

ANSO Highlight is to share the new ideas, methodologies, datasets and technologies of sustainability research by summarizing the latest progress and achievements of scientific projects funded by ANSO and ANSO partners. Through this publication, we would like to stimulate active collaboration and communication among ANSO members and partners.

Investigating the Transcontinental-scale Distribution of Antibiotic Resistance Along the Belt and Road Countries

Summary

"Investigating the transcontinental-scale distribution of antibiotic resistance along the Belt and Road countries" is a collaborative research project launched by ANSO in January 2020. This project is led by the Institute of Urban Environment, Chinese Academy of Sciences, in collaboration with Pakistan Academy of Sciences (PAS), National Science and Technology Development Agency (NSTDA, Thailand), Nanyang Technological University (NTU), Center for Progress and Development of Iran (CPDI), Slovenian Academy of Sciences and Arts (SASA) and Polish Academy of Sciences (PAN). The three-year project is tackling the increasingly serious problem of environmental antibiotic resistance to further promote sustainable development (SDGs 3) and to improve well-being across the region.

Period: January 1, 2020-December 31, 2022

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➡ P2-P6

Application and Demonstration of New Carbohydrate Agricultural Formulations in the Belt and Road Countries

Summary

"Application and demonstration of new carbohydrate agricultural formulations in the Belt and Road Countries" (ANSO-CAF) is a cooperative research project launched by ANSO in July 2020. The project is led by Dalian Institute of Chemical Physics, Chinese Academy of Sciences, in cooperating with the Institute of Polymer Chemistry and Physics, Academy of Sciences of the Republic of Uzbekistan; Patuakhali Science and Technology University, Bangladesh; Bangabandhu Sheikh Mujibur Rahman Agricultural University, Bangladesh and Koneru Lakshmaiah Education Foundation, India, and others. The project aims to apply the carbohydrate agricultural formulations in Central and South Asia for achieving the Sustainable Development Goals (SDGs 2- Zero Hunger).

Period: January 1, 2020 - December 31, 2022

PI: YIN Heng

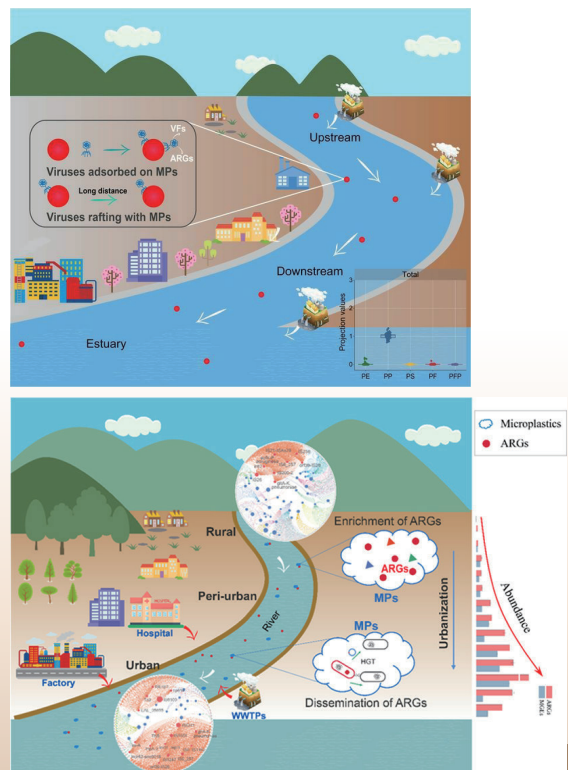
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➡ P7-P12

Investigating the Transcontinental-scale Distribution of Antibiotic Resistance Along the Belt and Road Countries

Objectives

Environmental pollution with antibiotic resistance has become a pressing environmental health problem for human beings, and is a frontier research topic in environmental health. With the construction of the Belt and Road, economic, cultural and personnel exchanges will become more frequent, increasing the dissemination risk of environmental antibiotic resistance. In order to jointly cope with the increasingly serious problem of the environmental dimension of antibiotic resistance and to enhance human health and well-being, this project will employ advanced technologies such as high-throughput quantitative PCR to elucidate the large-scale distribution characteristics of antibiotic resistance genes (ARGs) in the environment along the Belt and Road countries, to further analyze the relationship between ARGs and the potentially influential factors including economics, trade, population, climate and so on, and to train scientific researchers for antibiotic resistance research in countries along the Belt and Road countries, so as to help to halt the emergence and spread of ARGs and to build a community with a shared future for mankind.



Research Content

This project aims to survey countries along the Belt and Road region so as to confirm or determine the sampling sites and quantities, as well as local scientific research personnel expertise on techniques related to environmental resistance research. The collaborating institutions are mainly in charge of sample collection, pre-treatment, DNA extraction, physical and chemical properties analysis, etc. The Institute of Urban Environment, Chinese Academy of Sciences is responsible for sequencing and analyzing antibiotic resistance genes (ARGs) and potential pathogens by high-throughput quantitative PCR, thus comprehensively assessing the transcontinental-scale distribution pattern of environmental ARGs in the Belt and Road countries, and revealing the occurrence and transmission of ARGs in combination with the local cultural, climate, social economy and other parameters.

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Urbanization drives antibiotic resistance on microplastics in Chinese river

By American Chemical Society



Credit: Pixabay/CC0 Public Domain

Microplastic pollution of waterways has become a huge concern, with the tiny pieces of plastic entering food webs and potentially having harmful effects on animals and people. In addition, microplastics can act as breeding grounds for antibiotic-resistant bacteria. Now, researchers reporting in *Environmental Science & Technology* have analyzed antibiotic-resistance genes (ARGs) on five types of microplastics at different locations along the Beilun River in China, finding much higher abundances in urban than rural regions.

In rivers, major sources of microplastics include textile fibers from laundering, water bottle fragments, and films from bags and wrappers. Also prevalent in rivers are antibiotic-resistant bacteria and ARGs, introduced through wastewater discharge and urban or agricultural runoff.

Microplastics can act as a favorable surface for bacteria to colonize and grow into biofilms, where they can spread ARGs among themselves. Li Cui and colleagues wanted to examine the prevalence and diversity of ARGs on microplastics in the Beilun River, which flows from mountainous rural areas through Chinese and Vietnamese cities before entering the Beibu Gulf.

The researchers immersed samples of five kinds of microplastics in the Beilun River at 14 sites with different urbanization levels. After 30 days, they collected the microplastics and used high-throughput quantitative polymerase chain reaction to analyze almost all types of ARGs and the mobile genetic elements that help spread them among bacteria.

The detected ARGs conferred resistance to almost all major classes of antibiotics used in humans and animals. The abundance of these genes and genetic elements increased by about 1,000 times from rural to urban areas. In addition, the diversity of ARGs increased. Of the five types of plastics, polypropylene had the highest abundance of ARGs and the greatest risk of spreading the genes, possibly because of its larger surface area and ability to release dissolved organic matter. These results indicate that urbanization introduces many new ARGs into rivers from sources such as sewage, the researchers say.

➤ Explore further

Earthworms could help reduce antibiotic resistance genes in soil

More information: Ruitong Li et al. Impact of Urbanization on Antibiotic Resistance in Different Microplastics Isolated from a Large-Scale Whole River Analysis, *Environmental Science & Technology* (2021), DOI: 10.1021/acs.est.1c01395

Journal information: *Environmental Science & Technology* (2)

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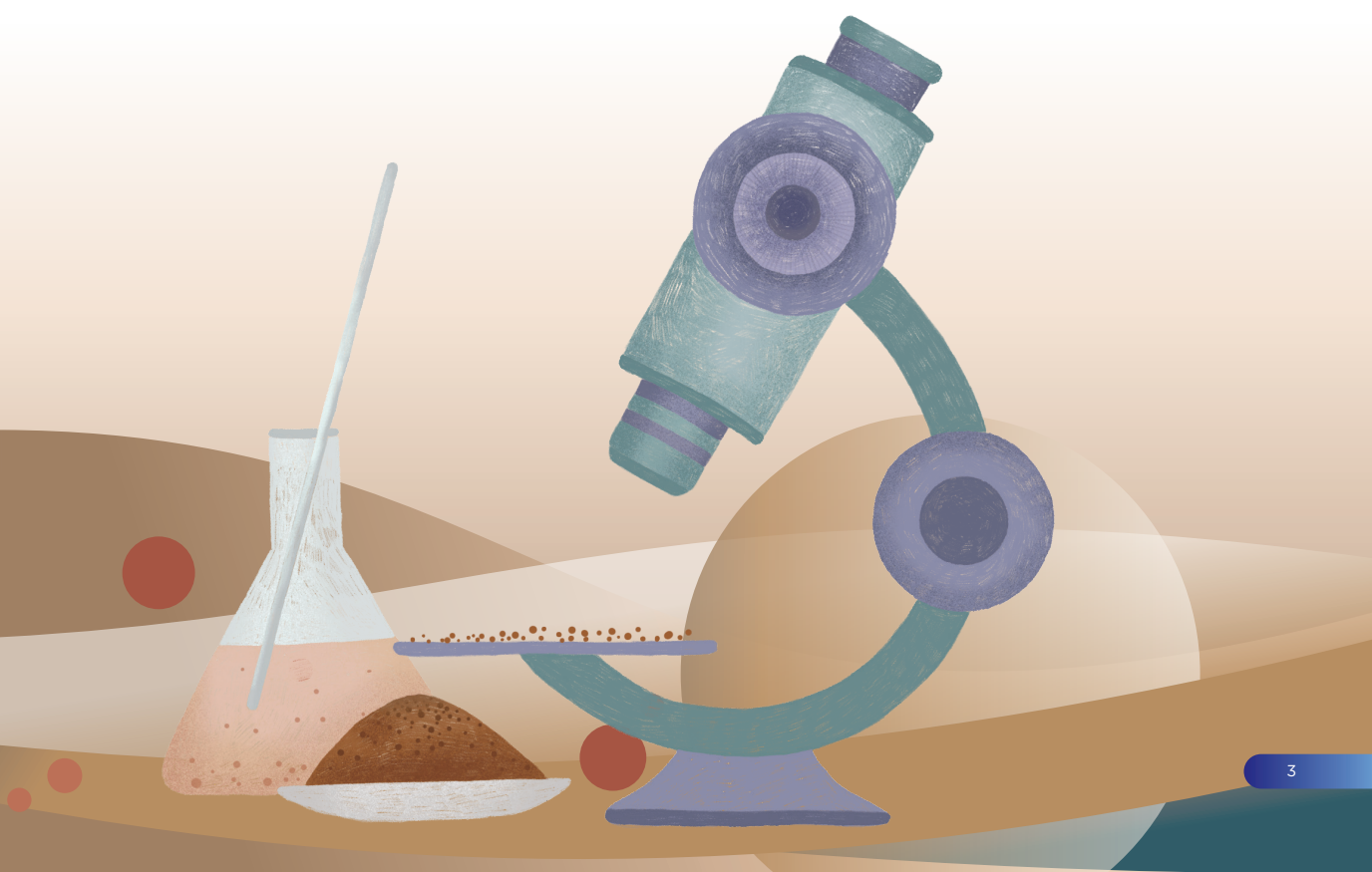
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Main Progress

Research on the antibiotic resistome and viral communities in microplastics at watershed scale

Using high-throughput quantitative polymerase chain reaction (HT-qPCR) and metagenomic analysis, the antibiotic resistome and viral communities in different microplastics (MPs) were investigated, revealing an urbanization-dependent distribution pattern of antibiotic resistance genes (ARGs) and the high diversity and potential environmental risk of viruses in MPs along the whole watershed. This work highlights the necessity of controlling MPs and ARGs pollution in urban areas and monitoring the potential risk associated with both viral transmission and MPs pollution. Our study was reported in at least seven media, including Science Daily, AAAS, News National US.

Progress of international cooperation

There was no evidence from the ARGs and metagenomic analysis data that aquaculture is a major driver of environmental Antimicrobial

Resistance (AMR-similar term as ARGs) in Central Thailand, and AMR was easily controlled by treated urban water pollution.

The culture-independent D₂O-Raman approach detected and linked a large portion of metabolically active indigenous bacteria to multiple antibiotics in their native environments, illustrating the great potential risks of these active cells to the spread antibiotic resistance via food chain.

We performed a comprehensive spatial and seasonal assessment of water quality and antibiotic resistance (AR) conditions in a Malaysian river catchment. The standardized “effect sizes” (Cohen’s D) for AR monitoring were used to improve the comparability of field studies. The combinations of DO and prospective modeling can be used as surrogate markers for predicting antibiotic resistance “Hot Spots” in rivers.



International seminar on "Environmental Antibiotic Resistance"

An international seminar on "Environmental Antibiotic Resistance" was co-organized with the United Nations Environment Program (UNEP) on November 19th, 2021. "One Health" was raised by the panelists to give advice on how to reduce the diffusion and spread of antibiotic resistance in the environment. This seminar promoted cooperation and exchanges among experts from countries along the Belt and Road, and provided new opportunities for joint efforts to tackle the severe challenges posed by antibiotic resistance to the environment and human health. The seminar was held both online and offline, attracting hundreds of attendees around the world and nearly 5000 viewers on the live streaming platform.



Highlights

- Urbanization predominantly contributed to both the abundance and dissemination of ARGs in riverine MPs. And, MPs types were found to significantly affect the distribution and dissemination risk of ARGs.
- Microplastic is an important vector for the attachment and dissemination of viruses. PP has the highest viral diversity and environmental risk among five types of MPs.
- Environmental antimicrobial resistance is associated with fecal pollution in Central Thailand's coastal aquaculture region.
- In the native environment of Nigeria, these active cells have great potential risk to spread antibiotic resistance via the food chain.
- In low-and middle-income countries where limited data are available, the combinations of DO and prospective modeling can be used to guide the local intervention.

Future Plans

- To map the large-scale spatial pattern of environmental ARGs in the countries along the Belt and Road and decipher the key factors associated with the occurrence and dissemination of ARGs. Train a group of professionals in environmental ARGs studies and establish related research facilities.
- To organize and participate in at least one international symposium on the environmental dimension of antibiotic resistance, invite scientists from the Belt and Road countries, as well as

European countries and the United States to advance our understanding of ARGs, promote protection of public health and the environment, and stimulate broader international cooperation.

- To publish no less than 2 progress reports and news reports.

Publication

Articles:

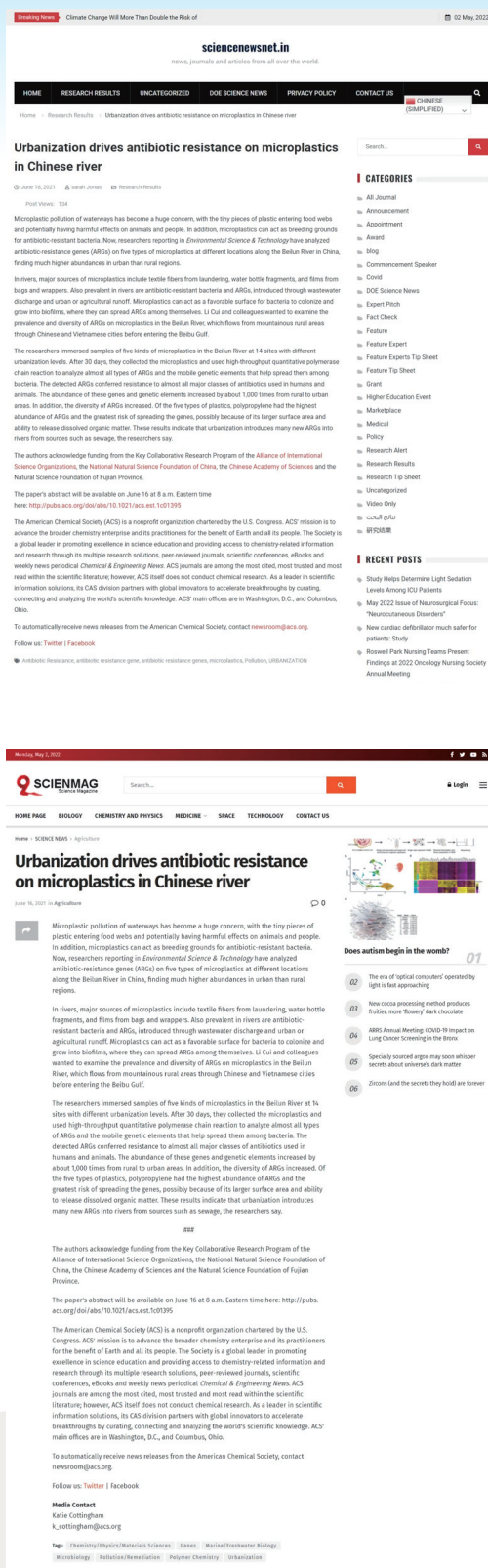
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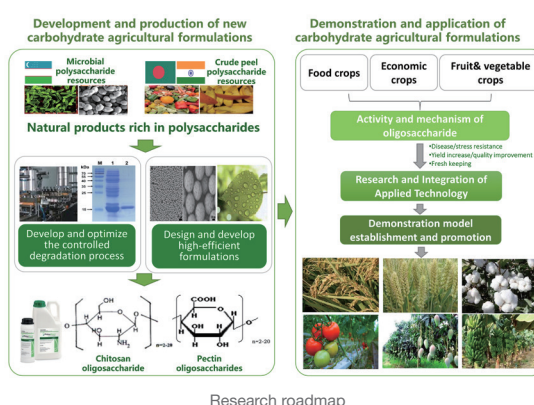
Yang, K., Chen, Q.L., Chen, M.L., Li, H.Z., Liao, H., Pu, Q., Zhu, Y.G., Cui, L. Temporal Dynamics of Antibiotic Resistance in the Plastisphere during Microbial Colonization. *Environ. Sci. Technol.* 2020, 54(18), 11322-11332.



Application and Demonstration of New Carbohydrate Agricultural Formulations in the Belt and Road Countries

Objectives

Excessive use of chemical pesticides has caused serious environmental and food safety problems. These practices are common in most countries along the Belt and Road. Carbohydrate agricultural formulations (CAFs) derived from natural products have been shown to induce plant resistance and promote plant growth. Taking into account the safety issues of pesticide use in countries along the Belt and Road, the ANSO-CAF cooperative research project aims to apply the CAFs in Central and South Asia. The development and application of carbohydrate-based agricultural formulations is expected to increase crop yields and incomes while reducing the amount and frequency of conventional chemical pesticides, contributing to local economy. It is also expected that through cooperation with local industrial companies, we will promote the export of our technical products, build a green agricultural network, carry out large-scale cooperation, and train researchers and local application technicians in the field of green agricultural preparations and planting, and improve the ability of sustainable agricultural development in countries along the Belt and Road.



Methodology

This project has applied enzyme engineering and glyco-engineering technologies to establish a process flow for the enzymatic hydrolysis of microbial polysaccharide resources and crude peel resources to prepare oligosaccharides. On the other hand, with the obtained polysaccharides and oligosaccharides, nanotechnology has been used to develop high-efficiency formulations suitable for local agricultural production applications. Moreover, plant protection and crop cultivation technology have been applied to carry out research on the application technology of carbohydrate agricultural formulations. Finally, demonstration bases were established to promote the products and technologies in surrounding areas. The project was completed with the cooperation of China, Uzbekistan, Bangladesh, and India. The Chinese side focuses on the enzymatic hydrolysis and utilization

of crude polysaccharide resources. Sino-foreign cooperation to preparing nano-polysaccharide materials and researching the application technology and system. Foreign teams mainly carry out field demonstrations and promotions of CAFs.

Main Progress

This project used the abundant polysaccharide resources of the Belt and Road countries as raw materials, established a new process for optimizing the production of pectin oligosaccharides, and realized the scale-up production of 200L pectin oligosaccharides. The product recovery rate reached 88%, and the degree of polymerization (DP) of the obtained oligosaccharides was distributed in the range of DP2-DP11. The project also realized the separation and purification of DP2-DP7 single pectin oligosaccharides from pectin degradation products and established relevant product quality standards.

Moreover, a low-cost preparation method for pectin nanoparticles has been established through Sino-foreign cooperation, pectin nanoparticles and a smart response carbohydrate slow-release agent embedded with agricultural fungicides were successfully prepared. The obtained pectin nanoparticles can effectively attach to the plant surface and promote crop growth. The smart response carbohydrate nanoparticles can release the grafted oligosaccharide plant immune inducer and the encapsulated fungicide in the structure in response to the reactive oxygen species (ROS) messenger substances generated during the interaction between plants and pathogens, realizing the coupling of two plant disease control strategies. The related CAFs showed good effects on rice, tomato, banana, mango, and other crops. Large-scale application demonstrations have been carried

polymerization
(DP)

DP2
|
DP11

pectin
oligosaccharides

purification

DP2
|
DP7

out on various crops such as tomato and rice in partner countries. Field experiments have shown that the prepared oligosaccharides can promote crop growth, promote fruit ripening, and finally increase yield. Through cooperation with domestic and foreign enterprises, we have realized the export application of CAFs products. Their application in Solanaceous crops showed the effects of protecting flowers and fruits, improving the fruiting ability, and prolonging the harvest period.

In addition, the project has organized several bilateral meetings and one multilateral academic meeting through online communication. Through this project, we have trained a doctoral student from Bangladesh, and the student was successfully graduated and selected as an outstanding international graduate of the University of Chinese Academy of Sciences.



The field application of CAFs on Garden pea (Promote the germination of pea crops and the growth of seedlings. the pea plants in the oligosaccharide treatment group grew vigorously, with large and dark green leaves, AOS: Alginate oligosaccharides, COS: Chitosan oligosaccharides, OGA: Oligogalacturonic acid)



The field application of CAFs on rice (Enhance the resistance to rice blast and sheath blight, and increase yield by 9%, AOS: Alginate oligosaccharides, COS: Chitosan oligosaccharides, OGA: Oligogalacturonic acid)



The field application of CAFs on cauliflower (Promote growth and maturity, and have a good control effect on leaf spot disease, AOS: Alginate oligosaccharides, COS: Chitosan oligosaccharides, OGA: Oligogalacturonic acid)

pectin
nanoparticles

Highlights

- The enzymatic production process and quality control method for large-scale preparation of pectin oligosaccharides have been established, which provides an important material basis for the application and scientific research of new oligosaccharide products.
- A new type of smart response carbohydrate nanoparticles was developed, which can simultaneously release fungicides and plant resistance inducers to realize the combination of two plant disease control strategies. The synergy of the two strategies can better achieve plant disease control.
- In Bangladesh, India, Malaysia, Myanmar, and other countries, the field application of CAFs on representative crops has been achieved, and application technologies on crops such as rice, potatoes, and peas have been established. These technologies improve disease and stress resistance of crops, increase crop yields, and improve product quality.

Future Plan

- Conduct field activity evaluation of several new CAFs for various crops in the “Belt and Road” countries, establish locally appropriate application technologies.
- On the basis of establishing a single application technology, field application demonstration for local characteristic crops in combination with the existing local planting technology will be carried out. Establish green planting technology systems for different crops in different countries, and carry out large-scale demonstration applications.
- Train local technicians on CAFs products and applied technologies as well as promote these products and technologies; at the same time, continue to carry out multilateral academic exchanges and establish a green agricultural cooperation network.

Publication

Articles:

Chunlai Zhang, Wenxia Wang, HengYin. The application and prospects of biological agricultural preparations in the “Belt and Road” countries’ planting industry. “Belt and Road Initiative Innovation Development Report 2021”, Science Press, 2021.

Ruixin Li, Maolong Li, Jinxia He, Hongguo Xie, Wenxia Wang, Meng Zhang, Gustavo Cabrera-Barjas, Pierfrancesco Morganti, Heng Yin. Preparation of Pectin Nanospheres and Its Effect on Wheat (*Triticum aestivum* L.) Seed Germination and Growth. *Journal of Plant Growth Regulation*. 2021. Doi.10.1007/s00344-021-10505-0

Santosh Kumar Bose, Yanqiu He, Prianka Howlader, Wenxia Wang, Heng Yin. The N-glycan processing enzymes beta-D-N-acetylhexosaminidase are involved in ripening-associated softening in strawberry fruit. *Journal of Food Science and Technology-Mysore*. 2021. 58. 621-631.



No	Patent Name	Intellectual Property	Status	Application Number
1	A recombinant polysaccharide degrading enzymes, coding gene and application thereof	Invention	Initial review qualified	202011402573.9
2	Polysaccharide soil regulator and application thereof	Invention	Initial review qualified	202011463580.X
3	ROS responsive pesticide slow-release preparation with induced resistance, and preparation method and application thereof	Invention	Initial review qualified	202110915163.2
4	ROS and GSH dual responsive pesticide slow-release preparation, and preparation method and application thereof	Invention	Initial review qualified	202110915159.6
5	Pesticide drug-carrying particle with spiky morphology, and preparation method and application thereof	Invention	Initial review qualified	202111510062.3
6	Suspended seed coating agent containing alginate oligosaccharide and its preparation and application	Invention	Initial review qualified	202111483432.9
7	Fruit crack inhibitor containing oligosaccharide and using method thereof	Invention	Initial review qualified	202111484450.9

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