JOURNAL OF PURE AND APPLIED MICROBIOLOGY, October 2011. Vol. **5**(2), p. 737-742

Biochemical Characterization of Noval Copper Tolereting Microbe from Wet Waste of Copper Industry

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(Received: 15 December 2010; accepted: 18 January 2011)

The present study put forth the isolation and biochemical characterization of one of the bacterial strain- *Klebsella* spp. which was isolate from copper industrial effluent. The isolate show tolerance to various concentrations of heavy metal Cu. The metal uptake property of the isolate can be applied for the heavy metal removal and recovery from industrial effluent.

Key words: Hindustan Copper Smelter, Effluent, Heavy metal, pH, Heavy metal tolerance, Antibiotics, etc.

Today microbes do have importance in the management and sustenance of environment (Vidali 2001). Many reports present the role of microbes in detoxification of contaminated sites, may be due to their interaction with heavy metals or degradation of chemicals like halogenated and organic compounds (Lloyd 2002, Gadd 2000, Iwamoto 2001, Nicolson *c*, Lloyd *et.al.* 2001, Leahy and Colwell 1990). Microbes from different ecosystems were reported to play significant role in maintaining the ecosystem as well as played varied roles to decontaminate the environment (Raychaudhuri *et.al.* 2008, Adarsh *et.al.* 2007, Choudhary *et.al.* 2008).

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The present study based on the characterization of one of the unknown isolate from a contaminated site of Khetri at Jhunjhunu district of Rajasthan where effluent of Hindustan Copper Smelter increased copper concentration beyond bioavailability level. The basic objective was to screen out the potential of this unknown microbe and characterize it to exploit its properties in heavy metal accumulation for environmental pollution monitoring.

MATERIAL AND METHODS

Collection of effluent

Effluent of copper industry was collected from Hindustan Copper Smelter, Khetri at its first discharge point in narrow mouth bottles and brought to the laboratory and was analyzed for various physico-chemical parameters. Copper concentration in the effluent was determined by AAS (Atomic Absorption Spectrophotometer).

Physico -chemical Characterization

Physico- chemical analysis is the prime consideration to assess the quality of effluents for its best usage and to know the pollutional strength and its effect on the ecological conditions (James

and Evanson1979). Industrial waste is also analyzed for various parameters to decide upon what physical, chemical or biological treatment should be given to make them suitable for discharge either in land for irrigation or in other water bodies.

Isolation of pure strain

Luria broth medium (Casein enzyme hydrosylate-1%, Yeast extract-0.5% and NaCl-0.5%) was used as a selective medium for isolation of metal accumulating microbe, the inoculum being the effluent which was collected from copper industry. From a mixed population of microbe was isolated by speared plate technique and pure isolate was obtain by repeated streak plate method on Luria agar medium (Casein enzyme hydrosylate-1%, Yeast extract-0.5%, NaCl- 0.5% and Agar1.5%).

Characterization of bacteria

For the identification of the unknown isolate various criterion was adopted to determine the shape, structure, optimum growth condition and chemical reactions on different chemically defined media. These identifications were based on Bergey's manual of bacteriology.

Morphological Characterization

Morphological characterization of the isolate was based on the light microscopy. Other than the simple and Gram staining, the detailed characterization was conducted through various other staining procedures for the detection of capsule and acid-fastness. A light microscopy was conducted as per standard operational procedures (SOP) at 40 X and 100 X magnifications on Olmpus microscope.

Physiological Characterization

Physiological characterization includes determination of optimum pH, temperature, growth profiling under enriched copper concentration as well as sensitivity for three different antibiotics to obtain optimum growth conditions and tolerance capability of microbe against the heavy metal-Cu.

pH Profiling

pH is a limiting factor, which governs bacterial growth. To determine the optimum pH, LB medium was adjusted to different pH ranging from 5-10 and were inoculated with 1% overnight grown inoculum. The growth was measured in term of O.D. at 660 nm in Systronics UV-Vis Spectrophotometer.

Temperature Profiling

For the determination of optimum temperature 1% inoculum was seeded into LB medium and overnight incubation was done at different temperatures such as 5,10, 15, 20, 25, 30, 35 and 40 ºC. The growth was measured in term of O.D. at 660 nm in Systronics UV-Vis Spectrophotometer.

Antibiotic Sensitivity

Antibiotic sensitivity was checked against three different antibiotics namely *Stereptomyces*, *tetracycline* and *Kenamycine.*

Metal Tolerance

The tolerance capability of isolate was checked for various concentrations of copper. For this purpose 100mM stock of $CuSo_4$ was prepared and various dilutions i.e. 20, 40, 60, 80 to 100mM were made and LB medium meant for the growth of isolate was supplemented with these various concentrations of metal copper.

Biochemical Characterization

Biochemical characterization was done to assess the presence of various exonucleases and endonucleases enzymes namely, gelatinase, hydrolysase, catalase, urease, oxidase, etc. All these tests were performed on chemically defined media. On the presence and absence of these enzymes, identification of the unknown isolate was done.

RESULTS

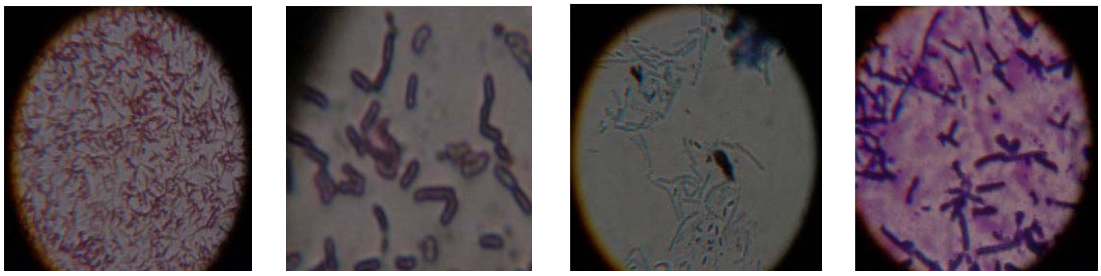
Physico-chemical characterization of effluent

Various physical and chemical parameters such as pH, acidity, alkalinity, sulphate, T.D.S.,

Table 1. Physico chemical assessment of Hindustan Copper Smelter effluent

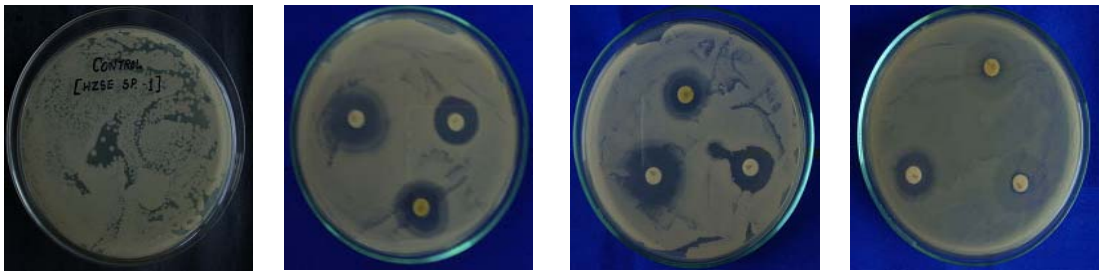
S.No	Physical/ Chemical characters	Result
1.	Temperature	23°C
2.	Colour	Milky
3.	T.D.S	0.0593
4.	pH	7.4
5.	Acidity	Nil
6.	Alkalinity	Nil
7.	Sulphate	0.0593
8.	Copper	20.64

Note:- Result of Sulphate ,TDS and Cu in ppm



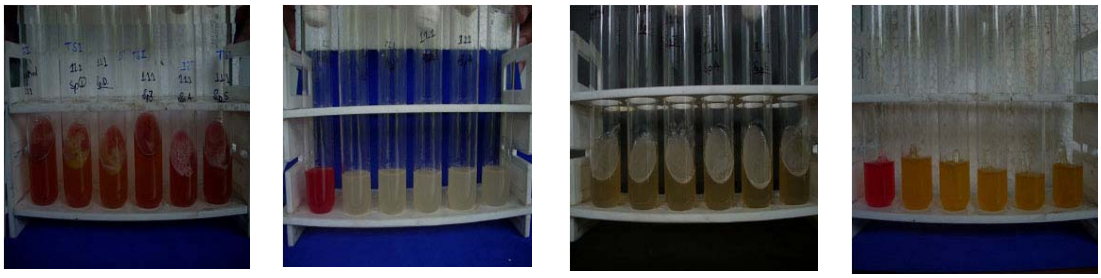
A. Gram positive isolate | B. Gram positive isolate [100X] | D. Non capsulated isolate | D. Non acid fast isolate

Plate 1. Photo



A. Control | B. RP 3 | C. RP 6 | D. RP 7

Plate 2. Antibiotic sensitivity of the isolate



A. Triple sugar ion positive | B. MR positive | C. Sim negative | D. Sucrose fermenting isolate

Plate 3.

and the concentration of copper was determine (Table 1). It was observed that Cu concentration was above bioavailability level i.e. 20.06 ppm. This high concentration of copper has adverse effect on environment. This concentration of Cu is also leached out with waste so, the mechanism of metal accumulation by microbes will help to recover the metal from waste.

Morphological characterization of the isolate

The preliminary characterization was based on morphological and biochemical properties of the isolate. The isolate was rod shaped as demonstrated by light microscopy. As depicted from staining results presented in Table 2, the isolate was found to be Gram's negative in nature. Other structural features like absence of capsule and it was not acid fast microbe. These structural features were clearly put forth by the staining procedures.

Physiological Characterization

The physiological properties of the isolate were concluded in terms of pH,

Table 2. Morphological characterization based on various staining procedures.

S.No	Tests	EF-I	EF-II	EF-IV	EF-V	EF-VI
1.	Simple Stain	Rod	Rod	Rod	Rod	Rod
2.	Gram's Stain	- ve	-ve	- ve	- ve	- ve
3.	Capsule Stain	- ve	-ve	- ve	- ve	- ve
4.	Acid-fast Stain	- ve	-ve	- ve	- ve	- ve

Table 3. Growth of isolate in various pH range.

S.No.	Isolate	pH5.0	pH6.0	pH7.0	pH8.0	pH9.0	pH10.0
1.	EF-I	0.052	0.488	0.488	0.409	0.137	0.355
2.	EF-II	0.030	0.395	0.563	0.311	0.206	0.264
3.	EF-IV	0.065	0.347	0.366	0.076	0.132	0.401
4.	EF-V	0.171	0.045	0.190	0.780	0.010	0.264
5.	EF-VI	0.035	0.061	0.990	0.061	0.060	0.253

Table 4. Growth of the isolate in various temperature

S. No.	Isolate	5°C	10°C	15°C	20°C	25°C	30°C	35°C	40°C
1.	EF-I	0.00	0.010	0.310	0.330	0.450	0.580	0.599	0.293
2.	EF-II	0.00	0.050	0.630	0.660	0.710	0.810	0.880	0.610
3.	EF-IV	0.00	0.031	0.130	0.145	0.390	0.480	0.630	0.360
4.	EF-V	0.00	0.078	0.079	0.170	0.042	0.178	0.248	0.136
5.	EF-VI	0.00	0.204	0.036	0.076	0.137	0.147	0.204	0.115

Table 5. Antibiotic sensitivity of isolate

S.No.	Isolate	*Streptomyces* (cm)	*Kenamycine* (cm)	*Tetracycline* (cm)
1.	EF-I	2.35	1.60	1.20
2.	EF-II	2.10	1.75	2.30
3.	EF-IV	2.30	1.80	1.35
4.	EF-V	2.10	1.95	2.35
5.	EF-VI	2.90	2.55	2.85

Table 6. Metal tolerance capacity of isolate in various enriched copper concentrations (LB + $CuSO_4$)

S.No.	Isolate	20mM	40mM	60mM	80mM	100mM
1.	EF-I	0.357	0.244	0.136	0.131	0.063
2.	EF-II	1.745	0.904	0.259	0.190	0.014
3.	EF-IV	1.272	1.272	0.821	0.760	0.544
4.	EF-V	0.248	0.076	0.076	0.042	0.017
5.	EF-VI	0.204	0.147	0.137	0.076	0.036

temperature, antibiotic sensitivity and tolerance against heavy metal- copper. These physiological conditions are reported as the functions in a living organism.

pH profiling

The isolate was found to grow within a pH range of 5-10 with optimum growth at pH 7.0 (Table 3).

Temperature profiling

The temperature range found suitable for growth was between 5 -40 ºC. The growth was measured in term of O.D. At 660 nm in Systronics UV-Vis Spectrophotometer but the optimum temperature being at 35 ºC (Table 4).

Antibiotic sensitivity

The isolate showed antibiotic sensitivity against all three antibiotics but it showed maximum sensitivity against *Streptomyces* (Table 5).

Metal tolerance

The isolate was found to demonstrate tolerance for the various concentrations of metal copper were shown in table 6. The minimum tolerance was for 100mM concentration by all five samples of the isolates.

Biochemical Characterization

A detail biochemical characterization was done on various chemically defined media to determine the presence of various exo and endonucleases enzymes (Table 7). The isolate was found to be citrate, catalase, gelatinase negative and urease positive and had carbohydrate fermentation ability for various sugars i.e. sucrose, dextrose and mannitol, except lactose.

DISCUSSION

The presence of various exonuclease and endonuclease enzyme indicated the possible defense mechanism in the isolate which participate in existence and adaptability of the organism against heavy metal. Similarly, Adarsh *et.al.* (2007) studied the metal microbe interaction of three bacterial isolates from East Culcutta Wetland.

The pH and temperature profile of an isolate would indicate the adaptability of the isolate to different habitats. These factors have an effect on survivability since they have roles in enzymatic function as well as overall metabolic efficiency. The isolate being able to withstand wide range of pH and temperature was capable of surviving in adverse conditions.

The metal tolerance data would definitely facilitate their application in remediation of heavy metals. Gadd (2000) studied the potential of microbial mechanism of metal mobilization and immobilization. Iwamoto (2001) studied bioremedial practices and perspective. These metals are commonly found in the mine effluents, battery industries, tanneries, etc. These tailing could be removed by application of this isolate. The data indicates the potential for the removal of these toxic metals from the site and moreover certain processes can be followed for the recovery of economically important metals from the microbes. Chowdhury *et.al.* (2008) reported metal accumulator microbes from wetland of East Culcutta and Kennedy *et.al.* (2008) discovered some novel enzymes from this microbes with the help of biotechnological applications.

CONCLUSION

The preliminary characterization of the isolate regarding its morphological, biochemical and physiological characters showed that the isolate was *Klebsella spp.* The strain was further confirmed on specific medium *i.e.* on *Klebsella* agar medium. The property of the isolate for the heavy metal tolerance would explain the role of this group of microorganism in the application for leaching and removal of the toxic metal which dumped into ecosystem. Thus the future prospect lies in the application of this isolate for purposes like heavy metal remediation and commercial production.

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