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Review Article

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

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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 bio-technology 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 $mM \cdot L^{-1}$ 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].

$$As_2S_2 + 14H_2O \xrightarrow{A.\ ferrooxidans} 2H_3AsO_3 + 2HSO_4^- + 2OH^+ + 18e^- \quad (1)$$

$$H_3AsO_3 + H_2O \xrightarrow{A.\ ferrooxidans} H_3AsO_4 + 2H^+ + 2e^- \quad (2)$$

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

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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].

$$Fe^{2+} + O_2 + 4H^+ \xrightarrow{A.\ ferrooxidan} 4Fe^{3+} + 2H_2O \quad (3)$$

$$As_2S_2 + 6Fe^{3+} \longrightarrow 2As^{3+} + 2S^0_{surface} + 6Fe^{2+} \quad (4)$$

$$H_3AsO_4 + Fe^{3+} \xrightarrow{A.\ ferrooxidan} FeAsO_4 + 3H^+ \quad (5)$$

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.

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