



Biodegradation of Industrial Pollutants by Plant Growth Promoting Halotolerant Bacteria-A Review

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Abstract Little information is there on biodegradation of industrial pollutants by halotolerant rhizosphere microorganism. Moderate halophile microorganism has wide application in degradation of organic pollutants under moderate salt condition. Their potential to fully degrade harmful chemicals remain unexplored. Conventionally known mesophilic organisms are found incapable for efficient removal of pollutants in saline concentration. Halophile or halotolerant microorganism are different and adapted to low-extreme salt condition. Therefore, these microorganisms are capable of bioremediation of hydrocarbon, dyes and phenol under saline condition. The previous studies have shown that halophilic microorganism can eliminate polycyclic aromatic hydrocarbon, petroleum hydrocarbon and phenolic compounds. In the present review we discuss recently work done on degradation of hydrocarbon, phenols, heavy metals, azo dyes, pesticide such DDT, lindane by halotolerant rhizobacteria.

Keywords: Biodegradation, Halotolerant, Organic Pollutants, Rhizosphere Bacteria

Cite This Article: Asmita Singha, and Singh Dileep Kumarb, "Biodegradation of Industrial Pollutants by Plant Growth Promoting Halotolerant Bacteria-A Review." *International Journal of Environmental Bioremediation & Biodegradation*, vol. 5, no. 3 (2017): 86-92. doi: 10.12691/ijebb-5-3-2.

1. Introduction

Environment is polluted due to several anthropogenic activities. Contamination present in saline condition has been little studied although there are several contaminated sites present at high or low temperature, high pressure or high salinity. (Marg & schime 2001 et al). Halotolerant are microorganisms that are capable to grow carry Out metabolic activities in moderate or extreme saline conditions. Microorganism is found to show growth below 2% nacl while halotolerant bacteria can grow in 2-10%. Halophile can be divided into slight halophiles (2-6%), moderate halophile (6-15%) and extreme halophile (15-30%). Currently halotolerant microbes can be of great potential in biotechnological research specifically in bioremediation. Industrial pollutants are often found in large amount in saline conditions especially in underdeveloped countries. Large amount of pollutants from industries such as harmful organic compounds and chemicals are produced. Organic pollutants removal by mesophilic microorganism has been extensively analyzed by nair et al. Using halophiles is an effective alternative for remediation with non-halophilic organism for contaminant degradation of saline soil or condition. Physiology of halotolerant bacteria allow to function properly in saline condition as they in bacteria stand to osmotic stress saline environment by actively allowing K⁺, glycerol other solutes to accumulate within the cytoplasm. Halophile organism could play an important role in treatment of hydrocarbon and phenol pollutants. Typical non-halotolerant bacteria

grow in medium having salt concentration less than 1%. Bacteria having optimum growth in salt concentration more than 1% are called halotolerant. Chlorinated hydrocarbon has wide variety of application because of their biocidal properties. Degradation of several pollutants by halophiles and halotolerant have received less attention. Several rhizobacteria have vast potential to degrade various hydrocarbons and phenols. Thus, such rhizobacteria have promising rule in biological treatment of hydrocarbon and phenolic contaminated sites.

2. Effect of Salt Concentration

Degradation rate effected by salt concentration in various ways. If salt concentration is less than, microbial activity will decrease, and dissolved oxygen will decrease. However halophilic bacteria like *halofores*, *halobacterium* show higher degradation. Some moderate halophile degradation rate decreases at high salinity. *Halomonas* strain show maximum degradation of DDT at moderate salt concentration. Several researched shows that degradation rate is affected in presence of Salt concentration can be lowered if Microorganisms are immobilized. Since 90% biological degradation of pollutants at moderate salt concentration is carried out by halotolerant salt concentration is carried out by microorganism. Various studies are being done to know the phylogenetic and physiology of these microorganism. Bioaugmentation with immobilized cells of microorganism for wide range of organic pollutants degradation at salt concentration of up to 200gm/l.

3. Plant Growth Promoting Bacteria

Soil salinity is reducing area and agriculture land by 1-2% every year that can be used for agriculture mainly in arid and semi-arid region. Plant growth promoting rhizobacteria are nonharmful rhizobacteria which are beneficial, and they play important part in health and nutrition of plants by going through a lot of mechanism. These process include siderophores synthesis which make iron soluble in soil and then make it available to plants, phytohormones production especially indole-3-acetic acid. These bacteria also produce precursor of phytohormone ethylene the enzyme 1-aminocyclopropane-1-carboxylic acid (ACC). deaminase that hydrolyzes ACC [1]. Previous pollutant. research shows that microorganism which show PGP activity levels ethylene hormone and help plants to grow in presence several stress conditions like salinity, heavy metals pollutant and other harmful chemicals [2] Previous research shows that plant that have PGP activity levels ethylene hormone and help plant to grow in presence of several stress conditions like salinity, heavy metals pollutants and other harmful chemicals. [2]. These bacteria can grow and survive in osmotic stress and help in growth of plants. Plant growth promoting rhizobacteria can be useful to alleviate saline stress in plants and improve phytoremediation in soil having large amount of industrial pollutants. Haloalkaliphilic bacteria can be naturally found in soils of oil located at saline-alkaline sites. Isolation of rhizosphere bacteria from such natural habitats can be utilized for lowering the saline -alkaline stress in plants that grow in such environment. Therefore, plant growth promoting bacteria which live in root extrudes such plant are rhizosphere can be efficiently used to degrade industrial pollutants. These bacteria have several enzymes which help in degradation of organic pollutants.

4. Impact of Soil Salinity on PGP Halotolerant Rhizobacteria

Sometime metabolic processes require high concentration of K and low concentration of Na for optimal growth get affected because of high level of Na accumulation. Under excessive of Na⁺ and Cl⁻ concentration in rhizosphere there are competitive interaction with other nutrient ions (K⁺, NO₃⁻ an H₂PO₄⁻) for binding sites and transfer of protein in bacterial cells. In presence of saline stress, osmotic pressure in rhizobacteria increases. The primary salinity gives rise to numerous secondary ones such as oxidative stress characterized by accumulation of oxidative species which produce several antioxidants enzymes that help in bioremediation process.

5. Effect of Osmolarity on Halotolerant Rhizobacteria

Several rhizobacteria show osmolarity in presence of NaCl. Microbes are adapting various mechanism to

counteract water flow. The cytoplasm develops osmotic equilibrium to high ion concentration by developing high salt concentration similar to that of surrounding media. A halotolerant strain *Pseudomonas fluorescens* MSP-393 which shows plant growth promoting properties synthesis osmolytes such alanine, glycine, glutamic acid, serine, threonine and aspartic acid by denovo mechanism which occur in cytosol which help to maintain salt tolerance [56]. These bacteria produce a large amount of protein in response to osmotic stress under high salt concentration which further processes gene expressions in these bacteria [57]. Different genes which are expressed in presence of salt are found in halotolerant *pseudomonas pseudo alcaligenes*. These proteins are produced from enzymes present in halotolerant rhizobacteria which act as a catalyst to initiate degradation process.

Table 1. Different halotolerant Rhizobacteria and plants in which they are found

Rhizobacteria	Plants
Bacillus safensis, Ochrobactrum pseudogregnonense	Wheat (T. aestivum)
Pseudomonas putida, and P. fluorescens	Wheat (T. aestivum)
Bacillus pumilus, Ochrobactrum sp.	
P. pseudoalcaligenes, B. pumilus	Rice (Oryza sativa)
B. subtilis, Arthrobacter sp.	Rice (O. sativa)
Azospirillum sp.	Wheat (T. aestivum)
Streptomyces sp.	Wheat (T. aestivum)
Pseudomonas sp., Bacillus sp., Variovorax sp.	Wheat (T. aestivum)
Azotobacter chroococcum	Avocado (Persea gratissima)
P. pseudoalcaligenes, P. putida	Maize (Z. mays)
Brachy bacterium saurashtrense, Brevibacterium casei,	Chickpea (Cicer arietinum)
Bacillus, Burkholderia, Enterobacter, Microbacterium, Paenibacillus	Pea nut (Arachis hypogaea)
Pseudomonas sp.	Common bean (Phaseolus vulgaris)
Azospirillum sp.	Tomato (Lycopersicon esculentum)
P. putida	Eggplant (Solanum melongena)
B. megaterium	Cotton (Gossypium hirsutum)
A brasilenes P. mendocina, B. subtilis	Radish (Raphanus sativus L.)
Bacillus sp., Ochrobactrum sp.	Barley (Hordeum vulgare)

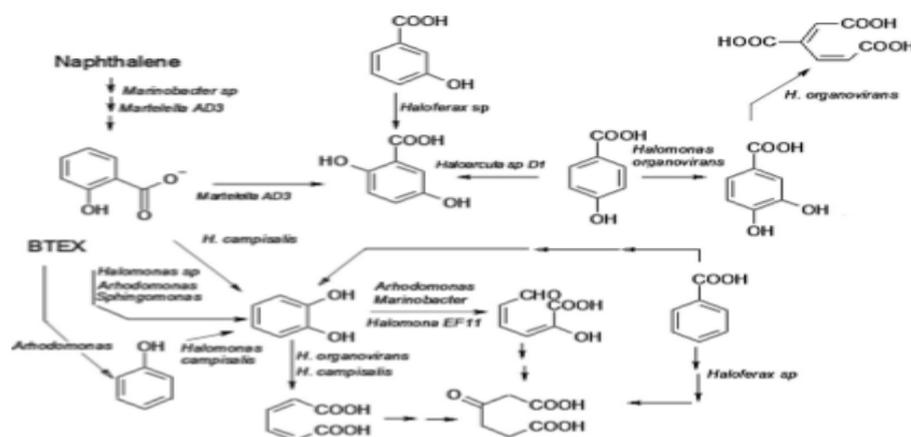


Figure 1. Biodegradation of organic pollutants by halotolerant microorganism

6. Petroleum Hydrocarbon

This compound is important source of pollutants in ecosystem. Little knowledge is available on degradation of hydrocarbon in saline conditions.

Generally, it is reported that extreme microorganism such as halophile or halotolerant, barophile, acidophile has limited degradation capacity. Metabolism and diversity decreases as concentration of salt increases which reduces metabolization of hydrocarbon. Degradation rate is studied using microbial consortia which show high rate of degradation. Capacity of biodegradation of microbial consortia is not characterized. Degradation of petroleum hydrocarbon was degraded at high salinity of about 15% nacl by oil bearing stratum. Bacterial consortium which was isolated from North Sea was metabolize petroleum hydrocarbon at salt concentration of 0-20% nacl which include marinobacter ssp and bacillus ssp. Bacteria such as rhodococcus, micrococcus and arthobacter grew in wide saline condition from 0.25-25% nacl but hydrocarbon metabolization occurred upto 15% nacl. Halotolerant *bacillus clausii*, degrade petroleum hydrocarbon upto 60%. *Bacillus subtilis FP-133* (Setyorini et al., 2006) [58], *Paenibacillus peoriae* NRRLBD-62, *Paenibacillus polymyxa* SCE2 (Alvarez et al., 2006) [59], *Halobacillus sp.* SR5-3. These other halotolerant bacteria showing PGPR properties which are identified to degrade hydrocarbons to large extent. Higher capacity to degrade aromatic hydrocarbon was shown by microbial mat in comparison to aliphatic compounds in 7.5% nacl. *Haloabacterium sp* show great capacity of degrading C10-C30 alkanes in a medium containing high salt concentration of approximately above 15%. *H. salinarium*, *H. volcanii* found to degrade large number of hydrocarbon. Hydrocarbon degradation and bacterial growth was strongly affected by decrease in salinity. paraffin and other petroleum derivative degradation is carried out by *Dietzia maris* in a medium containing 10 % nacl concentration at which bacteria has most optimum growth.

7. Crude Oil

Crude oils are defined as mixture of hydrocarbon. Microbial mats rich with cyanobacteria showed 21%. Degradation by aerobic heterotrophic bacteria primarily

degrade oil by hydrocarbon degradation the halotolerant actinomycete *Streptomyces* grows 30% salinity using crude oil as sole carbon source. Several bacteria which are Halotolerant in nature of genera *halomonas*, *Bacillus* and and *Dietzia* were isolated which are found to be potential to degrade crude oils in Saline condition as sole carbon source. DHT is a bacillus strain which are isolated from soil polluted with oil contaminants produced useful bio surfactant and enzymes when cultured in presence of crude oils, diesel oil, naphthalene as carbon source in presence of 1-10% nacl. Rhizopheric *pseudomonas sp C450R* aliphatic in nature and produced bio surfactants in presence moderate salty conditions. Wang et al in 2010 identified *Amycolicococcus subflavus DQS309A* a halotolerant actinomycete from china polluted site which can remove crude oils in condition containing of 1-10% nacl. *Bacillus cereus*, *comamonas*, *Bacillus altitudinis* are few bacterial strain which are isolated from various waste oil polluted sites are halotolerant having PGPR properties are identified to degrade crude oils alone or in consortia. *Acinetobacter*, *Arthobacter*, *Bacillus*, *Geobacillus*, *mycobacterium*, *Pseudomonas*, *rhodococcus*, *sphingomonas* and *xanthomonas* are halotolerant bacterial species which are isolated from sludge containing oil contaminants using PCR which help in phytodegradation of crude oils. Research efforts are being made on physiology of microorganism that are able to convert hydrocarbons into less harmful components in ecosystem having saline conditions and the metabolic process which help during degradation in saline conditions.

8. Aromatic Compounds

This compound contains aromatic ring. Several naturally occurring aromatic compounds are found in plant and in its root, such as vanillin, ferulic and cinnamic. Aromatic compounds present in environment as by product of agrochemicals of pharmaceuticals. They are indirectly present in atmosphere as intermediate product of degradation of hydrocarbons and PAHs. Taxonomic characterization of halotolerant bacteria was done by test which use benzoate as substrate (De Moral et.al 1988; Gracia et. at 1987; Ventosa et. al 1982) [60]. Earlier it was found that *bacillus subtilis sp* was found to grow at salt concentration 1. 65M. This strain is found to transform benzoic acid by ortho cleavage. Halotolerant rhizobacteria are found to biodegrade several harmful pollutants such

benzoic p-hydroxybenzoic acid, p-coumaric, and p-aminosalicylic acid which are aromatic in nature isolated from water of saline polluted sites. All isolates belonging to *halomonas* were moderately halophilic whereas bacillus strain was halotolerant. A new species of *halomonas organivoran* is able to degrade wide aromatic acids. Genus *marinobacter* can degrade wide range of hydrocarbons at high salinity. Similarly, halotolerant such as *bacillus subtilis*, *coagulans* and *bacillus candida* are able to degrade petroleum hydrocarbon and crude oils. Some isolated of *virgibacillus*, *oceanobacillus*, *halobacillus*, *bacillus subtilis*, *bacillus coagulans* are found to produce natural vanillin from ferulic acid (Peng chen, Lei Yan et al 2016) [61]. Few isolates of halophilic archaea such as *halofores sp* have been found which is able to degrade aromatic acids (Emerson et al., 1994) [62]. This strain belongs to family *halobacteriaceae* and grows at 2M nacl. 3 phenylproponic acid has an aromatic ring and aliphatic side chain which is degraded by *halofores sp D1227*. *Bacillus ligniniphilus* L1 degrade alkaline lignin which is an aromatic acid product. *Haloarcula sp D1* identified from polluted sites was found in presence of 4-hydroxybenzoic acid through aerobic pathway [3]. 4HBA was metabolized through gentisate pathway involving hydroxylation. *Halobacteriaceae* family can degrade p-hydroxybenzoic acid.

9. Phenolic Compounds Pollutants

Phenolic substance is major source of contaminated water by industrial pollutants such as pharmaceuticals, pesticide and oil industries. Degradation of phenolic pollutants in saline condition was pointed out by woolard and Irving (1994, 1995). Approximately above 95% of phenol was removed by rhizobacteria from wastewater which contain 0.2 to 0.13g/l phenol and 10% nacl. Halotolerant *bacillus cereus* degrade phenol at concentration as high as 2000mg/l. Maximum degradation rate was obtained at initial phenol concentration of 800mg/l. Halotolerant phenol degrading *halomonas sp* which has PGP properties is identified from great Salt Lake and several phenol contaminated sites. This strain has ability to remove phenol in medium containing phenol as sole carbon source. Another *halomonas campisali*. other rhizobacteria strain which is alkaliphile in nature and is found efficient to degrade wide variety of phenol and catechol in presence of phenol as carbon source and nacl concentration of 150g/l. Another strain *halomonas EF11* is also alkaliphile halotolerant bacteria reported in 2004 which grows in presence of phenol. This strain is found to transform phenol through meta and ortho pathway at high and low C/N Ratio. A moderately halophilic phenol degrading gram positive bacillus strain named *Thalassobacillus devorans* isolated. The genus *marinobacter* described by Gauthier et al (1992) [64]. *Marinobacter* are halophilic bacteria associated to proteobacteria. Nichlson and fatherpure recently studied bacterial culture at salt plains of Okhalama in presence and absence of benzene. This strain is able to grow in presence of phenol as sole carbon and energy source. Tyrosol is a phenolic compound that naturally found in wastewaters as sole carbon and energy

source. Tyrosol is converted into p-hydroxyphenyl acetic acid with a novel halotolerant bacillus strain YASI under moderate saline condition as it shows at 3-6% nacl Toluene was degraded easily while benzene, ethylbenzene, xylene took 2-3 weeks to degrade. This strain of halotolerant rhizosphere pseudo *monas alcaligenes*, bacillus degrade toluene, ethylbenzene and xylene to large extent. *Marinobacter* species are tolerant to wide range of nacl concentration. *Bacillus cereus* halotolerant bacteria isolated from solar salt in southern India can grow between 4-6% nacl concentration. Chamkha (et al 2008) [65] have isolated strain which resemble *Geobacillus pallidus* obtained from tyrosol enrichment of water from oil field of Tunisia. Benzene, toluene, ethylbenzene (BTEX) compounds exit in ecosystem after oil components released from wastewater. Similarly, *marinobacter hydrocarbonoclasticus* isolated from Mediterranean seawater grow at nacl concentration from 0.1 to 3.5 M with optimum at 0.06 M. Ghevariaya 2002 increased chrysene degradation to 86% by halotolerant *Achromobacter xylosoxidans*. Similarly effect of halotolerant *staphylococcus xylosus* on activated sludge as inoculum for treatment of waste water at various salt concentration. Similarly, rhizobacterial halotolerant in nature which show PGP properties were identified such as *Bacillus salinus* and *Bacillus simplex* which can grow in 1-3M nacl (Abou-elela et al 2010) [63] are anaerobic salt-tolerant bacteria. Another halotolerant bacterium which is utilized in bioreactor involving anaerobic pathway is *haloanerobium lacusrosei* showing optimum growth till 3% nacl.

10. Halogenated Hydrocarbon

These hydrocarbons are those which involve aliphatic and aromatic compounds having more than one hydrogen atoms acting in presence of halogen group. These hydrocarbons are highly toxic in nature because they remain in environment for a long time. Hydrocarbon having chlorine atom among this group are generally used because of their chemical and thermal stability. They are also main source of halogenated hydrocarbon. 2,4-dichlorophenoxyacetic acid is an herbicide which is used in large amount. In 1996 Maltseva et al found a halotolerant bacterium belonging to family *halomonadaceae* which degrade 2,4-D, benzoic acid and 4-chlorophenol in presence of 0.7-01. M nacl. Halotolerant species *penicillium chryogenum* was able to degrade 2,4 Dichlorophenoxyacetic acid. *Penicillium chryogenum* is found to grow in solid medium containing concentration up 100mg/l of 2,4 -D with sucrose. This strain can be potential degrader of benzoic acid and 4-chlorophenol. Phenol which are chlorinated in nature are widely used as agricultural antibiotics. Maltseva and oriel isolated halotolerant strain *Nocardiodes sp.* strain M6 which is alkaline in nature was able to grow on 2,4- D along with 2,4,5 and 2,4,6 trichlorophenol as sole carbon source. One of the several mechanisms involved in degradation of chlorinated phenols is – (i) Monooxygenases catalyze hydroxy acid at ortho-position of chlorinated phenol rings which further transform chloroacetic that may be degraded further via ortho and meta cleavage. Many bacteria that are halotolerant

in nature have been isolated such as *Pseudomonas knackmussii*, *rhodococcus opacus* IG, *Alcaligenes sp. A7-2*. Several arthobacter DCP degrading bacteria have been identified such as *pseudomonas sp*, *rhodococcus erythropolis*. Wang et al reported removal of 2,4-DCP is possible by immobilized cells of *bacillus insolitus*. Halotolerant *sphingomonas chlorophenolicum* L-1 catalyzed conversion of polychlorinated phenol to TeCHQ via removal of chloride ion. A new detoxification mechanism for 4-chloro-2-aminophenol has proposed by 2 halotolerant bacillus species. Halotolerant archaea of genus *halobacterium* and *haloforex* are found to be highly adapted to grow in presence of high concentration of toxic compounds like lindane and DDT (Oesterhelt 1998) [66]. Result of analysis by GC-MS shows that these bacteria have potential to partially decompose all these compounds.

11. Azo Dyes

Azo dyes form biggest group of dye which are involved in paper and textile manufacturing (Saratale et al. 2011) [67]. Dye generally show resistant to oxygen attack. Azo dyes which is drained in water are generated by bath dye producing and consuming industries. Biological removal of these dyes is possible by both aerobic and anaerobic pathways. *Gracilbacillus sp GTY* has been reported to decolorize acid dye B in medium containing 3-5% NaCl of harmful dyes. *Pseudomonas aeruginosa* and *bacillus circulans* consortia has efficiency to decolorize about 100mg/L of reactive black dye with degradation potential of 94% in presence of 4% NaCl by *Pseudomonas aeruginosa* and *bacillus circulans* consortia has efficiency to decolorize about 100mg/L of reactive black dye with degradation potential of 94% in presence of 4% NaCl by anaerobic pathway. The removal of dye occurred by biosorption with enzyme onto microbial cells of rhizobacterium *exiguobacterium sp*. This consortium is able to decolorize several dyes such as violet 13 and red 11 with discoloration efficiencies of 80-90%. Another dye red 120 was degraded by halotolerant *bacillus lentus* BI377 species under aerobic conditions (Oturkar et al 2011). Degradation of dye in aerobic and anaerobic microenvironment by microbes or consortia of microorganism which degrade dyes in medium under saline conditions are investigated to transform all harmful recalcitrant compounds for halotolerant microorganism. The wide range of salinities for dyes decolorization is an interesting characteristic for an eventual application to decolorization of textile effluents that are generally saline and slightly alkaline in nature because textile pollutants contain a complex mixture of harmful dyes. Several halotolerant strains have been identified which have ability to degrade dye.

12. Heavy Metals

Heavy metal pollution due to increase in industrialization has become a serious issue. Common heavy metal pollutants are copper, zinc, cadmium, lead. Priyalaxmi et al (2014) [68] stated that microbial load of mangrove

ecosystem states that bacterial population was higher and actinomycetes were large in number. Two bacterial strain PB-5 and RSA-4 had cadmium resistance. The potent strain RSA-4 was identified as halotolerant *bacillus safensis* by phylogenetic analysis. Similarly, several other halotolerant rhizobacteria were identified which were able to remove heavy metal such as *pseudomonas aeruginosa*, *proteus vulgaris*, *acinetobacter radioresistens*. *Pseudomonas aeruginosa* remove 75-85% of soluble cadmium.

13. Summary

Halotolerant microorganism has capacity to degrade wide variety of organic pollutants in presence of moderate amount of NaCl. All ecosystem on earth are submitted to pollution. Previous studies on microbial degradation of organic pollutants in saline condition is limited. This work shows that some halotolerant rhizobacteria are able to metabolize several pollutants. Further research should be made to the mechanism of degradation and discover the enzymes which are responsible for degradation and metabolism regulation. From application point of view, scientist have faith to search various tolerant range of bacteria that can live in low to moderate environment. In addition, these halotolerant bacteria produce enzyme which can have wide range applications. Excess of salt in soils is found to put adverse effect on the growth of plants and plant hormone.

Several microbes present with rhizosphere plants also get adversely affected due to salinity. Ecologically compatible and environmental friendly techniques are in large demand because of their efficiency to under agricultural soil which is saline in nature. Application of such potential microorganisms, PGPRs can be primary alternative to many of the traditional agricultural technique. Microbial enzyme which are used in industry go through one of major problem of stability. Enzymes are harmless, biodegradable and act as good catalysts but few of them are unstable in industrial processes. Halotolerant enzymes as per research are thought to be comparatively stable in these processes. Because of this all such organism may highly beneficial in environmental biotechnology for removal of organic effluents. For all this reason it is essential to focus on more depth research on growth conditions and of these types of microorganism which can be used as model system of degradation.

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