Available online on 15.06.2019 at http://jddtonline.info

Journal of Drug Delivery and Therapeutics

Open Access to Pharmaceutical and Medical Research

Open Access Research Article

Copper sulfate-resistant bacteria isolated from Blue Soil Hills, Sagada, Mountain Province, Philippines

Reyes Alvin T.*, Estes Jojie G., Reyes Alfred T., Bermudez Jay L., Atayde Jimbo D., Aguilar John Paul T., Baltazar Luigi S., Calapardo Michael E. and Vallada Roval L.

College of Fisheries-Freshwater Aquaculture Center, Central Luzon State University, Science City of Muñoz, Nueva Ecija

ABSTRACT

The objective of this study was to isolate copper sulfate-resistant bacteria in Blue Soil Hills, Sagada, Mountain, Province, Philippines. Three distinct bacterial colonies were isolated from the soil sample. The isolates were different from one another based upon colonial characteristics and growth patterns in solid and liquid medium. All three bacteria were gram-positive and were able to grow in medium supplemented with various concentrations of copper sulfate. More luxurious growth was observed in medium with highest supplementation (333.33 p pm). The isolated bacteria could be potential bioremediation agents in soil and water heavily contaminated with copper.

Keywords: Copper sulfate, bioremediation, Blue Soil Hills, Sagada, Mountain Province

Article Info: Received 05 May 2019; Review Completed 06 June 2019; Accepted 09 June 2019; Available online 15 June 2019

Cite this article as:

Reyes AT, Estes JG, Reyes AT, Bermudez JL, Atayde JD, Aguilar John Paul T, Baltazar LS, Calapardo ME, Vallada RL, Copper sulfate-resistant bacteria isolated from Blue Soil Hills, Sagada, Mountain Province, Philippines, Journal of Drug Delivery and Therapeutics. 2019; 9(3-s):478-481 http://dx.doi.org/10.22270/jddt.v9i3-s.2881

***Address for Correspondence:**

Reyes Alvin T., College of Fisheries-Freshwater Aquaculture Center, Central Luzon State University, Science City of Muñoz, Nueva Ecija

INTRODUCTION

Blue Soil Hills, also known as Kaman-utek Hills is one of the prime tourist destinations in Sagada, Mountain Province, Philippines because of the bluish-green coloration of the soil. The colors are deeper when the soil is moistened or after raining. According to the local tourism office, the bluish-green coloration of the soil is due to high copper sulfate content.

It is well known that the most important uses of metals and metalloids in various industries have been as biocides and antimicrobials. Copper (Cu) along with mercury (Hg), silver (Ag), arsenic (As) and antimony (Sb) are the most commonly used metals or metalloids. Copper is involved in aerobic respiration specifically in electron transport chain[1]. This metal is highly toxic to prokaryotes and eukaryotes at a high dose[2]. Copper compounds in agriculture are used as antimicrobial, algicidal, pesticidal and antifungal agents[3]. According to Grass et al. (2011), inorganic and organic copper compounds have been used as treatment for a number of human diseases such as syphilis, tuberculosis and anemia[4]. Most recently, the use of copper antimicrobial solid surfaces in hospitals have resulted to reduce microbial contamination and transmission of hospital-acquired infections[5,6,7]. The rapid killing of bacteria on solid copper surfaces is caused by membrane rupture, coupled with generation of Reactive Oxygen Species (ROS) leading to further cellular destruction, including degradation of plasmid and chromosomal DNA[4].

The objective of this study was to isolate copper sulfate-resistant bacteria in Blue Soil Hills, Sagada, Mountain Province, Philippines.

MATERIALS AND METHODS

Collection of Soil Sample

Composite soil sample was collected in Blue Soil Hills, Sagada, Mountain Province, Philippines. About 10 g of the collected soil sample was transferred in Erlenmeyer flask containing 150 mL of Trypticase Soy Broth (TSB). The flask was incubated in room temperature for 48 hours to allow the luxurious growth of bacteria.

Isolation of Bacteria

Two series of 10-fold dilutions (10^{-5} and 10^{-6}) of the TSB-soil suspension was made in sterile distilled water. One hundred microliters (100 µl) of the diluted suspension was spread into Trypticase Soy Agar (TSA) plates. The plates were incubated at room temperature for 24 hours.

Cultural Characterization of Bacterial Colonies

Bacterial colonies grown in TSA plates were differentiated based on their appearances on the medium. Colony characters such as shape, margin, elevation and texture were

considered. Colonies that exhibit different cultural characters were purified in TSA plates. Purified isolates were grown in TSA and TSB for the identification of growth patterns.

Gram Staining

The purified isolate was streaked on TSA plate and incubated at 37 ˚C for 18 to 24 hours. A smear was prepared by mixing a small amount of growth with a drop of distilled water. The smear was air-dried and fixed by heat. The glass slide was labeled properly. The dried smear was stained with crystal violet for 1 minute and was rinsed thoroughly with tap water. Afterwards, the smear was covered with Gram's iodine for 1 to 2 minutes and was washed with tap water. The smear was decolorized by dripping 95% ethanol and was washed immediately. Then, the smear was counterstained with safranin for 45 seconds and was washed by tap water. The slide was examined under microscope. Gram-positive bacterium should be colored blue while Gram-negative bacterium should be colored red. Cell shape was also observed under the microscope.

Growth in TSA Supplemented with Various Concentrations of Copper Sulfate

TSA plates were supplemented with various concentrations of copper sulfate (Table 1). The purified isolates were streaked in the supplemented TSA plates. The plates were incubated at room temperature for 48 hours and bacterial growth was observed.

Table 1: Preparation of the TSA-supplemented plates and its corresponding copper sulfate concentration.

Volume of TSA (mL)	Weight of Copper Sulfate Added (g)	Working Concentration of Copper Sulfate (ppm)
15	0.1	6.67
15	0.2	13.33
15	0.3	20.00
15	0.4	26.67
15	0.5	33.33
15	0.6	40.00
15	0.7	46.67
15	0.8	53.33
15	0.9	60.00
15	1	66.67
15	2	133.33
15	3	200.00
15	4	266.67
15	5	333.33

RESULTS AND DISCUSSION

Cultural Characterization of Bacterial Colonies

Three distinct bacterial colonies were isolated from Blue Soil Hills, Sagada, Mountain Province, Philippines. The isolates were different from one another based upon colonial characteristics and growth patterns in solid and liquid medium (Table 2).

Table 2: Colonial characteristics and growth patterns of the three bacterial isolates from Blue Soil Hills, Sagada, Mountain Province, Philippines.

Isolates	Shape	Margin	Elevation	Texture	Growth Pattern	
					Agar	Broth
Isolate A	Round w/ scalloped margin	Undulate	Convex	Mucoid	Filiform	Membranous
Isolate B	Irregular and spreading	Curled	Umbonate	Dull and granular	Filiform	Ring
Isolate C	Round	Erose	Flat	Smooth	Effuse	Membranous

Gram-staining and Shape of Cells

All three bacteria were gram-positive wherein cells were stained blue. Cell shape of isolates A and C was spherical while isolate B had rod cells (Figure 1).

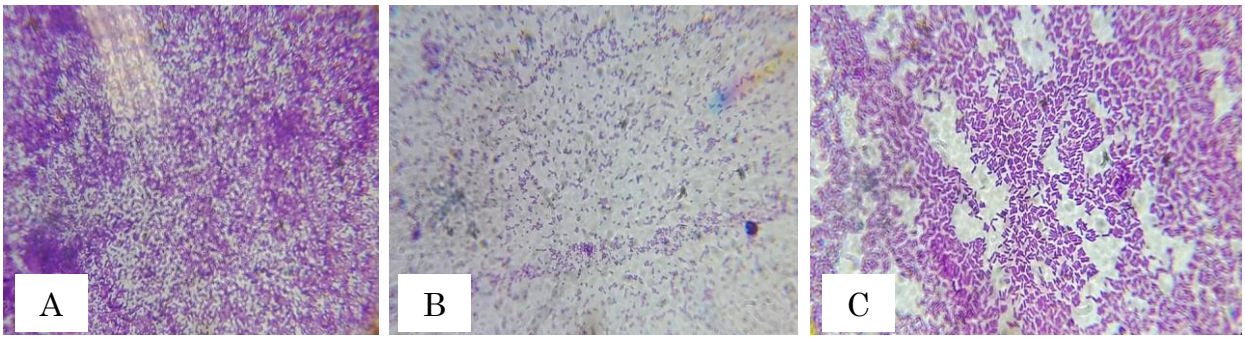

Figure 1: Gram-reaction of the three bacterial isolates (A) Isolate A; (B) Isolate B; (C) Isolate C.

Growth in TSA Supplemented with Various Concentrations of Copper Sulfate

All of the isolated bacteria were able to grow in medium supplemented with various concentrations of copper sulfate (Table 3). More luxurious bacterial growth was observed in TSA with highest supplementation (333.33 ppm) (Figure 2)

Table 3: Growth observation in TSA plates containing various concentrations of copper sulfate.

Volume of TSA (mL)	Weight of Copper Sulfate Added (g)	Working Concentration of Copper Sulfate (ppm)	Growth Observation
15	0.1	6.67	+
15	0.2	13.33	+
15	0.3	20.00	+
15	0.4	26.67	+
15	0.5	33.33	+
15	0.6	40.00	+
15	0.7	46.67	+
15	0.8	53.33	+
15	0.9	60.00	+
15	1	66.67	+
15	2	133.33	+
15	3	200.00	+
15	4	266.67	+
15	5	333.33	+

Note: + means presence of bacterial growth

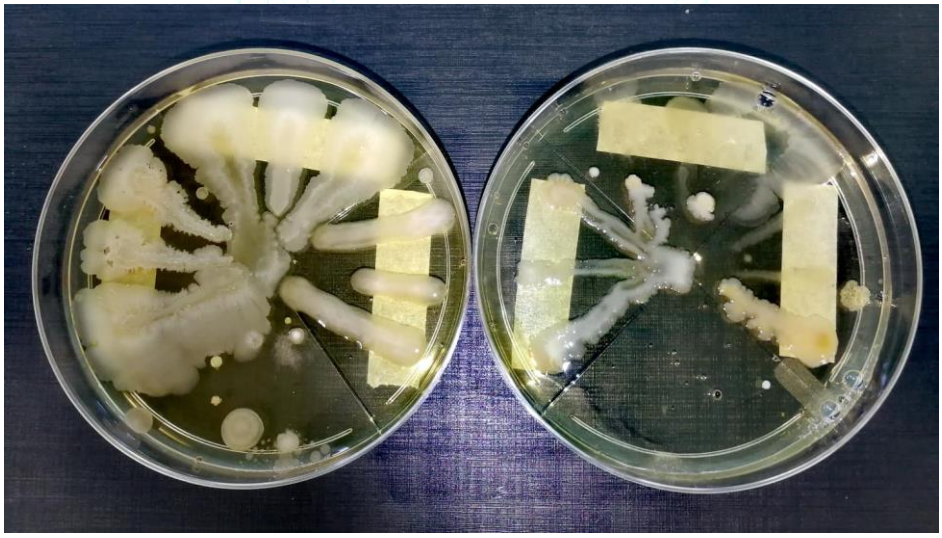

Figure 2: Isolates grown in TSA supplemented with 5 g (A) and 0.1 g (B) copper sulfate.

Bacteria possess copper homeostasis and copper resistance mechanisms because this essential metal is highly toxic and involved in host defense. In *Escherichia coli*, the cue and cus systems have components that could modify the charge in ionic copper and efflux it[8]. In some *E. coli* strains, a plasmid-borne copper resistance system, pco, confers copper resistance[9,10,11]. Moreover, the AcrD and MdtABC in *E. coli* could efflux copper and other antimicrobials when NlpE, an outer membrane lipoprotein is overexpressed[12]. In *Salmonella* spp., enterobactin and TolC are involved in copper detoxification[13]. Gram-positive bacterium such as *Enterococcus hirae* imports copper into the cytoplasm via CopA, an ATPase and binding of excess cytoplasmic copper by a copper chaperone (CopZ), which donates it to either a copper export ATPase (CopB) or CopY, which is a copper-responsive repressor of gene expression for the *E. hirae* cop operon[14].

Mining activities, extensive use of copper in industries and crop production are major sources of copper pollution of soils and water. Copper pollutions pose a serious health threat to human and in the biodiversity of the ecosystems[15]. The development of methods to remove toxic heavy metals such as copper from water and soils is currently an area of intensive research[16,17,18]. The isolated copper sulfate-resistant bacteria from Blue Soil Hills, Sagada, Mountain Province, Philippines could be potential bioremediation agents in soil and water heavily contaminated with copper. Biological removal of pollutants is attractive because it is considered cost-effective and eco-friendly[18,19,20].

CONCLUSION

This study was able to isolate three bacteria that could withstand copper sulfate concentration as high as 333.33 ppm. The isolated copper sulfate-resistant bacteria from Blue Soil Hills, Sagada, Mountain Province, Philippines could be potential bioremediation agents in soil and water heavily contaminated with copper.

REFERENCES

1. Solioz M, Abicht, H K, Mermod M, Mancini S, Response of Gram-positive bacteria to copper stress, J. Biol. Inorg. Chem., 2010; 15, 3-14.
2. Gaetke LM, Chow CK, Copper toxicity, oxidative stress, and antioxidant nutrients, Toxicology, 2003; 189, 147-163.
3. Russell PE, A century of fungicide evolution, J. Agric. Sci., 2005; 143, 11-25.
4. Grass G, Rensing C, Solioz M, Metallic copper as an antimicrobial surface. Appl. Environ. Microbiol., 2011; 77, 1541-1547.
5. Casey AL, Adams D, Karpanen TJ, Lambert PA, Cookson BD, Nightingale P, Miruszenko L, Shillam R, Christian P, Elliott TSJ, Role of copper in reducing hospital environment contamination, J. Hosp. Infect., 2010; 74, 72-77.
6. Marais F, Mehtar S, Chalkley L, Antimicrobial efficacy of copper touch surfaces in reducing environmental bioburden in a South African community healthcare facility, J. Hosp. Infect., 2010; 74, 80-82.
7. Mikolay, A, Huggett S, Tikana L, Grass G, Braun J, Nies DH, Survival of bacteria on metallic copper surfaces in a hospital trial, Appl. Microbiol. Biotechnol., 2010; 87, 1875-1879.
8. Munson GP, Lam DL, Outten FW, O'Halloran TV, Identification of a copper-responsive two-component system on the chromosome of *Escherichia coli* K-12, J. Bacteriol., 2010; 182, 5864–5871.
9. Tetaz TJ, Luke RK J, Plasmid-controlled resistance to copper in *Escherichia coli*, J. Bacteriol., 1983: 154, 1263-1268.
10. Williams JR, Morgan AG, Rouch DA, Brown NL, Lee BTO, Copper-resistant enteric bacteria from United Kingdom and Australian piggeries, Appl. Environ. Microbiol., 1993; 59, 2531-2537.
11. Brown NL, Barrett SR, Camakaris J, Lee BT, Rouch DA, Molecular genetics and transport analysis of the copper resistance determinant (pco) from *Escherichia coli* plasmid pRJ1004, Mol. Microbiol., 1995; 17, 1153-1166.
12. Nishino K, Yamasaki S, Hayashi-Nishino M, Yamaguchi A, Effect of NlpE overproduction on multidrug resistance in *Escherichia coli*, Antimicrob. Agents. Chemother., 2010; 54, 2239-2243.
13. Pontel, L. B., Scampoli, N. L., Porwollik, S., Checa, S. K., McClelland, M. & Soncini, F. C. (2014). Identification of a Salmonella ancillary copper detoxification mechanism by a comparative analysis of the genome-wide transcriptional response to copper and zinc excess. Microbiology 160, 1659-1669.
14. Solioz M, Abicht HK, Mermod M, Mancini S, Response of Gram-positive bacteria to copper stress, J. Biol. Inorg. Chem., 2010; 15, 3-14.
15. Atlas RM, Bartha R, Microbial ecology: Fundamentals and applications. 4th edn. Benjamin/Cummings Science Publishing, USA, 1997; 656 pp.
16. Andreazza R, Pieniz S, Wolf L, Lee M, Camargo FAO, Okeke BC, Characterization of copper biosorption and bioreduction by a highly copper resistant bacterium isolated from copper-contaminated vineyard soil, Sci. Total Environ., 2010; 408:7:1501-1507.
17. Chen XC, Wang YP, Lin Q, Shi JY, Wu WX, Chen YX, Biosorption of copper (II) and zinc (II) from aqueous solution by *Pseudomonas putida* CZ1, *Colloid. Surface B*., 2005; 46:101-107.
18. Chen H, Yang B, Chen X, Identification and characterization of four strains of *Acidithiobacillus ferrooxidans* isolated from different sites in China, Microbiol. Res., 2007. doi:10.1016/j.micres.2007.09.002.
19. Camargo FAO, Bento FM, Jacques RJS, Roesch LFW, Frankenberger WT, Uso de microrganismos para a remediação de metais, *Tópicos Especiais em Ciência do Solo*, 2007; 5:467-496.
20. Chen XC, Wu WX, Shi JY, Xu XH, Wang H, Chen YX, Adsorption of copper and zinc on *Pseudomonas putida* CZ1: Particle concentration effect and adsorption reversibility, Colloid. Surface B., 2007; 54:46-52.