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Characterization of Phenol-Degrading Bacteria: A Review

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Abstract

Phenol and its higher homology are aromatic molecules that originate from industries waste and occur naturally in our environment. Biodegradation is a major mechanism of removal of pollutants from a contaminated site. This review focuses on the recent researches on characterization of phenol degrading bacteria around the world. Scientist interested to have their own isolate because there is no individual organisms or groups of organisms that were universally appropriate and applicable for bioremediation because place of origin does play a role in determining the important properties of microorganisms. The compatibility to the application site as well as the biochemical and physiological potential of the microbes should be considered. Degradation of phenol occurs as a result of the activity of a large number of microorganisms with bacteria as the major player. Characterization of phenol degrading bacteria has resulted in bringing out the possible biological mechanism to remediate the phenol contaminant in the environment.

Keywords: Screening, isolation, characterization, bacteria, fungi, yeast, phenol biodegradation

INTRODUCTION

Environmental biology is an area that covering any biological organism, product or process that are beneficial to environment especially in control or reduction of pollutant and power use. One of the commonly discussed topics in environmental biology is bioremediation that is related to a technology that apply biological system, usually microorganisms to clean up a polluted contaminated site. The bioremediation industry promise a turnover around 20 billion dollar globally (Bains, 2004).

Updated on Thursday, July 25th, 2013, The United State Environmental Protection Agency (EPA) has published the current list of 126 Priority Pollutants. It is a set of chemical pollutants that were regulated and have a developed analytical test methods. Among the commonly found wastes are arsenic, benzene, chloroform, cadmium, chromium, lead, phenol, PCB's, trichloroethylene and toluene. The pollutant penetrate ecosystem by various situation either accidentally released to environment such as through leaking of the containers, crashes of toxic waste tanker, landfill leach ate and runoff the excessive pesticide from agriculture farm or resulted from the activity and development of industries. The cost for hazardous waste removal is very high. A report prepared by United States Government Accountability Office mentioned that their agencies spent almost $30 billion from 1986 to 2008 for all environmental cleanup and restoration activities (GAO, 2013).

Physical, chemical and biological methods have been widely applied to remove contaminant from the polluted area. However, amongst the technique, biological removal offers the cheapest and safest through bioremediation (Han *et al*., 2012; Bijay*et al.,* 2012).

There are various strategies in bioremediation which include; using native (indigenous) microbes at the contaminant are placed, improving microbes living conditions to the environments where there are only a few toxin-degrading microbes, so the condition will help the microbes increase in numbers and lastly by adding microbes which have been selected that have capabilities to degrade contaminants to the polluted environment which also known as bioaugmentation (Kazuya W. et al, 2001; Mrozik & Piotrowska-Seget, 2010). Accomplishment of bioaugmentation relies on the survival of the microbes added and the cells could be more resistant to the new environment by immobilization in a carrier such as calcium alginate (Santos *et al*., 2009), gellan gum (Moslesmy et al., 2002) and polyacrilamide (Chen et al., 2002).

Phenol Pollution

Phenol is among the common toxic environmental pollutants that characterized as first priority pollutants by European Union (Soudi & Kolahchi, 2011; Clodio et al., 2009). Phenol occurs naturally and also originated from industrial effluents such as pharmaceutical, food industries, oil refineries and coal conversion process (Yemendzhiev *et al.,* 2008; Passos *et al*., 2010). The phenolic compound also released in wastewater from steel industries, petrochemicals, polymeric resins and dye manufacturing units. It is an aromatic molecule that is soluble in water and organic solvents and could diffuse across a cell's membrane of an organism. The studies showed that constant administration of phenol by animals could cause pathological changes. Phenol discharged to water body will endanger aquatic life even at concentration of 500-2500 mgL^{-1} (J Yan *et al*., 2005). Phenol can cause health effects in both short-term (less than 14 days) and long term (after 365 days) exposure (OhioEPA, 2002). A human could suffer with anorexia, weakness, headache and muscles pain in long-term exposure to phenols vapors, meanwhile, acute or chronic ingestion of phenol may cause burning of mouth, diarrhea, sweating, sore throat, vomiting and abdominal pain (Michalowicz & Duda, 2007; Bull, 2007). The toxicity may depend on the degree of hydroxylation (-OH) and methoxylation of the ring. Based on the toxicity and risk to organism and environment, phenol requires appropriate treatment of waste containing phenol before being released to the nature. The ideal solution for pollution abatement nowadays is bioremediation, the most helpful technology that uses biological systems for treatment of contaminants (Desai et al., 2010). The biodegradation of phenol reduction is chosen as the cost of this technique is lower and offers complete mineralization (Shweta & Dhandayuthapani, 2013; Agarry et al., 2008). Even though, this recent technology is a multidisciplinary approach, but its central thrust depends on microbiology.

The use of genetically modified organisms capable to degrade phenol or newly designed phenol is still limited. Hence, exploration of the biodiversity of wild strains from natural ecological environments is currently the preferable and most appropriate approach to search for the capable candidate. Phenol had a bactericidal action and thus cause problem in microbial action. For that reason, research on the screening of strains and the isolation and characterization of microorganism that possess phenol degradation characteristic with better phenol tolerance is very important. A lot of researchers from various counties tried to discover the suitable microorganism because different locations require local sources. This is because local microorganisms are better suited with local conditions and would not cause ecosystem disaster as in the case of imported untested microorganisms. The discoveries have led to the application of microorganisms in bioremediation of phenol by individual microorganism or consortium (Abd. El-Zaheret *et al*., 2011). The microorganisms are possible to degrade phenolics by biological oxidation with the help of phenol oxidizing enzyme and microbial metabolism (Margesin & Schinner, 2001; Margesin et al., 2004). Bioremediation technology has been applied in field pilot or full scale. Therefore, in order to recondition the polluted location, microbial bioremediation strategies can be used *in-situ* and *ex-situ* bioremediation*.* Both methods relies on the relative abundance, structure, catabolic versatility and biotic/abiotic interactions of the microbial communities (aerobic/anaerobic) that are indigenously present, amended or stimulated at contaminated sites such as industrial waste treatment plant (Desai et al., 2010). This review summarizes the recent available information on type of microorganism, its characterization, phenol degradation capabilities and procedures used for research on this topic. Organisms endowed with phenol degrading capacities include number of bacteria and fungi including yeast. Recently, various types of aerobic bacteria, fungi and yeast reported in phenol degradation. Where as, only a few phenol degrading anaerobic organisms have been involved via one pathway (Basha *et al*., 2010). While, aerobic degradation is via meta or ortho pathway (Basha et al., 2010; Nair et al., 2008; Omokoko *et al*., 2008).

Phenol Degrading Bacteria

Since 1975, diversity of bacteria that can aerobically decompose phenol has been reported such as: *Bacillus stearothermophilus* (Buswell, 1975), *Pseudomonas putida* (Hill & Robinson, 1975; Yang & Humphrey, 1975; Bettman & Rehm, 1984; Hinteregger *et al*., 1992; Gotz & Reuss, 1997; Reardon *et al*., 2000), *Pseudomonas cepacia* G4 or *Burkholderiacepacia* G4 (Folsom *et al*., 1990; Solomon *et al*., 1994; Muller, 1994; Schroder *et al*., 1997;), *Pseudomonas resinovorans* (Dikshitulu *et al.,* 1993; Yang & Lee, 2007), *Arthrobacter* (Baradarajan *et al*., 1995); *Acinebacter* sp. (Paller *et al*., 1995; Abd-El-Haleem *et al*., 2003), *Alcaligeneseutrophus* (Leonard & Lindley, 1998), *Ralstoniaeutropha* (Leonard *et al*., 1999), *Nocardioidess* (Cho *et al*., 2000), *Pseudomonas pictorum* (Annadurai *et al*., 2000), *Pseudomonas aeruginosa*, *Pseudomonas fluorescens* (Oboirien *et al*., 2005; Ojumu *et al*., 2005). A study on *Desulfobacteriumphenolicum* sp. that degrade phenol anaerobically was reported by (Bak & Widdel, 1986). The studies on phenol degrading bacteria continue until the recent years.

An *Acinetobacter sp.* isolated from wastewater and activated sludge of a petroleum chemical plant,showed ability in phenol degradation in research done by Ren et al., 2008. With 16S rDNA sequence, out of ten isolates, four (PD4, PD5, PD8 and PD9) identified as *Acinetobacter*sp. Other five isolates (PD1, PD2, PD6, PD7 and PD39) known as *Pseudomonas* sp., and one (PD3) as *Comamonas*sp. The researchers choose *Pseudomonas* sp., PD39 as the representative to study in details. In medium containing 800 mgL^{-1} of phenol, the strain grown better in pH 7.0, at temperature 30 ºC. It also could metabolize phenol at up to 1200 mgL^{-1} and removing 99.96% of 637 mgL^{-1} in industrial wastewater within 72 hours (Ren et al., 2008).

Using batch culture with synthetic phenol (100 –500) mgL^{-1}, Agarry et al. (2008) studied the capability of an indigenous *Pseudomonas fluorescence* in bioremediation. Observation at multi cultivation times revealed that, phenol was totally degraded at varies cultivation times. The lag phase increased from 0 to 66 hours when the initial phenol concentration was increased from 100 mgL^{-1} to 500 mgL^{-1}. Monod kinetic model have been used to show the inhibition effect of phenol with 500 mgL^{-1} as the initial phenol concentration. The Ks increased and Rsmax decreased when phenol was increased. Haldane model estimated the biokinetic constants and showed that the *Pseudomonas fluorescence* had a good potential in bioremediation of phenol waste effluents.

A strain *Acinetobacter* sp. ISTPCP-3 was identified based on 16S rDNA sequence analysis and phylogenetic characteristics as well as morphology and biochemical tests. This strain was isolated from paper mill effluent and sediment core of pulp. It had high pentachlorophenol (PCP) degradation capability. *Acinetobacter* sp. ISTPCP-3 is mesophile because the best growing temperature was 30°C. It preferred pH 6.5–7.5, and pH 7 was the optimum and able to degrade 200 mgL^{-1} PCP while 250 mgL^{-1} was inhibitory to its growth. Application of gas chromatograph–mass spectrometric (GC–MS) revealed thatthis strain degrade PCP via *ortho* ring-cleavage with the formation of 2-chloro-1,4-benzenediol and 2,3,5,6-tetrachlorohydroquinone (Sharma *et al*, 2009).

According to biochemical characteristics and 16S rRNA sequence analysis, Shourian et al. (2009) isolated *Pseudomonas* sp. assigned as *Pseudomonas* sp. SA01 from pharmaceutical disposal wastewaters plant. Within 30 hours of incubations, the strain able to degrade 700 mgL^{-1} and up to 1000 mgL^{-1} of phenol with short lag phase however higher phenol concentrations (>1000 mgL^{-1}) inhibit the growth of this bacteria. The optimization experiments resulted with optimum degradation at pH 6.5 and with addition of casein and mannitol and as auxiliary carbon and nitrogen sources it could remove phenol in 20 hours of incubation. The SA01 strain metabolizes the phenol via a meta-cleavage pathway.

Cordova-Rosa et al. (2009) reported about indigenous consortium of phenol-degrading bacterial with *Acinetobacter calcoaceticusvar. Anitratus,* originated from wastewater treatment plant of an industrial coal. The consortium was able to survive in high phenol concentrations (1200 mgL^{-1}) with faster degradation rate compared to a single culture of the *A. calcoaceticus*. Using continuous and batch systems of a bioreactor, a high phenol biodegradation (above 95%) was obtained but incubation in coke gasification wastewater, showed that phenol still in degrade after 10 days incubation at pH 9–11.

Majority isolation of phenol-degrading bacteria were done from industrial waste, but Sandhu et al. (2009), was isolated ten bacteria from leaves of *Fraxinus pennsylvanica* that able to mineralize phenol. The isolate originated from trees near to (5–6 m) of a swine production facility in Hamilton County, Iowa. *Rhodococcus, Alcaligenes* and *Acinetobacter* were the identified genera according to 16S rDNA sequence analysis. According to the amplified fragments of the large subunit of a multicomponent phenol hydroxylase from these isolates signify it classed as a single kinetic class, have a moderate affinity for phenol that consistent with the predicted levels of phenol in the phyllosphere. The degradation pathway determined by amplification of for catechol 1,2-dioxygenase and catechol 2,3-dioxygenase genes via PCR supported with a functional assay for catechol 2,3-dioxygenase activity. The results proved that the gram-negative strains and also *Rhodococcus* degrade phenol by ortho pathway. The results exhibited that the leaf surface has taxonomically distinct communities of phenol-degrading bacteria based on the identified genes of phenol catabolic pathways.

Liu et al. (2009), reported about phenol-degrading bacterium from the activated sludge and phenol-contaminated soils in Northwest of China. The high biodegradation capability strain named as strain XA05 and FG03. XA05identified as *Acinetobacter* sp. and FG03 was closely related to the *Sphingomonas* sp according to DNA sequencing and homologous analysis of 16s rRNA gene. Both strains were immobilized in polyvinyl alcohol (PVA) at the ratio of 1:1, via repeated freezing and thawing. The experiments indicated, the immobilized and free cells have 95% phenol degradation efficiencies with 800 mgL^{-1}in 35 hours. The immobilized cells showed higher degradation rate constant in wider pH range and less temperature effect contrast to free cells. The immobilized cells can be reused in not less than 20 cycles and acquire better storage stability.

Also in China, microbes isolated from soil of natural forest of bamboo and pine, able to decomposed phenolics compounds based on their ability to grow in a medium containing *p*-coumaric acid, anphenolicallelochemicals. Identification process was done through biochemical analysis and 16S or 18S rDNA sequencing. Both methods recognize the four isolated strains as *Pseudomonas putida*4CD1, *Pseudomonas nitroreducens*4CD2, *Pseudomonas putida*4CD*3 and Rhodotorulaglutinis*4CD4 (Zhang et al., 2010).

Li et al. (2010) discovered the ability of a psychrotrophic organism, *Pseudomonas putida* LY1 to degrade 200 mgL^{-1} phenol at a range of temperature from 25 to 35ºC, with 25ºC as the optimum temperature. High initial phenol concentrations (800 mgL^{-1}) inhibited the growth of this strain. Haldane model was used to correlate the growth kinetics at low concentrations of phenol. Based on the report of other organisms, the Haldane parameters of psychotropic *P. putida* LY1 were almost within the range.

Application of *Pseudomonas aeruginosa* to remove phenol and the benzoic acid was done by Razika et al. (2010). The microbe preferred phenol compared to benzoic acid as the carbon source but not higher that 80 mgL^{-1}. Besides, Zheng et al. (2010) and Hank et al. (2010) also reported the biodegradation activity of *Pseudomonas aeruginosa*. Zheng et al. (2010) reported that *Pseudomonas aeruginosa* strain HSD38 able to degrade 500 ppm of initial concentration of phenol but incapable to tolerate higher than 700 ppm.

Chakraborty et al. (2010) did an investigation on the native bacterial *Arthrobacter citreus* strain ESDSPB2 isolated from the waste of a coke processing plant for phenol degradation. The optimal conditions for phenol elimination were at pH 7, temperature 30ºC and 0.25 % of glucose. The strain *Arthrobacter citreus*is able to completely mineralize up to 5mM of initial concentration of phenol and tolerate up to 22mM of initial concentration of phenol.

A strain TW1 originated from industrial wastewater treatment plant of Coke Company (Cairo, Egypt) was isolated by Essam et al., (2010). Morphological and physiological analysis revealed it was gram negative, single and cluster short-rod, motile and strictly aerobic, and partial 16S rRNA sequencing classified TW1 in the beta group of *Proteobacteria* as *Alcaligenes*. Strain TW1 was tolerate to various PAHs and monocyclic aromatic compounds. Interestingly, the Haldane model finely described it utilized up to 1200 mg phenol as a sole source of carbon and the growth kinetics.

Castillo et al. (2011), explores the potential of the bacterial communities in cork-processing wastewaters. Minimal number of bacteria was found when four pool samples were analyzed by DGGE but there were similar bands in all profiles might showing common microorganisms presence in all pools. Among the cultivable aerobic bacteria were isolated and further tested for their tolerance towards phenol and two chlorophenols. 16S rDNA sequencing identified the most resistant strains were group as gram negative (*Enterobacter, Acinetobacter, Serratia* and *Stenotrophomonas*) and some gram positive bacteria (*Arthrobacter* and *Bacillus*) that were usual (chloro)phenol degraders. From the identified strains, three of the identified capable to tolerate up to 10 mM phenol and 1.5-2.0 mMchlorophenols. All strains had phenol hydroxylase activity. Some of the strains seemed used meta fission pathway to degrade phenol whereas with some might use both pathways. Acridine staining, epifluorescence microscopy and by SEM were applied to detect the formation ofbiofilms onto the surface of residual small cork particles.

Mohite et al. (2011) reported on the isolation of two bacterial strains OCS-A and OCS-B from an oil-contaminated soil. Both were aerobic, phenol tolerance and degraded about 90% of 100 mg/L phenol in 80 h via meta cleavage pathway. The isolates were identified as *Proteus mirabilis* and*Citrobacterfreundi* according to the morphological, physiological and biochemical characteristics referred to Bergey's manual of systematic Bacteriology.

Isolation of microorganism from wastewater of coking industries attracted many scientists in China. Cao et al. (2011) also identified bacteria that responsible for phenol biodegradation from activated sludge of aerobic and anaerobic pools. 28 species from 20 genera obtained and belong to beta and gamma-Proteobacteria. Based on 16s rDNA sequence, the efficient degraders known as *Pseudomonas monteilii*GCS-AEJ-1 and *Pseudomonas plecoglossicida*GCS-AN-J-3, with degradation ability of 94.6% and 92.2% respectively.

16SrDNA gene sequence analysis and also the physio-biochemical characteristics and were applied by Yu *et al*., 2011 to classify the phenol-degrading bacterium. The identified *Bacillus cereus* B3, was originated from the coking wastewater of Coking Plant of Shougang in Beijing. *B. cereus* achieved the optimum growth conditions at 37ºC, pH 9.0 with 10% of inoculum. 800 mgL^{-1} was the maximum concentration of phenol for the strain. The addition of glucose inhibits the degradation of phenol.

Bacteria designated as *Acinetobacterlowffii* strain UW7 according to morphological, physiological andbiochemical properties, supported by a 16S rRNA gene sequence was originated from wastewater treatment plant in a coking chemical factory. The best growth and phenol degradation achieved at pH 7 and 30 °C with 25 g/l as the effective degradation rate but could tolerate up to 4.0 g/L. (Liu et al., 2011).

Another Asia country that involved in the isolation of phenol-degrading bacterium was Malaysia. In 2011, a research group reported about isolation of 115 bacterial from industrial sites and farms. The isolated strains were tested for phenol biodegrading capability in minimal salt media with 500 mgL^{-1}phenol. Out of thirty seven bacterial isolates exhibited phenol degrading capability but the best was identified as *Acinetobactersp.* strain AQ5NOL 1 according to partial 16S rDNA sequencing as well as BiologTMGN plates. High degradation rate were achieved in an optimum condition, $30^{o}C$, pH 7 and with addition of 0.04% (w/v) of $(NH_4)2SO4$ and 0.01% (w/v) NaCl and in the optimum condition, the strain could tolerate until 1500 mgL^{-1} (Ahmad et al., 2011).

Mohanty (2012) isolated *Pseudomonas sp.* NBM11 from phenol-contaminated soil from hospital waste. Taguchi method was applied to study the physiological parameters resulted with 30ºC as the optimum temperature and 7 as the best pH. Within 168 hours free cells of *Pseudomonas sp.* NBM11 completely degrades up to 1000 ppm of phenol while more than 1100 ppm will inhibit the growth. While, using immobilized cells, the isolated strain able to degrade up to 1000 ppm of phenol within 48 hrs. The microbe reported has 199bp genes that encode the 60.523 KD of phenol hydroxylase P3 component, a component of the enzyme phenol hydroxylase.

A *Thermoanaerobacterium*-rich sludge dominated by *Thermoanaerobacterium thermosaccharolyticum, Thermoanaerobacterium Aciditolerans, Desulfotomaculum* sp., *Bacillus coagulans* and *Clostridium uzonii* were used to test phenol degradation ability in palm oil mill effluent (POME) with 100 to 1000 mgL^{-1} of phenol concentration. 65% of 400 mgL^{-1} phenol was removed with acetic acid and butyric acid as the main metabolites. Response surface methodology (RSM) was applied to study phenol removal efficiency and the effects of oil palm ash, $NH_4NO_3$and $Fe2^+$ concentration on hydrogen production and. The result showed 200 mgL^{-1} $Fe2^+$, 300 mgL^{-1} $NH_4NO_3$and 20 gL^{-1} oil palm ash in POME will increased phenol degradation efficiency, with predicted 3.45 L hydrogen production and 93% phenol degradation efficiency. Highly reproducible results were obtained in a confirmation experiment under optimized conditions with 3.43 L hydrogen production and 92% phenol degraded. Meanwhile, continuous stirred tank reactor was used and 4.2 L hydrogen produce with 92% phenol degraded (Mamimin et al, 2012).

Chen et al. (2012) isolated *Ochrobactrum*sp. CH10 from soil of wetland in Beijing. Within 24 hours, the strain grows and efficiently degrades 100% of phenol in medium containing 400 mgL^{-1} phenol with pH 7.0 as initial pH at 30 ºC and with 5% of inoculum. For 900 mgL^{-1} phenol, 92.3% was degraded within 44 hours and 82.2% for 1000 mgL^{-1} phenol in 48 hours. The degradation kinetics was followed Haldane's model with parameter; (maximum specific rate) upsilon (max), = 0.126 per hour, K(s) (half-saturation constant) = 23.53 mgL^{-1}and K (I) (inhibition constant) = 806.1 mgL^{-1}.

In another part of China, Liu *et al.*, 2012, successfully isolated a bacterial strain as an excellent bacterial candidate for the biotreatment of high-strength phenol-containing industrial wastewaters. Sample of activated sludge taken from a coke plant in Wuhan, China. The bacteria known as *Pseudomonas aeruginosa*WUST-C1 based on the 16S rDNA sequence alignment. Beside phenol, the strain could grow on pyrocatechol, alpha-naphtol, hydroquinone, naphthalene, iso-quinoline and indolein an aerobic condition. *P. aeruginosa*WUST-C1 was unaffected by ampicillin and chloromycetin. In 36 hours, WUST-C1 completely degrade 1200 mgL^{-1}phenol. The cell growth kinetics of WUST-C1 was investigated at 35 °C with a range of 50 to 1600 mgL^{-1}as the initial phenol concentrations ranging from described by Halden equation. The Haldane parameters suitable to grow WUST-C1 on phenol were, µmax= 2.47 h per hour, K(s) (half-saturation constant) = 48.7 mg L^{-1}, and K(I) (inhibition constant)= 100.6 mg L^{-1}(R2 = 0.988).

Sun et al. (2012), isolated phenol-degrading bacteria from activated sludge from an aeration tank of the cooking wastewater treatment plant in China. Five strains identified as *Pseudomonas* spp. through physiological, morphological and biochemical test. The strains capable to degrade 55.5% to 79.6% of initial 500 mgL^{-1}phenol within 24 hours incubation at 28ºC and 170rpm agitation.

Another group of scientist from China screened and isolated four phenol degraders from activated sludge in coking plant. Strains identified as *Sphaerobacter, Acinetobacter baumannii, Comamonas testosterone* and *Novospingobium Naphtalenivorans* based on morphological, physiological and 16S rDNA identification. The highest degradation efficiency owned by *Comamonas testosterone* with high tolerance up to 2000 mgL^{-1} of phenol and degrades 100% of 1000 mgL^{-1} of phenol within 48 hours. The optimum conditions for phenol degradation by the best strain was in initial pH 7.5-8.5, at 30-40ºC and 150r x min^{-1} of shaking rate (Chen et al., 2012).

Meanwhile, He Xi-Pu et al., 2012, isolated a strain named F5-2 that has high capability to utilized phenol as the sole source of carbon was isolated from the wastewater of a paper mill plant. According to the morphology, biochemical characteristics, and the 16S rDNA sequence, this strain known as *Arthrobacter*sp. 1500 mgL^{-1} is the highest phenol concentration for the strain which could tolerate in temperature range 20-40ºC, pH 5.0-9.0, salinity of 0-40 gL^{-1} with shaking rate of 200 r x min^{1}. The *Arthrobacter*sp. F5-2 degrades 96.13% of the phenol within 57 hours with the initial phenol concentration is 800 mgL^{-1} and 2% of inoculum size of the culture medium via ortho-pathway.

A paper reported by Gracioso et al. (2012), explained their work on isolation and characterization of a phenol-degrading bacterium, *Achromobacter* sp. strain C-1, a gamma *Proteobacteria* from an industry in Cubatao, Brazil. 16S rRNA gene sequences showed it had 99% similarity to *Achromobacter xylosoxidans*. Physiology and morphological characteristics showed it is a gram- negative, short-rod or coccobacillary bacterium, non-motile and strictly aerobic on medium containing phenol, catechol, m-cresol and o-cresol. The phenol degradation pathway of phenol or glucose for *Achromobacter* sp. strain C-1 was determined by two-dimensional SDS-polyacrylamide gel (2D SDS PAGE). Nine protein spotted on the gel were exclusively induced from phenol-cultures of strain C-1. Using peptide mass fingerprinting, three phenol-degrading enzymes (4-hydroxy-2-oxovalerate aldolase, hydroxymuconic semialdehyde dehydrogenase and phenol degradation meta-pathway protein) were identified. The result supported by tandem mass spectrometry of selected peptides and also analysis of the metabolite produced that suggests the phenol degradation was via the β-ketoadipate pathway using catechol 2, 3 dioxygenase enzyme.

Using physiological, biochemical and 16S rDNA methods, Liu et al. (2012), identified a strain that used phenol as carbon source as *Achromobacter*. The strain could completely degrade 600 mgL^{-1}phenol within 67 hours and tolerate up to of 1000 mgL^{-1} of initial concentration. The degradation rate was maximized at pH 7, 30ºC with 0.3% salinity.

Bhattacharya *et al*, 2012,demonstrated that the three from 28 isolates identified by 16S rDNA sequencing as *P. aeruginosa*MTCC 1034*, P. fluorescens*MTCC 2421 and *B. cereus* ATCC 9634 isolated from Bangalore industrial effluent were capable to degrade phenol. The optimum conditions for growth and phenol degradation were 30°C, pH 7.0, 0.1% of glucose and peptone. A degradation of 74%, 76% and 83% was obtained for *B. cereus* ATCC 9634, *P. fluorescens*MTCC 2421 and *P. aeruginosa*MTCC 1034respectively within 48 hrs.

Astrain RTE1.4, originated from effluents of a chemical industry was grown well in medium containing phenol and its derivative, named guaiacol, 2,4-dichlorophenol and pentachlorophenol. This bacterium was identified as *Acinetobacter* sp. by 16S rRNA gene analysis supported with biochemical, morphological and physiological characteristics. It decomposes phenol at 200 to 600 mg/L at wide pH range from 5 to 9 and temperature 25-37ºC. The phenol degraded by the ortho-pathway (Paisio, 2013).

Halophilic microorganism for biotreatment of phenol identified as *Modicisalibactertunisiensis, Halomonasorganivorans* and *Arhodomonasaquaeolei.* These three bacteria grown on hypersaline media with 100 g L^{-1} of total salts. The strains habor genes encoding catechol 1,2-dioxygenase and protocatechuate 3,4-dioxygenase that have been cloned from *Halomonasorganivorans* (Bonfa' et al., 2013).

Elahwani et al. (2013) isolated six phenol degrading bacteria and identified one as *Alcaligenes*sp. AM4 using 16S rDNA sequencing. The Plackett Burman, a statistical experimental design was applied to optimize the medium and culture conditions. The optimal predicted formula was 240mg L^{-1} of phenol, 0.15 of inoculum for 37.5ml of culture volume containing 500 mg L^{-1} NH_4SO_4, 750 mg L^{-1} K_2HPO_4 and KH_2PO_4, 300 mg L^{-1} $MgSO_4$ and 250 mg L^{-1} of NaCl.

Kumari et al., 2013 isolated *Pseudomonas aeruginosa* from activated sludge plants in South Africa. It was identified based on morphology, biochemical as well as 16S rRNA analysis. 600 mg L^{-1} was the maximum concentrations for the isolated *P. aeruginosa* with spesific growth rate at 0.209.

Awan et al., 2013 collected soil samples from industrial area of Lahore, Jahang, and from Gomal University D.I. Khan. The locally isolated soil bacteria were tolerate up to 2.5 g/100 ml phenol. The degrade ability of these bacteria by was optimum at 35oC. and pH 7 with shaking speed of 120 rpm.

A phenol degrading *Alcaligenesfaecalis* was isolated from coke oven wastewater of Durgapur steel plant, India. It was identified via 16s rDNA analysis, biochemical, morphological study. This isolate tolerated at high phenol concentration (2100mgL^{-1}) (Subhasis et al., 2013)

Finally, Maza-Marquez et al*.* (2013) recently reported that, the bacterial community of an olive washing water (OWW) storage basin was characterized and cultivated. In order to select high phenol-degrading strains, effluent pollutants reduction media have been applied containing increasing amounts of polyphenols, Two isolate, *Raoultellaterrigena* and *Pantoeaagglomerans* were identified through 16S rRNA gene sequencing and shows ability to degrade 93% of phenol and at the same time reduce the BOD by 91%, COD by 89% and colour by 62%.

CONCLUSION

Information from this review has made a list of works done by scientists around the world on isolation and characterization of bacteria that capable to degrade phenol since phenol is one of environmental contaminant and its persistent and toxic effect. A lot of attempts were made to isolate, screen and select the most efficient candidate in phenol biodegradation as well as the optimization of parameters to support the growth and the rate of biodegradation like pH, agitation rate and temperature. Certain biodegradation process induced with the presence of auxiliary carbon and nitrogen sources. Overall, the optimum conditions for growth and phenol degradation for the majority of mesophilic microorganisms were at 30°C-37°C in neutral pH with 150rpm agitation with the addition of 0.1% of glucose and peptone for certain bacteria especially *Pseudomonas.* The degradation rate varies among the microorganism between 75% up to 100% depends on the phenol concentration within the incubation period. Some bacterial isolates were tolerated at higher concentrations (100-1500mgL^{-1}) that vary the degradation kinetics. Majority of the studies on biodegradation of phenol degradation have been performed aerobically. Based on the findings, confer that many kind of bacteria have a remarkable potential for its application in bioremediation and waste management, including the detoxification of phenolic wastes.

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