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Research Progress on the *Actinomyces arthrobacter*

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Abstract

The genus *Arthrobacter* was established in 1947 by Conn & Dimmick. So far, more than 70 recognized species of the genus *Arthrobacter* have been certified. Its special functions have been widely known by researchers, such as, in agricultural, in medical, in industrial, and in environmental areas, etc. What deserves to be mentioned is that some species of genus *Arthrobacter* have showed the function of degrading pesticides, fixing nitrogen, producing beneficial enzyme, treating sewage, and so on. Recently, the applications of the genus *Arthrobacte*r, especially the VBNC (viable but non-culturable) bacteria of this genus in the field of contaminated environment repair attract people's attention and some related research results have also been obtained. The functions that we have known are waiting for us to study about mechanism, deeply. And, we can look forward to discovering more potential functions and applications of this genus in ecological environment. In the meantime, these discoveries must bring more new changes and knowledge.

Keywords

***Arthrobacter* sp., Application, Environment, VBNC, Rpf**

1. Introduction

The genus *Arthrobacter* was established by Conn and Dimmick, which is one of the most common genera in soil [1]. Most species of the genus are gram-positive bacteria, aerobic, having an obvious growth cycle, no spore formation, no fermentation, mainly rest in the soil, sewage, etc. In peptone medium, because of making use of

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glucose and other sugar compounds, some of them can produce a small amount of acids, while, others do not produce acids. Generally, their G + C content of DNA is 59% - 70%; the representative species of the genus is *Arthrobacter globisformis*. Many members of this genus have been isolated from various environments, such as air, oil, fresh water, tobacco leaves, human skin, activated sludge, polluted soil, and so on [2]. With the scientific research progress, it has been shown that functions of this genus can be applied to many fields, which are agriculture, medicine, industry, especially environmental rehabilitation fields, etc. They could fix nitrogen, remove sulfur and phosphorus, be used as biological flocculants, medicaments. Some poisonous contaminants and chemical substances difficult to degrade (such as pesticides, PCBs, heavy metals) also can be degraded by some bacteria belonging to the genus *Arthrobacter* [3]-[6]. In this study, we also introduce some VBNC bacteria isolated from soil, sewages, identified as *Arthrobacter* by adding Rpf (resuscitation promoting factor) that are secreted by *Micrococcus luteus*, and study on some functions in environmental areas. All these have been showed that the genus *Arthrobacter* bacteria are a very active member in the ecological environment, and also become the indispensable part of today's society; these discoveries must bring more new changes for us.

2. Application of the *Actinomyces arthrobacter* in Several Fields

2.1. Application in Agriculture

Existence of phosphorus is widespread in our daily life, like detergent, pesticide, compound fertilizer, etc. And the conventional method of extracting phosphorus is from phosphate rock. Without any doubt, this method makes available phosphate rock reduced. Nowadays, a new approach of combining ABC (animal bone charcoal) with beneficial bacteria not only can enhance sustainability of planting industry by reusing P, but also facilitate the recycling of P from food industrial waste [7]. The *Arthrobacter* bacteria are just important one of these beneficial bacteria.

There is widespread use of pesticides in our environment, such as, atrazine, a kind of herbicides, which has become one serious contaminant due to its hard degradation. Up to now, there are many papers whose research results were related to biodegradation of pesticides. Several papers among them described some *Arthrobacter* spp. could degrade atrazine. For example, Jackie and his partners, not only did they isolate the *Arthrobacter nicotinovorans* HIM from a sandy dune soil in New Zealand, they also identified the atrazine-degradation genes *atzABC* in a plasmid and discovered the genes had obvious homology with the genes of *Pseudomona*s sp. ADP, which also had an effect on degrading atrazine [8]. In addition, another *Arthrobacter* species (DAT1) was isolated from an agricultural soil in Hebei Province, China, by Wang *et al*. It is capable of growing by utilizing atrazine as a sole nitrogen source. These papers elucidate the genus *Arthrobacter* can be applied to deal with stuff, which are difficult to degrade in pesticides [9].

Besides, Jiang *et al*. collected soil samples from Japan's Okinawa prefecture, after composting treatment, separated one *Arthrobacter* sp. strain of HS-G_8 having fixing nitrogen ability with no nitrogen medium. And the normal physiological and biochemical identification showed the strain HS-G_8 was a new species of the genus *Arthrobacter* [10]. Pisarska *et al*. also isolated several endophytic *Arthrobacter* species from leaves of maize [11]. The isolated strains were identified as *Arthrobacter nicotinovorans* and *Arthrobacter nitroguajacolicus*, respectively. Two strains, *Arthrobacter nitroguajacolicus* A18 and A34 having the nitrogenase reductase gene-*nif* H, were determined. In addition, nitrification and denitrification functions of the *Arthrobacter* species have also been reported. From these articles reported, we can conclude some bacteria of the genus are closely related to growth of the plants. In a word, the genus *Arthrobacter* can have huge application prospect in agriculture area and more functions are worth discovering and studying.

2.2. Application in Medicine

XOD/XDHs, xanthine oxidase and dehydrogenase, as we all know, are of great necessity in the innate immune system, cardiovascular diseases and antimicrobial agents. Besides, XOD (xanthine oxidase) is widely used in clinical assay, especially in the determination for liver diseases. Xin *et al*. successfully extracted and purified a XOD from *Arthrobacter* sp. They also determined the optimal inducer, pH and temperature for XOD [12]. Combined with the previous studies, we have realized the DFA III (di-d-fructofuranose 1,2':2,3' dianhydride) has a potential of improving the occurring of osteoporosis and iron deficiency anemia. Several inulin fructotransferase (DFA III-producing) from *Arthrobacter* species also have been discovered. For example, Kazutomo

isolated and purified a kind of inulin fructotransferase from the *Arthrobacter ureafaciens* D13-3 [13]. Besides the above, we can also use the recombinant L-arabinose isomerase from *Arthrobacter* sp. to transfer D-galactose to D-tagatose, which is currently being tested as one kind of drug to anti-diabetic and obesity control.

But, the discoveries of Imirzalioglu *et al*. alarm us that bacteria of genus *Arthrobacter* may bring some diseases to people [14]. In the research, there was a patient whose skins presented an erythema resembling the erythema migrans manifestation of Lyme disease, without a history of tick bites. They collected some skin swabs and soil samples in the area where the patient possibly acquired infection. After a series of experiments, a soil bacterium, *Arthrobacter mysorens* was isolated from the collected skins and soil samples. This results described infections with *Arthrobacter mysorens* might be underestimated, microbiological diagnostic techniques also should be applied in cases of patients with unclear erythema.

Maybe, for some diseases, which are difficult to cure, we can find a breakthrough from the genus *Arthrobacter*. In the meantime, we have to be vigilant, some diseases could be caused by bacteria belonging to genus *Arthrobacter*.

2.3. Application in Industry

Recently, marine microorganism has become a new resource of enzyme preparation. As a novel type of attractive sweetener, DFA III (difructose anhydride III) usually is added to baked foods, beverage, candy, pharmaceutical formulations. Now, the inulin fructotransferase can be used in the mass production of DFA III. Zhao *et al*. isolated *Arthrobacter aurescens* strain SK 8.001 from soil. The strain can produce IF Tase (inulin fructotransferase). The main product of the enzyme was just DFA III [15]. Wang *et al*. isolated 12 strains from marine environment, using the sea water medium. Related experiments gave obvious evidence: surface of shell is best place to screening chitonsanase-producing bacterial strain [16]. The microbe, *Arthrobacter* sp. XWI-1002 was selected for further experiments. XWI-1002 could produce chitosanase in the presence of chitosan.

The sugar industry often uses dextranase, which is of great importance. It can hydrolyze dextran deposits and can be added to toothpaste or other dental products to prevent dental caries, and usually be used to manufacture blood substitutes. But the problem is that the molds which produce dextranase for industry present safety issues, so it is necessary for us to find other new bacteria producing dextranase. A new bacterium named *Arthrobacter oxydans* KQ11 was isolated by Wang *et al*. from the ocean, which can produce dextranase in low temperature, short production time and stability under the alkaline conditions [17]. These mean, as long as the *Arthrobacter oxydans* KQ11 can be put into industry, it will probably bring us enormous economic and social benefits.

Coal and oil are used widely in our modern society, the emissions of sulfur into the atmosphere lead to the increasing of acid rain. And the consumption of adopting the methods of physical and chemical to remove sulfur is too high. On the contrary, the cost of biological desulfurization is cheaper, relatively. According to this, biological method is the better choice with no doubt. Researchers have discovered some bacteria of the genus which can remove sulfur in fossil fuel, and the efficiency is higher than other desulfurization bacteria [18].

Previous studies have shown that some kinds of *Arthrobacter* species, such as *Arthrobacter ureafaciens*, *Arthrobacter nicotianae*, *Arthrobacter oxidans* can produce nicotine dehydrogenase, which could degrade nicotine to improve quality of tobacco slice and smoke of burley tobacco.

Apparently, all of the above expounds a fact, that is, the genus *Arthrobacter* has a broad application prospects in industrial sector.

2.4. Application in Environment

There is a lot of coking wastewater every year in our world, containing CA (carbazole), DBF (dibenzofuran), DBT (dibenzothiophene) and naphthalene etc. These organic compounds possess toxicity and resist to degrade. Shi *et al*. studied degradation ability of *Arthrobacter* sp. W1. The strain can degrade the four kinds of organic matter simultaneously and quickly [19]. PAEs (phthalic acid esters) are a group of refractory organic compounds, which are mainly used to increase plasticity and intensity of the products in manufacturing PVC. Because some of the phthalates and their metabolites are alleged to be carcinogenic, teratogenic and endocrine-disrupting substances, now, PAEs have become ubiquitous environmental pollutant. BBP (n-butyl benzyl phthalate) is just a phthalic acid ester. Yang *et al*. found a BBP degrading bacterial strain—*Arthrobacter* sp. HS-B2 from contaminated sludge in a river of Hubei province, which grew well on BBP as the sole source of carbon and can degrade BBP effectively under certain conditions [20].

Today, dynamite, propellants and smokeless powder are almost made from NG (nitroglycerin). But NG is explosive and flammable. Because of inappropriate handling, NG has existed in soil and groundwater at various locations. This is a problem to be solved by using microbes. Johana *et al*. isolated a strain, *Arthrobacter* sp. JBH1 from contaminated soil [21]. The strain can grow in the medium, which NG is the sole source of carbon and nitrogen. And a series of column experiments showed NG can be completely mineralized by JBH1 combined with a porous media.

For the past few years, biological phosphorus removal has been more and more popular than other means. Among these bacteria, some *Arthrobacter* sp. showed the highest phosphorus accumulating capacity. This means that the genus *Arthrobacter* has great potential prospect in sewage treatment and aquaculture application. The bacteria of this genus could also degrade 4-bromophenol, 4-chlorophenol and some heavy metals, such as, Cr (VI). All these research achievements send us a vital message: the genus *Arthrobacter* is playing an important role in environment [22] [23].

2.5. Application in Other Aspects

Besides the above mentioned applications of the genus *Arthrobacter*, they can also have application in wine making, producing amino acids. The degradation mechanism of *Arthrobacter* sp. HW08 was studied by Yan *et al*., which was demonstrated as a promising way to deal with SW (swainsonine) poisoning [24]. They found one intracellular enzyme of *Arthrobacter* sp. HW08 could degrade SW efficiently. This discovery shows a possible application of HW08, which can protect animals from SW poisoning in the livestock industry. There must be other features and applications waiting for our discovery.

3. Discovering of the *Arthrobacter* in VBNC State and Their Roles in the Environment

The results of the current study indicate cultivable bacteria only accounts for 0.01% - 10% of the total numbers of bacteria in the nature, most of them are belonging to the uncultivated microorganisms, or in the VBNC state [25]. In the past ten years, we have used a new approach, namely using the Rpf protein (resuscitation promoting factor) to promote the VBNC state bacteria to recovery, in which the VBNC bacteria to become culturable. By this way, many of the VBNC state bacteria which sensitive to Rpf were separated from soil environment and some of the wastewater biological treatment system. Those isolates have been published in the following GenBank database and note that "the VBNC state strain was isolated in MPN system by using Rpf": http://www.ncbi.nlm.nih.gov/nuccore/AB847906, and so on), which includes many new species of the genus *Arthrobacter*.

Rpf was discovered by Mukamolova and co-workers, a protein secreted by *Micrococcus luteus*. Early research about Rpf focuses on the medical field, finding it can resuscitate the VBNC state cells of *Micrococcus luteus* and some species of the genus *Mycobacterium*. Little work has been done to explore the potential environmental applications. Ding and his team find Rpf also stimulates the growth of VBNC bacteria in the ecological environment early, including the *Arthrobacter*. On the studies of their environmental functions also achieve some results, as described below [26].

Most recently, we have selected some VBNC bacteria strains from activated sludge, soil, sewage treatment system by using the culture supernatant of *M. luteus* or by cloning the *Rpf* gene and expression in *Escherichia coli*, containing Rpf protein. Among these recovering bacteria, there are some strains belonging to *Arthrobacter* based on their physiological, biochemical characteristics, and a BLAST search on the basis of 16S rRNA gene sequences. Then, we did some researches on their applications in environmental remediation. Results have been achieved as follows.

In general, according to our research, nearly 50 bacteria have been isolated from the effective samples by using the MPN (most probable number) system. Among them, the genus *Arthrobacter* is one of the most important genera that can degrade PCBs [27]. We also isolated a variety of novel *Arthrobacter* strains in VBNC state from urban wastewater treatment system and discovered these strains had a strong biological deodorization, nitrification, denitrification and other functions after renewal cultivation [28]. One *Arthrobacter* sp. strain, LC13^{T}, was selected by using kaolin suspension as active evaluation system, the flocculation efficiency of which is up to 80% [29]. And, we constructed two bioflocculant-producing bacterium M3 (*Arthrobacter*) and M7 (*Chryseobacterium*), using these VBNC state bacteria as screening flocculant producing source. The crude bioflocculant

MAC37 was also obtained by purification and solidification of the flocculant, which M3 and M7 produced. Adhesive wastewater treatment was treated by the multiple microorganisms fermentation [30]. PCBs (polychlorinated biphenyls) contaminated soil had been spread with enrichment culture in the inorganic salt medium with biphenyl as the sole carbon source. Then, we isolated six bacteria belonging to five genera (*Rhodococcus*, *Arthrobacter*, *Chryseobacterium*, *Alcaligenes*, *Achromobacte*) from the treatment group (with Rpf). Recently, some VBNC state bacteria isolated from the pharmaceutical wastewater, a novel actinobacterium *Gordonia jinhuaensis* ZYR 51^T was the first discovered VBNC state *actinomyces* from pharmaceutical wastewater bioreactor using resuscitation promoting factor, the VBNC state *actinomyces* and the resuscitation promoting factor (Rpf), between both of the special relationships become very wonderful [31].

4. Conclusions

There are now a lot of substantial evidences demonstrating that the *Actinomyces arthrobacter* is widely employed in many fields in modern society. Especially VBNC *Arthrobacter* spp. strains, they have strong repairing effects on ecologically environment polluted, provide a new scientific evidence for the vital role that microorganism plays in the field of environmental pollution repair and environmental protection, bring a new approach to dealing with the serious environmental pollution.

With the development of modern biological technology, a variety of molecular biology research methods provide more approaches to knowing more VBNC bacteria. Also, DGGE, FISH, Microarray and other molecular biological analytic technique have been applied in this area. We can find handling methods from these VBNC bacteria to deal with some environmental problems and problems in other fields. Undeniably, there are still many problems waiting for us to solve, such as, gene expression form of the VBNC *Arthrobacter* spp. strains at different stages, mechanism of producing special enzyme what we need, bacteria in aerobic or anaerobic conditions of the fermentation process, synergy of the genus *Arthrobacter* and other genera, etc. On the other hand, so far, the VBNC strains are aroused by Rpf from *Micrococcus luteus*. Next, the focuses of our research are how to find a Rpf-like gene isolated from the *Arthrobacter* sp. strain and if the gene has similar function, perhaps, VBNC state bacteria may be able to unravel many mystery roles of bacteria in the ecological environment.

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