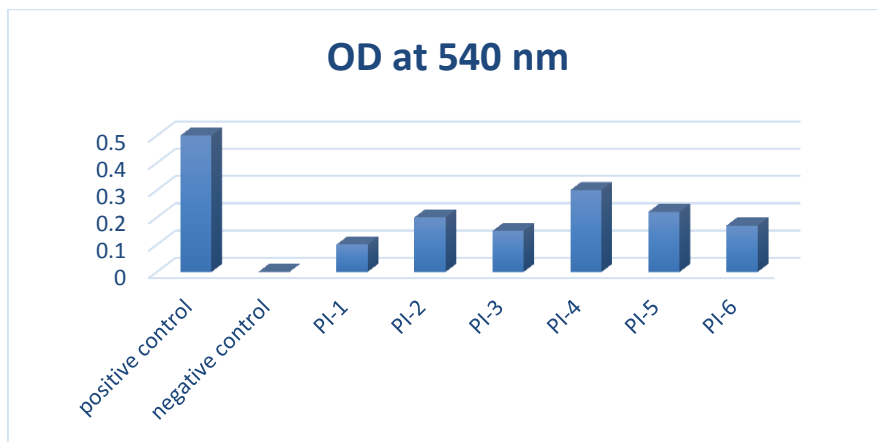


Fig.5. A= +ve control and -ve control and B= samples showing paint leaching

The graph was plotted on the basis of OD value taken. Comparison of positive and negative control with six isolates indicated that PI-4 was showing more degradation (Graph 1).



Graph 1. Isolates showing rate of degradation

Molecular identification and phylogenetic analysis

Molecular identification was done by amplifying genomic DNA followed by sequencing of 16s rRNA gene. Isolate was identified by The Basic Local Alignment Search Tool (BLAST) analysis of obtained 16s rRNA sequence. Further we concluded PI-4 isolate as *Brevibacterium* sp by phylogenetic analysis.

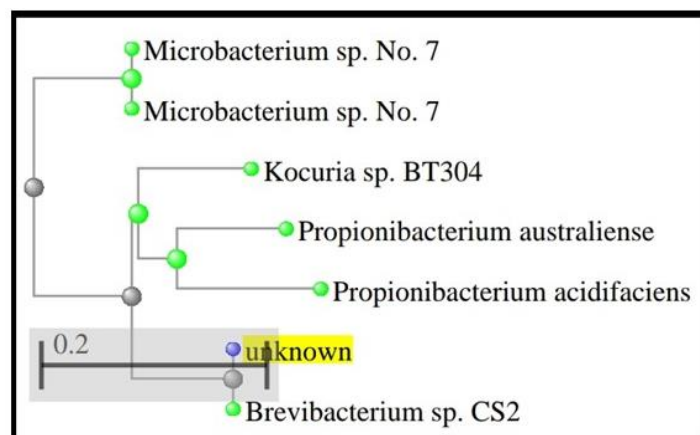


Fig.6. Phylogenetic tree of PI-4 isolate

Conclusion

The present study was an effort to obtain paint degrading bacterial isolate from wall scrapings. It concludes that microorganisms such as bacteria, not only cause discoloration of paint surfaces but also, they can directly cause degradation of the materials through their metabolic activities. Several microorganisms are well known for their paint degrading activity. As evidenced from the literature survey, there is a great diversity of bacteria and fungi that are capable of growing on paint coating. The presence of various polymer compounds used in paint manufacturing makes it resistant to degradation and continue to be a potential hazard to the environment as well as humans. Use of nonabrasive and environmentally safe methods, to reduce the impact of microbial activities can further reduce the damage as well as help in bioremediation of paint contaminated water, soil and environments to clean up.

Declarations

Source of Funding

This research did not receive any grant from funding agencies in the public, commercial, or not-for-profit sectors.

Competing Interests Statement

The authors declare no competing financial, professional and personal interests.

Ethical Approval

Based on Institutional guidelines.

Consent for publication

Authors declare that they consented for the publication of this research work.

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