EXCELLENT PUBLISHERS

International Journal of Current Research and Academic Review

ISSN: 2347-3215 Volume 3 Number 10 (October-2015) pp. 364-372

www.ijcrar.com

Influence of Heavy Metals Tolerance and Antibiotic Resistance in Pattern in *Halomonas organivorans*

A.B. Parameshwar, S. K. Anil, S.YJayachandra, Mohan Reddy and M.B.Sulochana*

Department of Biotechnology, Gulbarga University, Gulbarga Karnataka, India -585106

****Corresponding author***

KEYWORDS

Halophiles, Polyextremophiles, Heavy metals, Antibiotic Resistance

A B S T R A C T

Halophiles are microorganisms that adapt to moderate and high salt concentrations, present investigation is to assess the heavy metal tolerance, and Antibiotic resistance in Halophilic bacteria,*Halomonas organivorans* was subjected for study. Biochemical and morphological studies indicate that, strain is Gram negative, grow optimum at 10% salt concentration, pH 7-11 and temperature at 37^0 C. Metal tolerance tests with mercury (Hg), iron (Fe), lead (Pb) and silver (Ag) were performed with the selected strain. The metal tolerance was found up to 5mM concentration. These isolate was also associated with resistance to antibiotics ampicillin, Tetracycline, chloramphenicol, kanamycin, erythromycin, streptomycin, rifamicin and other antibiotics. There is close relation between the resistance to heavy metals and antibiotics, bacterial survival become multiple stress and can tolerate and strain could be used in bioremediation.

Introduction

In a worldwide sense pollution of soils, sediments and water with heavy metals has been an unfortunate by-product of industrialization. Micro-organisms adapted to live under chemical and physical extremes that are usually lethal to cellular molecules. Bacteria live in environments that gradually undergo physical and chemical changes, bioavailability of heavy metals is essential for the growth of certain microbes, accumulation at high S concentrations in soil or water is toxic to all branches of life.

Extreme environmental habitat exhibit the diverse groups of microorganisms that are capable of growing in harsh conditions, their molecular diversity make these organisms to survive in extremities of Salt, Pressure, pH, Heavy Metal contaminated environment and temperature, such organisms are called Polyextremophiles.

The ability to adapt rapidly to changes in their environment is essential for the survival of all microorganisms.

Microorganisms have evolved several mechanisms to tolerate the presence of heavy metals (by either efflux, complexation, or reduction of metal ions) or to use them as terminal electron acceptors in anaerobic respiration. (Cheng, 2003)Other resistance mechanisms include active efflux, complexation, reduction and sequestration of the heavy metal ions to a less toxic state (Nies, 1999). These tolerance mechanisms are generally plasmid driven, which greatly contributes to dispersion from cell to cell (Collard *et al*., 1994, Valls and de Lorenzo, 2002).

Metals, such as Hg, Pb, and Ag, have no biological functions and are toxic to microorganisms even at minimal concentrations. Both nonessential and essential (at higher concentrations) metals can block functional groups of important molecules and transport channels for required nutrient ions, damaged cell membranes and DNA structure, and alter enzyme specificity leading to disruption of the cellular functions. As a consequence of the environmental exposure toheavy metals, microorganisms have evolved metal resistance strategies, including exclusion by permeability barrier, cellular sequestration, enzymatic transformation, reductionof metal to less toxic forms, and efflux of the metal ionsfrom the cell. Genes responsible for heavy metal resistanceare mainly located on plasmids; therefore, these determinantsmight easily transfer within an ecological systemsuch as soil (Nies 1999, Bruins 2000,).Metals can cause deleterious oxido-reduction reactions with proteins or other compounds (e.g. H_20_2 and iron also known as Fenton reaction), generating toxic compounds (e.g. OH. OH^-) that alter macromolecular structures such as proteins, membranes and DNA, leading to cell death (Stadtman ER 1990)

The present study was focused on the resistance and tolerance factors in halophiles is contributing mainly for adaptation in harsh environmental conditions most often polluted environment is rich in heavy metals and other toxic materials. Hyper saline conditions and antibiotic resistance is co resistance and tolerance factor is plasmid oriented.

Materials and Method

Bacterial Culture

We have isolated the *Halomonas organivorans*. (NCBI Accession No. JQ906721) was grown on Halophilic media containing 10gm Peptone, 10gm Yeast extract, 20gm $MgSo_{4,}$ 2gm KCl, 3 gm tri-sodium Citrate, on Petri plates, incubated at 37°C for 24 hrs . The *H.organivorans* was then inoculated into Halophilic broth and incubated at 37°C in a shaking incubator.

Morpho-Physiological and Biochemical Characters of the Isolates

The characters of the organisms were studied following standard microbiological methods. Morphology, vegetative cell and spore characters were observed under a phase contrast microscope (100X objective) from 12 h old culture grown on rotary shaker at 100 rpm, 30 ± 1°C.

The physiological and biochemical characters viz. indole production oxidase, catalase, urease hydrolysis, acid from glucose, mannitol, arabinose, xylose, citrate, and propionate utilization and tyrosine hydrolysis were studied. Assay of casein, gelatin, starch hydrolysis etc. were also checked.

Antibiotic Resistance

Response of the organisms to different antibiotics was tested on halophilic agar medium. The plates were surface seeded with bacterial suspension. Different antibiotic discs with effective concentrations were placed over the plates.

Inhibition of growth depicted by a clear zone formation around the discs indicated sensitive reaction, otherwise the organism was resistant to the antibiotic. Diameter of the inhibition zone was measured with an antibiotic zone scale.

Heavy Metal Ion Stress

Halophilic media were prepared with 1mM, 2mM, 3mM, 4mM, 5mM of heavy metals such as $HgCl_2$, $AgNO_3$ and Lead Acetate, the *Halomonas organivorans* strain was inoculated and kept for incubation at 37 ^{0}C, After 12, 18, 24, 36 hrs of incubation. Then the growth was measured spectrophotometer at 600 nm

NaCl and Common Salt Tolerance

Growth of the organisms on nutrient agar medium supplemented with different concentration of NaCl and common table salt (locally available) was checked. Highly diluted suspensions of the organisms were spotted on the plates, incubated at 30°C for 72 h and growth was recorded.

Effect of Inoculums Size

Halophilic media of pH 7.0 was prepared with different inoculums size of 0.5, 1, 1.5, 2 ml of *Halomonas organivorans* strain was inoculated aseptically and the growth was observed after 18, 24, 36, 48 and 72 hrs of incubation at 37 ^{0}C. Then the growth was measured spectrophotometer at 600 nm.

Effect of pH

Halophilic media was prepared with different pH 3,4,5,6,7,8,9,10,11,12 and *Halomonas organivorans* strain was inoculated aseptically, and growth was observed after 18, 24, 36, 48 and 72 hrs incubation at 37 ^{0}C. Then the growth was measured spectrophotometer at 600 nm.

Results and Discussion

The morphological characters of our isolate was gram negative bacilli, rod shape, non-spore forming and motile microorganism. The biochemical tests of isolated strain is as shown in the Table 2 and figure1. All these observations were compared with Bergey's Manual of Bacteriology, and was reported earlier in our laboratory, Halophilic bacteria grew better at the temperature of 28–37ºC and at pH 7.0–8.0(Graph 1-5) on medium supplemented with 5–20% NaCl concentration. Our results agreed with the study of Kushner*et al.*,(1998) who isolated the halophilic microorganisms from salt ponds of China and observed the optimum growth at the temperature of 35–40ºC and at pH 7.0–8.0 with 10% (w/v) NaCl. belongs and It was noticed in this study that some strains isolated from this particular environment did not grow with high concentrations of metals, or had difficulties in growing under laboratory conditions in culture media adjusted with metals, with the exception of Hg However, this effect is somewhat common, and most of the isolates from metal contaminated environments only proliferate under laboratory conditions when no metal or small amounts of metal are used (Gadd, 1990). The fact that the isolate did not grow when metal concentrations similar to those found in their environment can also be explained probably due to the fact that the composition of the media and other conditions of incubation were decisive for the determination not only of the metal tolerance but also for the determination of the optimal pH for growth (Horikoshi, 1999; Krulwich, 2000). However, since all strain was isolated from highly contaminated soils, they should have been stress adapted and able to proliferate, or at least survive in such an environment. Although some studies have shown detailed mechanisms of heavy metal tolerance for some bacterial species, for others these mechanisms are not yet very well known (Tsai *et al*., 2005). The ability to cope under this metal-contaminated environment may depend on genetic and/or physiological adaptation (Gadd, 1990). Therefore certain bacterial strains that are not adapted to live in metal contaminated environments may be sustained or induced by the activity of other bacterial strains or at a given concentration, a metal - may be toxic to one species while serving as a growth stimulant to others (Tsai *et al*., 2005). Heavy metals are more than likely able to affect the microbial populations in a given environment by reducing abundance and species diversity and selecting for a resistant population (Gadd, 1990). Tolerance to heavy metals may result from intrinsic properties of the microorganism, e.g. producing extracellular mucilage or polysaccharide or an impermeable cell wall (Gadd, 1990). Likely in this type of extreme environment, one of the most important factors that explains the presence or absence of a given population may well be the relationships that exist between the whole bacterial community. 24 antibiotics have been tested for the susceptibility test, *halomonas organivorans* has been observed to resistance against 12 antibiotics and sensitive to 12 antibiotics(Table 1).

Table.1 List of Antibiotics used for the Antibiogram

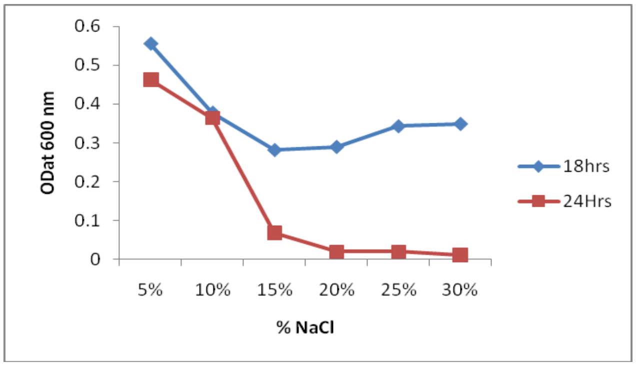
Sl. No.	Antibiotic	Resistance Sensitivity	Zone of inhibition(mm)
1	Amikacin	Sensitive	21mm
2	gentamicin	Sensitive	21mm
3	Erythromycin	Sensitive	25mm
4	Polymyxin	Sensitive	16mm
5	Clotrimazole	Resistance	Resistance
6	Rifampicin	Sensitive	20mm
7	Cefixime	Sensitive	19mm
8	Vancomycin	Resistance	Resistance
9	Nystatin	Resistance	Resistance
10	Methicillin	Resistance	Resistance
11	Pepracillin	Sensitive	17mm
12	Amphotericin	Resistance	Resistance
13	Streptomycin	Sensitive	19mm
14	Levoflaxacin	Sensitive	18mm
15	Oxacillix	Resistance	Resistance
16	Ciproflaxin	Sensitive	23mm
17	Amphicillin	Resistance	Resistance
18	Amoxyclav	Resistance	Resistance
19	Cefamandole	Resistance	Resistance
20	Azithromycin	Resistance	Resistance
21	Chlorophenicol	Sensitive	29mm
22	Itrconazole	Resistance	Resistance
23	Fluconazole	Resistance	Resistance
24	navobiocin	Sensitive	12mm

Table.2 Morphological and Biochemical Characterization of *Halomonas Organivorans* strain

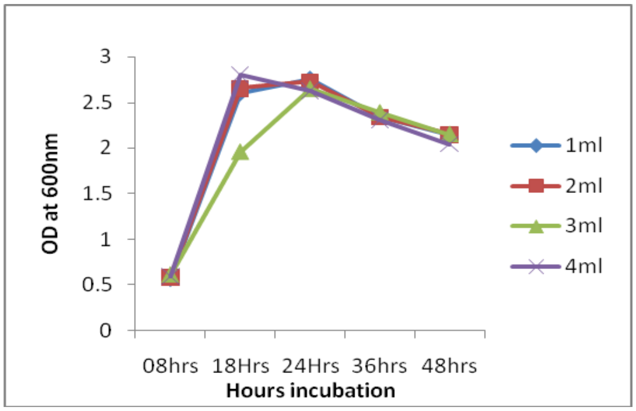
Test	Result
Glucose	Positive
D-ribose	Positive
Lactose	Negative
Sucrose	Negative
Starch	Negative
Mannitol	Positive
D-xylose	Negative
D-fructose	Positive
L-arbinose	Negative
Gelatin liquefaction	Positive
Citrate utilization	Positive
Nitrate reduction	Positive
Catalase	Positive
Oxidase	Positive
Colony Morphology	Round
Color	Creamy white
Margin	Entire
Elevation	Raised
Opacity	Opaque
Gram’s reaction	negative
Shape	Rods
Size	Short
Arrangement	Single
Spore	Non spore
Motility	Motile

Figure.1 *Halomonas Organivorans*

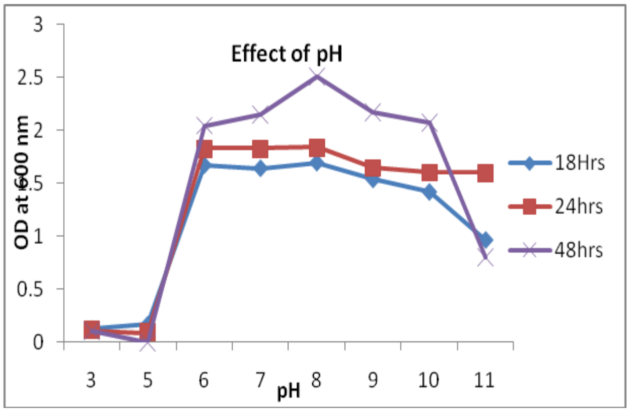
Graph.1 Effect of Nacl on Growth of *Halomonas Organivorans*



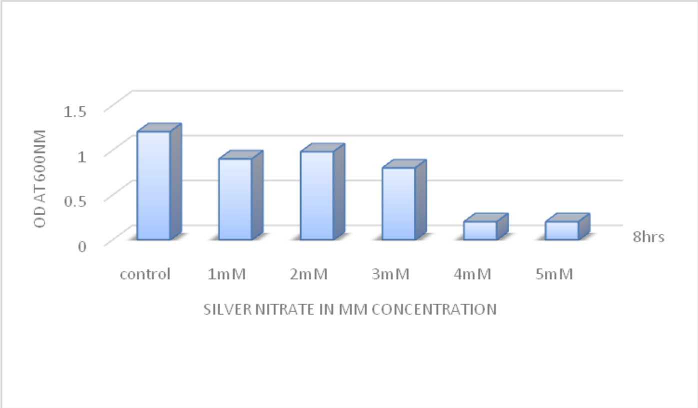
Graph.2 Effect of Inoculum on Growth of *Halomonas Organivorans*



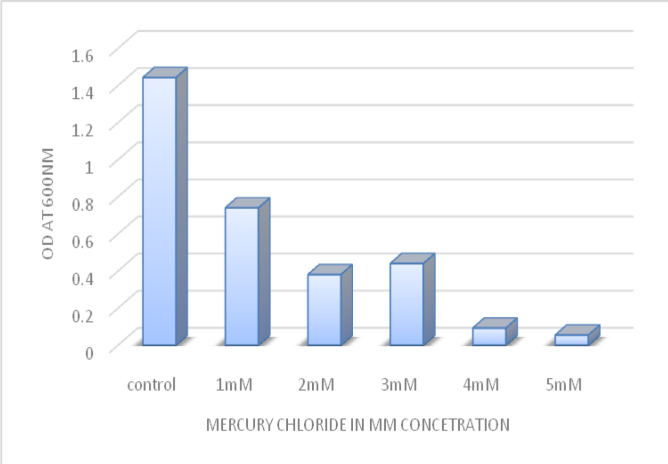
Graph.3 Effect of Ph on Growth of *Halomonas Organivorans*



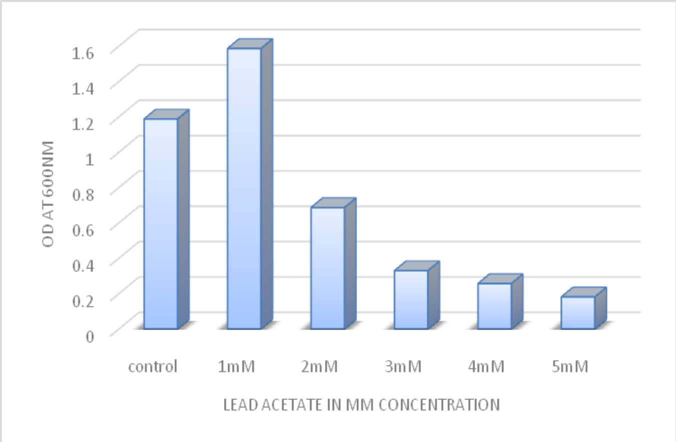
Graph.4 Tolerance of Silver Nitrate on *Halomonas Organivorans*



Graph.5 Tolerance of Mercury Growth of *Halomonas Organivorans*



Graph.6 Tolerance of Lead Acetate Growth of *Halomonas Organivorans*



Halomonas strain found in Maruiy lake, Egypt, is used for the copper bioremediation in addition resistant to other metals and is also resistant to group of antibiotics (Osman et al. 2010). Differences in sensitivity toward antibiotics and other inhibitors have been exploited to differentiate between the activities due to either group. Antibiotics targeting the protein synthesis machinery were also employed in such studies, anisomycin to selectively inhibit archaeal protein synthesis, and erythromycin or chloramphenicol which are known as inhibitors of the bacterial ribosome. The conclusions were similar to those obtained using taurocholate (Oren 1990c; Gasol et al. 2004).

Antibiotics have been used for the purpose of selecting for particular organisms in enrichment or maintenance cultures. Penicillin is most popular, but ampicillin and streptomycin have been used (Torreblanca et al. 1986; Montero et al. 1988; Wais 1988; Kulichevskaya et al. 1992). A combination of penicillin G, rythromycin, and cycloheximide were used to select for archaea at different pHs from subzero hypersaline methane seeps (Niederberger et al. 2010).

The *Halomonas organivorans* strain can tolerate the heavy metals such as Mercury, Led and Ag Ions upto 5 mM concentration and in addition resistant to most of the antibiotics. Mostly the resistance to antibiotics and heavy metals is mediated by its plasmid. Mercury is found to be more lethal for the growth of the strain than the silver and lead.

References

Chang, J.S., J. Hong, O.A. Ogunseitan and H.B. Olson, 1993. Interaction of mercuric ions with the bacterial growth medium and its effects in enzymatic reduction of mercury. Biotechnol. Prog., 9: 526-32.

Collard, J.M., Corbisier, P., Diels, L., Dong Q. &Jeanthon, C. (1994). Plasmids for heavy metal resistance in *Alcaligenseutrophus*CH34: Mechanisms and application. FEMS Microbiology Reviews 14, 405-414

D.,H Nies, “Microbial heavy metal resistance”. Applied microbiology and biotechnology vol. 51, pp.730-750. 1999.

Gadd, G.M. (1992). Microbial control of heavy metal pollution. Microbial control of pollution, *48th Symposium of the Society for General Microbiology, J.C. Fry et al.*, Cambridge University Press, 59-88.

Gasol JM, Casamayor EO, Joint I, Garde K, Gustavson K, Benlloch S, Díez B, Schauer M, Massana R, Pedrós-Alió C (2004) Control of heterotrophic prokaryotic abundance and growth rate in hypersaline planktonic environments. AquatMicrobEcol 34:193–206

G.M. Gadd, “Metals and microorganisms: A problem definition”. FEMS Microbiology Letters 100, pp. 197-204, 1992.

Hiroki, M. (1992). Effects of heavy metal contamination on soil microbial populations. *Soil Science and Plant Nutrition* 38, 141-147.

Kushner, D. J. and Kamekura, M., (1988) Physiology of halophilic eubacteria. In *Halophilic bacteria* (ed. Rodriguez-Valera, F.), CRCPress, Boca Raton, FL, USA,.

Krulwich, T. A. 2000. Alkaliphilic prokaryotes. *In* Dworkin*et al*. (ed.), The prokaryotes: an evolving electronic resource for the microbiological community, 2nd ed. Springer-Verlag, New York, N.Y.

Kulichevskaya IS, Milekhina EI, Borzenkov IA, Zvyagintseva IS, Belyaev SS (1992) Oxidation of petroleum hydrocarbons by extremely halophilic archaebacteria. Microbiology 60:596–601

M.R Bruins, S. Kapil, F.W. Oehme, "Microbial resistance to metals in the environment". Ecotoxicology and Environmental Safety vol. 45, pp.198-207, 2000.

SILVER S. Bacterial resistance to toxic metal ions – a review. Gene 179, 9, 1996.

Stadtman ER (1990) Metal ion-catalyzed oxidation of proteins: biochemical mechanism and biological consequences. Free RadicBiol Med 9: 315–325.

Montero CG, Ventosa A, Rodriguez-Valera F, Ruiz-Berraquero F (1988) Taxonomic study of nonalkaliphilichalococci. J Gen Microbiol 134:725–732

Niederberger TD, Perreault NN, Tille S, Lollar BS, Lacrampe-Couloume G, Anderson D, Greer CW, PollardW, Whyte LG (2010) Microbial characterization of a subzero, hypersaline methane seep in the Canadian High Arctic. ISME J 4:1326–1339

Nies, D.H., (1999). Microbial heavy metal resistance. *Applied Microbiology and Biotechnology* 51, 730-750. environment. Ecotoxicol. Environ.Saf. 45, 198, 2000.

NIES D.H. Efflux-mediated heavy metal resistance in prokaryotes. FEMS Microbiol. Rev. 27, 313, 2003.

Oren A (1990c) The use of protein synthesis inhibitors in the estimation of the contribution of halophilic archaebacteria to bacterial activity in hypersaline environments. FEMS MicrobiolEcol 73:187–192

Osman O, Tanguichi H, Ikeda K, Park P, Tanabe-Hosoi S, Nagata S (2010) Copper-resistant halophilic bacterium isolated from the polluted Maruit Lake, Egypt. J ApplMicrobiol 108:1459–1470

Torreblanca M, Rodriguez-Valera F, JuezG,VentosaA, Kamekura M, KatesM(1986) Classification of non-alkaliphilichalobacteria based on numerical taxonomy and polar lipid composition, and description of *Haloarcula*gen. nov.and*Haloferax*gen. nov.SystApplMicrobiol 8:89–99

Tsai, Y.-P., You, S.-J., Pai, T.-Y. & Chen, K.-W. (2005). Effect of cadmium on composition and diversity of bacterial communities in activated sludges. *International Biodeterioration& Biodegradation* 55: 285-291.

Valls, M. & de Lorenzo, V. (2002). Exploiting the genetic and biochemical capacities of bacteria for the remediation of heavy metal pollution. *FEMS Microbiology Reviews* 26, 327-338.

WaisAC (1988) Recovery of halophilic archaebacteria from natural environments. FEMS MicrobiolEcol 53:211–216.