

Review Article

The Medicinal use Bioleaching of Realgar and its Antitumor Effect *In Vivo* and *In Vitro*

Peng Chen¹, Ruixiang Xu¹, Zhengrong Wu¹, Yan Wei², Wenbin Zhao¹ and Hongyu Li^{1,2*}

¹School of Pharmacy, Lanzhou University, PR China

²Institute of Microbiology, School of Life Sciences, Lanzhou University, PR China

*Corresponding author: Hongyu Li, School of Pharmacy, Institute of Microbiology, Lanzhou University, Gansu Key Laboratory of Biomonitoring and Bioremediation for Environmental Pollution, School of Life Sciences, Tianshui Road No. 222, Lanzhou, 730000, PR China

Received: October 07, 2016; Accepted: November 03, 2016; Published: November 07, 2016

Abstract

Realgar, also called *xionghuang* in China, was an arsenic compound. Recently, previous study has shown that realgar was used to cure some serious blood diseases in clinic such as the Acute Promyelocytic Leukemia (APL) and Chronic Early Young Granulocyte Leukemia (CPL). However, some disadvantages such as high toxicity, low solubility and poor bioavailability limit their application of the potential anticancer agent in clinical. To overcome those obstacles above, *Acidithiobacillus ferrooxidans* (*A.ferrooxidans*) was used to process realgar. After bioleaching, the arsenic concentration in the solution was significant improved. Furthermore, Realgar Bioleaching Solution (RBS) have an obvious antitumor effect *in vivo* and *in vitro*.

Keywords: Realgar; Bioleaching; *A. ferrooxidans*; RBS; Antitumour

The Traditional Technology to Process Realgar

Traditional methods of processing realgar are grinding it in the solution [1]. That approach can make realgar particles at the scale of micron meters. In recent decades, a new instrument named high energy ball, was used to process realgar. the machine can make the realgar powder in nanometer size [2]. There is a research demonstrated that when realgar powder at nanometer size it would be more soluble and higher bioavailable than the raw realgar [3]. Furthermore, when the size of realgar particles smaller than 150nm the anticancer effect to cancer cells will be increased significantly. Thus, processing raw realgar in nano-sized can enhanced the anticancer effect to cancer cells and reduced toxic to normal cells [4]. However, those disadvantages such as high toxic and poor solubility are not resolved as yet. As an arsenic compound realgar still have moderate toxicity. The toxicity would lead to neurotoxicity and made the level of certain amino acid in the cell changed when treat with realgar [5,6].

The New Technology Bioleaching of Realgar

Nowadays, the poor solubility and high toxicity was solved when the bioleaching was utilized to process realgar. This biotechnology is using *Acidithiobacillus ferrooxidans* (*A. ferrooxidans*: a mesophile bacteria) to bioleach realgar. That bacteria is the key factor to overcome the disadvantage above. After bioleaching, the solubility of realgar enhanced importantly which is the reason why the arsenic concentration improved significantly. More important, the biotechnology is environmental and more efficient than the high energy ball. Therefore, using biotechnology to process realgar can resolve the above-mentioned problems.

The distinguishing feature of *A. ferrooxidans*

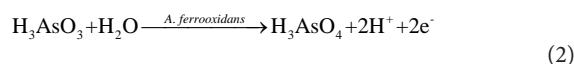
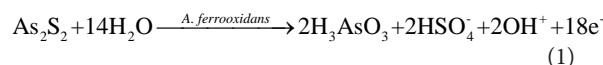
A. ferrooxidans is characterized with tolerance to high concentration of heavy metal ions such as arsenic ions, cupric ions, and nickel ions [7,8]. Another research showed that the InC of DMA^V (Dimethyl arsenate) for *A. ferrooxidans* in 9K medium was 32 mM·L⁻¹ that is why the bacteria can live in the waste water containing arsenic

ions [9]. The bacteria was cultured in 9K medium containing realgar [10,11]. When the bacteria grow to the exponential phase, transferred them into a fresh media containing that concentrations of realgar is thither than before [12]. After the After two months of inoculating under realgar stress the arsenic resistance of *A. ferrooxidans* was enhanced significantly [13].

A. ferrooxidans plays an important role in bioleaching. That is because the bacterium can get the energy by oxidizing ferrous ions and elemental sulfur for it growth [14]. With the growth of the microorganism the rate of realgar resolved increased markedly. Certainly the process of bioleach realgar was related to many factors in medium. Optimum condition of bioleaching realgar by utilizing *A. ferrooxidans* in the medium with pH 1.5, 35°C, 20% v/v inoculum of bacteria is a ferrous concentration of 1.0g/L and a 1.5% w/v pulp density [10]. The result showed that efficiency of bioleaching is efficiently under these conditions.

The mechanism of bioleaching

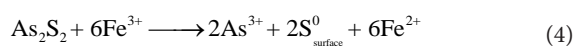
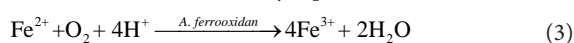
As a traditional Chinese medicine the effective parts of realgar is arsenic ions, whereas, poor solubility is a characteristic of realgar. After bioleaching, the solubility of realgar would increase significantly by the bio-oxidation of *A. ferrooxidans*. The bacteria play an important role in the procedure of bioleaching. In this paper, two aspects of the bacterium design: on the one hand, the direct action of the bacteria can increased the rate of realgar resolved; on the another hand the indirect action of bacteria impact the concentration of arseni by certain inorganic ions such as ferrous ions and ferric ions. The oxidative reactions include the two steps that may have occurred as described in Eqs. (1)and (2) [15,11].



The direct effect of bacteria is *A. ferrooxidans* can growth on the

surface of realgar powder. When use *A. ferrooxidans* to bioprocess realgar particles the surface of mineral powder was modified by the bacterium. That change of the particles has been observed by the SEM and EDS. There are many cracks at the surface of realgar particles compared with the sterile group. Meanwhile, the arsenic concentration improved significantly in 9K medium [16].

The indirect effect of *A. ferrooxidans* works by the family iron ions. Furthermore, a more detailed explanation for the oxidations is the bacteria get the energy by oxidizing ferrous ions and the ferrous ions was oxidized to ferric ions. The procedure was described in Eqs. (3) [17]. Then ferric can catalyze the realgar resolved in the medium. That is why the rate of realgar resolved increased evidently. These reactions can be characterized by Eqs. (3),(4). and (5). [11].



The Antitumor Effect of Realgar Bioleaching Solution (RBS)

The arsenic concentration of RBS is approximately 15 times than the sterile control [18]. Same as to the raw realgar powder arsenic compounds, RBS showed an evident anticancer effect *in vivo* and *in vitro*. More importantly, the effect is more efficiency and safety than the raw realgar [19]. Therefore, RBS will be potential antitumor drugs in the future.

The antitumor effect of RBS in the cancer cells and mice

And the experiment result indicated that the RBS has a marked antitumor effect *in vivo* and *in vitro*. Flow Cytometry (FCM) showed that RBS induced Sarcoma-180 cell (S180 cells) apoptosis. Because the result of FCM has demonstrated that cancer cells in the sub-G1 phase (the sub-G1 phase usually regard as the symbol of apoptosis in the cell cycle) takes up a higher percentage when treated with RBS. And the effect increased in a time- and concentration-dependent manner. Furthermore, RBS has the same effect in the Kunming mice. Two obvious curative phenomenons were found in the body of mice: for one thing, the weight of tumor growth less than the normal group; for another, the life of the mice was extended. More important, the distribution of arsenic in organ and tissue such as lung, liver, and tumour is higher than in another organ or tissue. That demonstrated that RBS has a high selective affinity to tumor in clinical trials [20]. Furthermore, RBS has the same effect for other cancer cell such as the H22 cells in the mice. The apoptosis of the cancer cells lead to the weight of mice is lighter and the growth of tumor is slowly than the normal saline group. And the live of mice is longer than the control group just as the mentioned before [21].

The antitumor effect of RBS in the *C. elegans*

C. elegans a model organism to evaluate the pharmacological activity of anticancer drugs. RBS can down-regulated ras gene in *Caenorhabditis elegans*. Ras pathway is the guarantee for the growth and other living activity of *C. elegans* [22,23]. When use the RBS to treat the worms the mutant phenotype was inhibited obviously. Meanwhile, the RBS has less toxicity in *C. elegans* compared with another arsenic compounds including white arsenic and realgar

[19]. Another study shows that the mechanism of the effect is RBS can increase Reactive Oxygen Species (ROS) in *C. elegans*. Previous research demonstrated that a higher concentration of ROS in organism can inhibit the formation of cancers cells [24]. When treat with RBS to *C. elegans* the levels of intracellular ROS accumulation in experimental group is two times more than the control [25]. All of the result above demonstrated that RBS has an obvious anticancer effect both *in vivo* and *in vitro* and it is a candidate medicine in the treatment of cancer.

Conclusion

Some disadvantages limit the application of realgar. Bioleaching, a new technology to process the agent through using *A. ferrooxidans*. The bacteria play an important role in the procedure of bioprocess. Direct effect was increased the resolved of realgar and the indirect effect improved the arsenic concentration obviously. More importantly, RBS showed an evident antitumor effect by inducing apoptosis *in vivo* and *in vitro*. Meanwhile, the effect is more efficient and less toxic than raw realgar.

Acknowledgment

This work was supported by Gansu Province Science Foundation for Distinguished Young Scholars (Grant No. 1308RJDA014), Technology Program of Gansu Province (Grant No. 1604FKCA110), Longyuan Support Project for Young Creative Talents (Grant No. GANZUTONGZI [2014] no.4), and the Fundamental Research Funds for the Central Universities of China (Grant No. lzujbky-2015-57), Technology Program of Lanzhou City (Grant No. 2015-3-142, Grant No. 2015-3-93, Grant No. 2015-3-97), National Natural Science Foundation of China (81403145; 81560715; 31660026), Sub-Project of National Science and Technology Major Projects for "Major New Drugs Innovation and Development" (2015ZX09501-004-003-008), the Agricultural Biotechnology Research and Application Development Project of Gansu Province (GNSW-2009-01).

References

- Jiang H, Ding JH, Zhang YH, Shi ST, Gao S, Gong HZ, et al. Study on water processing conditions of Realgar Journal of Chinese medicinal materials. 2009; 32: 26-28.
- Tian Y, Wang X, Xi R, Pan W, Jiang S, Li Z, et al. Enhanced antitumor activity of realgar mediated by milling it to nanosize. International journal of nanomedicine. 2014; 9: 745-757.
- Ma Q, Wang C, Li X, Guo H, Meng J, Liu J, et al. Fabrication of water-soluble polymer-encapsulated As₄S₄ to increase oral bioavailability and chemotherapeutic efficacy in AML mice. Scientific reports 6. 2016.
- Deng Y, Xu H, Huang K, Yang X, Xie C, Wu J. Size effects of realgar particles on apoptosis in a human umbilical vein endothelial cell line: ECV-304. Pharmacol Res. 2001; 44: 513-518.
- Huo T, Zhang Y, Li W, Yang H, Jiang H, Sun G. Effect of realgar on extracellular amino acid neurotransmitters in hippocampal CA1 region determined by online microdialysis-dansyl chloride derivatization-high-performance liquid chromatography and fluorescence detection. Biomedical chromatography: BMC. 2014; 28: 1254-1262.
- Wang Y, Chen M, Zhang Y, Huo T, Fang Y, Jiao X, et al. Effects of realgar on GSH synthesis in the mouse hippocampus: involvement of system XAG-, system XC-, MRP-1 and Nrf2. Toxicol Appl Pharmacol. 2016.
- Dave SR, Gupta KH, Tipre DR. Characterization of arsenic resistant and arsenopyrite oxidizing *Acidithiobacillus ferrooxidans* from Hutti gold leachate and effluents. Bioresour Technol. 2008; 99: 7514-7520.

8. Xu Y, Yang M, Yao T, Xiong H. Isolation, identification and arsenic-resistance of *Acidithiobacillus ferrooxidans* HX3 producing schwertmannite. *J Environ Sci (China)*. 2004; 26: 1463-1470.
9. Yan L, Yin H, Zhang S, Duan JG, Li Y, Chen P, Li HY. Organoarsenic resistance and bioremoval of *Acidithiobacillus ferrooxidans*. *Bioresour Technol*. 2010; 101; 6572-6575.
10. Chen P, Yan L, Leng F, Nan W, Yue X, Zheng Y, et al. Bioleaching of realgar by *Acidithiobacillus ferrooxidans* using ferrous iron and elemental sulfur as the sole and mixed energy sources. *Bioresour Technol*. 2011; 102: 3260-3267.
11. Zhang JH, Zhang X, Ni YQ, Yang XJ, Li HY. Bioleaching of arsenic from medicinal realgar by pure and mixed cultures. *Process Biochemistry*. 2007; 42: 1265-1271.
12. Leng FF, Li KY, Zhang XX, Li YQ, Zhu Y, Lu JF, et al. Comparative study of inorganic arsenic resistance of several strains of *Acidithiobacillus thiooxidans* and *Acidithiobacillus ferrooxidans*. *Hydrometallurgy*. 2009; 98: 235-240.
13. Leng FF, Sun SC, Wang YG, Jing YG, Wei Qw, Li HY. Arsenic bioleaching in medical realgar ore and arsenic bearing refractory gold ore by combination of *Acidithiobacillus ferrooxidans* and *Acidithiobacillus*. *Trop J Pharm Res*. 2016; 15: 1031-1038.
14. Li LL, Lv ZS, Zuo ZY, Yang ZH, Yuan XL. Effect of energy source and leaching method on bio-leaching of rock phosphates by *Acidithiobacillus ferrooxidans*. *Hydrometallurgy*. 2016; 164: 238-247.
15. Lázaro, Isabel. Electrochemical Study of Orpiment (As_2S_3) and Realgar (As_4S_6) in Acidic Medium. *ECS J Solid State Sci Technol*. 1997; 144: 4128-4132.
16. Chen P, Yan L, Wang Q, Li Y, Li H. Surface alteration of realgar ($As_{(4)}S_{(4)}$) by *Acidithiobacillus ferrooxidans*. *International microbiology: the official journal of the Spanish Society for Microbiology*. 2012; 15; 9-15.
17. Hansford GS, Vargas T. Chemical and electrochemical basis of bioleaching processes. *Hydrometallurgy*. 2001; 59: 135-145.
18. Zhang JH, Zhang B, Wang XQ, Pang RJ, Li HY. Enhancement of a bioleaching solution for dissolution rate and bioavailability of medicinal realgar, a poorly water-soluble arsenical compound (As_2S_3) by bacteria. *J Biotechnol*. 2008; 136: S499-S499.
19. Liu D, Zhi D, Zhou T, Yu Q, Wan F, Bai Y. Realgar bioleaching solution is a less toxic arsenic agent in suppressing the Ras/MAPK pathway in *Caenorhabditis elegans*. *Environ Toxicol Pharmacol*. 2013; 35: 292-299.
20. Xie QJ, Cao XL, Bai L, Wu ZR, Ma YP, Li HY. Anti-tumor Effects and Apoptosis Induction by Realgar Bioleaching Solution in Sarcoma-180 Cells *in vitro* and Transplanted Tumors in Mice *in vivo*. *Asian Pacific journal of cancer prevention: APJCP*. 2014; 15; 2883-2888.
21. Zhang X, Xie QJ, Wang X, Wang B, Li HY. Biological extraction of realgar by *Acidithiobacillus ferrooxidans* and its *in vitro* and *in vivo* antitumor activities. *Pharm Biol*. 2010; 48: 40-47.
22. Kayne PS, Sternberg PW. Ras pathways in *Caenorhabditis elegans*. *Curr Opin Genet Dev*. 1995; 5: 38-43.
23. Lee MH, Ohmachi M, Arur S, Nayak S, Francis R, Church D, et al. Multiple functions and dynamic activation of MPK-1 extracellular signal-regulated kinase signaling in *Caenorhabditis elegans* germline development. *Genetics*. 2007; 177: 2039-2062.
24. Dolado I, Nebreda AR. AKT and oxidative stress team up to kill cancer cells. *Cancer cell*. 2008; 14: 427-429.
25. Zhi de J, Feng N, Liu DL, Hou, RL, Wang MZ, Ding XX, et al. Realgar bioleaching solution suppress ras excessive activation by increasing ROS in *Caenorhabditis elegans*. *Arch Pharm Res*. 2014; 37: 390-398.