《生物科学杂志(ISSN 2958-4035)》
Journal of Biological Sciences (ISSN 2958-4035)

ChengZhu Science™

Serial in April, 2023

ChengZhu Science Research Center Limited

(Hong Kong Registration NO. 80561228)

Journal article DOI is registered via JiangXi Province ChengZhu Environmental Engineering Company Ltd, with Crossref membership (DOI prefix 10.58473)

出版人： 刘焕 香江出版社有限公司

Publisher: Liu Huan, Xiangjiang Publishing Company Ltd.



Copyrights Statements

Copying and Transferring is Forbidden!

版权申明

禁止复制、转载！

All the intellectual property (mainly including the original academic knowledges and brand logo) are prohibited to copy or transfer into other publications or websites. To cite this article, only short quote is acceptable, but copying or transferring any substantial part of this article is NOT allowed (Defined in <Copyright Ordinance> in Hong Kong). The original academic knowledge is the substantial part of an article as academic journal. For learning purpose, it is allowed to read our website in online video class only. This journal is published by Hong Kong Publisher, and the copyrights is regulated and protected by <Copyright Ordinance> in Hong Kong, China. This PDF document is accessible to public only through Hong Kong domain websites (natural-foundation-science.org), and its printed version is the formally published journal. Without permission, it is NOT allowed to print, issue and sale.

所有形式知识产权（主要包括原创型学术知识和品牌标识）禁止复制、转载到其他出版物和网站。如果需要引用这篇论文，仅仅允许简短引述，但是禁止复制、转载这篇论文中任何实质性部分（香港《版权条例》中定义）。作为学术杂志，这篇论文中的原创型学术知识即为作品的实质性部分。仅仅允许以学习为目的在线视频课堂阅读本公司网站。本杂志由香港出版社出版，其版权受中国香港《版权条例》监管和保护。此PDF文档仅仅通过香港主机网站向公众公开 (natural-foundation-science.org)，并且其印刷版本杂志为正式出版物。未经许可，不得印刷、发行、销售。

Article 14. Original review of specificity in the interaction between pathogen invasion and host organism

Author: Liu Huan (1983-), Master of Science (First Class Honours, 2009), The University of Auckland.

DOI: https://doi.org/10.58473/JBS0022

Formally published on 08/04/2023; Latest revised on 15/10/2026.

Abstract

This article mainly contains the below knowledge parts: 1.the host antibiotics/antibody and immunology enzymes against pathogen; 2.specificity in both gene sequences and enzyme expression by pathogens; 3.specificity in both gene sequences and enzyme expression by host; 4.bio-informatics technology in gene sequence database; 5.specificity in environmental conditions for disease breaking out; and 6. implications for future research development.

Key words: Specificity, Host-Pathogen Interaction, Pathology, Gene Screening, Bio-informatics, Functional Genomics Biology.

1.Introduction

There are three main parts with regards to the topic ‘specificity in the interaction between pathogen invasion and host organism’: firstly, the specificity in population genetics between invasive pathogen genotype and host genotype (including specific host cells, tissue, organ or individual); secondly, the specificity in metabolic biochemistry pathways between invasive pathogen phenotype and host phenotype, which is further discussed below; third, the specificity in cultural or ecological conditions (such as specific season, geography site, pathogen transmission pathways [4]) between pathogen invasion and host organism. This article aims to review the representative case reports in the three parts of specificity research firstly for study purpose, and then attempts to discuss the new theories and design new methods for future research purpose.

2.Host Antibiotics/Antibody and Immunology Enzymes Against Pathogens

The specific enzyme species functioning as the immunology inhibitors in host cells are reviewed by my previous article [36], which is further discussed in this review

paper. Host organism synthesizes specific enzyme species against invasive virus, which has been consequently and widely utilized to develop the clinic diagnose methods against specific virus species. For example, using both the monoclonal and polyclonal cultivation in Canine parvovirus developed by laboratory, a rapid diagnostic kit for Canine parvovirus (CPV) on the basis of specific antibody has been developed by using double antibody sandwich method, ELISA[17]. In total 47 stool samples collected from clinical cases have been tested with hemagglutination test and ELISA respectively, resulting in overall coincidence rate of 91.5%, but the coincidence rate of the two methods is 100% for the obvious positive and negative samples. The results can be judged with naked eyes within 30 minutes, with the advantages of simplicity and rapidity. Further more, there is no cross reaction in this ELISA test with Canine distemper virus (CDV), Canine infectious hepatitis virus (ICHV), Feline distemper virus (FPV) and Mink viral enteritis virus (MEV), revealing the specificity in the antibody against virus CPV.

Both S protein and N protein are the ideal biochemical indicators for SARS-CoV-2 vaccine development and antigen detection. Establishing a rapid, reliable and low-cost quantitative detection method for SARS-CoV-2 antigen to diagnose the early stage of infected patients is crucial for epidemic prevention and control. In this study, goats have been immunized with inactivated SARS-CoV-2 virus to prepare polyclonal antibody in sheep as a coating antibody against SARS-CoV-2. Targeting S protein or N protein as an antigen, hybridoma cell culture supernatant has been screened to obtain the antibody, with sheep anti-mouse enzyme labeled as a detection antibody. An enzyme linked immunosorbent assay (ELISA) monoclonal antibody screening system has been established for the antigen detection against SARS-CoV-2. The results have shown that a highly purified and specific sheep polyclonal antibody against SARS-CoV-2 is prepared with a titer of 1:51200, a concentration of 7.34 mg/mL, and an EC50 value of 0.143 μg/mL, which can specifically recognize SARS-CoV-2 whole virus. Both S protein and N protein have low crossing reactivity with common laboratory pathogens and proteins, so that they can be used to establish monoclonal antibody screening systems and dual antibody sandwich ELISA antigen detection methods [28].

Rabies virus (RABV) has a long history of causing rabies, causing the mortality rate of humans and animals to reach nearly 100%. Vaccine immunization is currently the most effective mean of rabies prevention and control, requiring that the titer of RABV neutralizing antibody in the serum after immunization is ≥0.5 IU/mL, which is the standard indicator recognized by the World Health Organization for achieving immune protection effects for rabies vaccines. RABV glycoprotein (G) is the main antigen that stimulates the host body to produce RABV neutralizing antibodies, with close correlation between the enzyme linked immunosorbent assay (ELISA) detection technique based on the specific antibodies of RABV G protein and the virus neutralization test. This study aims to establish a competitive ELISA (cELISA) method based on the Semliki forest virus-RABV G (SFV-RVG) expressing only one

structural protein of RABV G and monoclonal neutralizing antibodies RABV G protein, to simplify and develop the measurement of RABV neutralizing antibodies. The prerequisite for establishing a cELISA method is that the monoclonal antibodies used can well distinguish between positive and negative samples. Consequently, a monoclonal antibody has been screened from 8 strains of horseradish peroxidase (HRP) labeled as RABV G Protein monoclonal antibodies to establish a cELISA method for the detection of RABV neutralizing antibodies. Considering the specificity and sensitivity of cELISA, 7A3 strain is finally chosen as the optimal competitive monoclonal antibody. After comprehensive assessment, the criteria of sensitivity, specificity, repeatability and coincidence rate of this kit have all performed well [29].

Below this article summarizes several case studies with regards to the host enzyme expression in aquatic species under different environmental stimulus. Although these enzyme species are classified into non-specific category, which play important role in host resistance under abiotic environment stress conditions, these enzyme species are associated with host resistance against biotic stress (invasive pathogens) as well, which are commonly analyzed in host-pathogen interaction experiment, and further development is discussed in the final part of this article for addressing specificity. Additionally, these enzyme species are also vital for terrestrial plants to adjust metabolism pathway induced by multiple environment stress conditions, which will be discussed in detail in my next article[40].

For example, the breeding experiment of shrimp *Procambarus clarkii larvae* with the mass of (0.017 9 ± 0.0005) g has been hatched by the same parental generation for 42 days, fed by three kinds of food including biological flocs, feed and rotifer *Brachionus calyciflorus*. The effects of feeding methods on the cultivation of *Procambarus clarkii larvae* have been examined. The results have shown that the concentrations of total phosphorus, ammonia nitrogen, nitrite nitrogen and nitrate nitrogen are the lowest in the floc group during the whole culture period, which is significantly different from the other two groups, while there are no significant differences in the final biomass, weight gain rate and specific growth rate among the three experimental groups. The survival rate of the floc group is significantly higher than that of the feed group, but is not significantly different from that of the rotifer group; For the feed group, the activities of α- amylase and lipase are the highest, whereas those of floc group are the lowest. The activities of trypsin and pepsin in floc group are significantly higher than those in feed group and rotifer group, without significantly difference in activities of α-amylase, trypsin and pepsin from those of the other two groups; Both total antioxidant capacity (T-AOC) and catalase (CAT) activity of the floc group are the highest, but the content of malondialdehyde (MDA) is the lowest, in comparison to the feed group and rotifer group. Among the three kinds of bait, the biological flocs results in the best effect on the survival, growth, digestive enzymes and immune antioxidant capacity of the young shrimp [9].

High water temperature in summer changes the immune regulatory system in bivalves,

which is one of the important reasons causing massive death of oysters. In this paper [10], both *Crassostrea gigas* and *C. sikamea,* as well as their hybrid progeny have been selected as bio-samples to examine the high water temperature effects by counting the survival rate of F1 generation during the cultivation period, and the changes in the activities of four immune-related enzymes, including catalase (CAT), malondialdehyde (MDA), superoxide dismutase (SOD) and total antioxidant capacity (T-AOC), have been tested under high temperature stress. This research has compared the tolerance under high temperature mutation among *Crassostrea gigas*, *C. sikamea* and their hybrid progeny. The results have shown that: 1. With the increase of water temperature, the change of CAT activity in the mantle of hybrid oyster is negatively correlated with MDA. The CAT activity of heterozygotes is activated before 35 ℃ ($H>0$, $hp>1$), whereas the hybridization shows obvious disadvantage ($H<0$, $hp<-1$) in the 35 ℃ and recovery group; 2. The SOD activity of heterozygotes generally declines firstly and then increases, with the minimum value at 35 ℃ by insignificant differences ($P<0.05$); 3. T-AOC activity of heterozygotes has shown heterosis ($H>0$) before 30 ℃, compared with hybrid weakness ($H<0$) after 30 ℃. At the temperature turning point of 30 ℃, T-AOC activity decreases significantly. The results have shown that high temperature results in significant effect on the immune enzyme activity of *Oyster gigas*, *Oyster ursamoto* and their hybrid progeny. When the external temperature changes, the hybrid progeny responds faster than its parents, consequently providing the reference for oyster cross breeding [10].

In order to reveal the acute toxicity and physiological changes of *Babylonia areolata* under low and high pH stress, the activities of four families of immune enzymes in *Babylonia areolata* have been tested [11], including glutathione peroxidase (GSH-PX), catalase (CAT), peroxidase (POD) and acid phosphatase (ACP), with pH=8.0 as the control group and pH=5.0, 6.0, 7.0, 9.0 as the tested group respectively. The results have shown that pH stress at different time imposes significant effect on the activity of immune enzymes ($P<0.05$), but there is no significant effect on the activity of immune enzymes ($P>0.05$) among different stress levels; After being exposed to acute pH stress, the vitality of *Babylonia crenata* decreases and the movement of wall climbing turns to be slow after being cultured in low pH and high pH water for 6 hours, finally beginning to die after 48 hours; The activity of GSH-PX in each test group has shown the trend of ‘inhibition to induction’, while the CAT activity of each pH treatment group displays as the pattern of ‘induction to inhibition to induction’ with continuous extension of time; Both POD activity and ACP activity in low pH treatment group (pH=5.0, 6.0) have shown the trend of ‘induction- inhibition’. This study is of reference significance for the cultivation of *Babylonia crenata*, enriching the data in the immune system of shellfish [11].

The effects of both acute and chronic low salinity stress on the survival rate and non-specific immune enzyme activity of large yellow croaker (*Pseudosciaena crocea*) have been studied [12]. The results have shown that under acute low salt (15,8) stress, the activity of superoxide dismutase (SOD) in the liver of *Pseudosciaena crocea*

increases at first and then decreases, with ascending trend significantly on the first day ($p<0.05$) and then the trend descending gradually to significantly lower than that of the control group, whereas the activity of catalase (CAT) in the liver displays as declining trend firstly, then increasing and finally decreasing. More specifically, the activity of CAT decreases significantly on the first day, and then increases on the second day, but starts to decrease on the third day, which becomes significantly lower compared with the control group on the seventh day; The activity of acid phosphatase (ACP) in serum shows similar trend to SOD, which decreases and then gradually increases. It decreases significantly on the first day and then increases gradually, but finally is still significantly lower than the control group on the seventh day; The activity of alkaline phosphatase (ALP) in serum differs from the above enzyme species, which gradually increases from the first day to the third day during the experiment and is significantly higher than that of the control group, but it begins to decline to the level of the control group at the seventh day; The content of lysozyme (LZM) in serum fluctuates, with an overall trend of rising firstly and then falling. In comparison, after 14 days of chronic low-salt (8) culture, there is no significant difference in the non-specific immune enzyme activities of large yellow croaker in comparison to the control group ($p>0.05$). In addition, all groups of large yellow croakers do not show death rate during the experimental period, and only the activities and feeding of large yellow croakers in the acute low salt stress group are influenced to the limited extent, so large yellow croaker has shown high tolerance against chronic salinity reduction culture, but the sudden dropping in salinity significantly affects the non-specific immunity of large yellow croaker, which need to be avoided in the salinity of the culture environment [12].

The effects of different concentrations of marine red yeast (strain No.: RM) on the growth performance of *Litopenaeus vannamei* larvae, its immune enzyme activity and resistance to *Vibrio parahaemolyticus* have been studied [13]. Four additive concentrations of marine red yeast (1.0×10^8, 1.0×10^9, 1.0×10^{10} and 1.0×10^{11} cfu/kg) and a control group without the addition of marine red yeast have been compared and contrasted after 42 days of feeding experiment, with 5 replicates per concentration treatment. After the completion of the feeding experiment, the pathogen of *Vibrio parahaemolyticus* invasion experiment has been carried out for a period of 14 days. The results have shown that the overall survival rate of shrimp culture in each experimental group is more than 90%, and there is no significant difference compared with the control group ($P>0.05$); Except marine red yeast of 1.0×10^8 cfu/kg treatment group, other treatments significantly increase the final weight of *Litopenaeus vannamei* ($P<0.05$), and the growth rate and feed conversion efficiency in both 1.0×10^9 and 1.0×10^{10} cfu/kg added group are significantly higher than those of the control group. The effects of different concentrations and amounts of marine red yeast on the non-specific immune enzyme activity of shrimp serum vary greatly. At the level of 1.0×10^9 cfu/kg, the activities of both superoxide dismutase and total nitric oxide synthase in shrimp serum are significantly higher than those in the control group and other treatment groups ($P<0.05$); At 1.0×10^{10} cfu/kg, the activities of acid

phosphatase, alkaline phosphatase and lysozyme in shrimp serum are significantly higher than those in other groups ($P<0.05$). After being infected by *Vibrio parahaemolyticus*, the cumulative mortality of shrimp in each treatment group turns to be 53%, 27%, 20% and 33% respectively which are significantly lower than that in the control group (73%) ($P<0.05$). Compare with other treatment groups, the cumulative mortality of shrimp is the lowest at 1.0×10^{10} cfu/kg added. In general, adding appropriate concentration of marine red yeast into the feed of *Litopenaeus vannamei* can effectively improve the growth performance, serum non-specific immune level and resistance to *Vibrio parahaemolyticus*. Taking the factors of shrimp growth, non-specific immunity and resistance to *Vibrio parahaemolyticus* into comprehensive consideration, the optimum addition of marine red yeast (strain RM) in feed is chosen at 1.0×10^{10} cfu/kg [13].

3.Specificity in Both Gene Sequences and Enzyme Expression by Pathogens

This article summarizes that the theory of this specificity research in pathogens should included two parts: firstly, specificity study in pathogen helps to identify and classify the exact pathogen for disease prevention and remediation; secondly, the inoculation of mutant strains with deletion of pathogenic genes would trigger the immunology of host species against the corresponding wild pathogenic strains [25].

Bacterial leaf streak (BLS) is caused by Xanthomonas oryzae pv.oryzicola (Xoc) that infects rice Xanthomonas transports as toxic or non-toxic factors into the host cells through type III secretory systems (T3SS), triggering the host plant's disease resistance or susceptibility reaction. Among them, type III effectors (TALES) which play the role in transcriptional activators target specific genes of the host plant, affecting the pathogenicity of pathogen or host disease resistance against it by regulating the expression of targeted genes. The researchers have chosen Tn5 transposon to insert the genomic DNA of virulent strain GD41 of Xoc, obtaining the mutant strains of GD41, with a mutant strain GD21 of significantly weaken pathogenicity that is screened through the pathogenicity test. According to the experiments results, it is speculated that TALIXoc strain may produce GRP4 protein by recognizing the GRP4 gene promoter sequence and subsequently activating the GRP4 gene expression. GRP4 protein interferes with the variable splicing of NPR3 transcript and inactive NPR1, the key regulatory gene of salicylic acid signaling pathway, to regulate the expression of defense-related genes normally, thus inhibiting the disease resistance of rice against this pathogen strain [5].

Zhang (2022) has examined the effects of both VdxyL3 and VdITP1 genes from *Verticillium dahliae* on cotton disease resistance [6]. There are mainly four steps implemented in this research: Firstly, all the xylosidase genes and inositol transporter genes are identified from *Verticillium dahliae* DNA database by bioinformatics, with

genetic analysis of their domains and evolutionary relationships; Secondly, the expression patterns of six xylosidase gene sequences under root exudates environment of different resistant and susceptible cotton are separately analyzed by qRT-PCR Technology; Thirdly, both xylosidase gene VdxyL3 and inositol transporter gene VdITP1 are silenced by HIGS technology, investigating the disease index of the silenced plants against specific pathogen genes in 14 and 21 days after inoculation, with the test of the gene silencing efficiency and fungal biomass in plant stem by qRT-PCR Technology; Then the CAT enzyme activity, SOD enzyme activity and MDA content in the leaves of VdxyL3 silenced plants have been detected by spectrophotometry; Fourthly, the targeted VdITP1 gene region of both Arabidopsis plants and cotton embryogenic calli from T2 generation are further obtained by RNAi technology. According to the results, firstly, thirteen xylosidase gene sequences have been identified from the database of *Verticillium dahliae*, including nine glycoside hydrolase from family members 43, one is from family member 3 and three are from family members 31. In total 13 genes are distributed on 6 chromosomes without forming gene clusters. The results of qRT-PCR show that the targeted six genes are all capable of being induced by root exudates, with expression levels significantly increased firstly and then decreased after they are cultured under one or more root exudates environment for 6 or 12 hours. Among them, the expression of VdxyL3 gene is significantly increased after being induced by the root exudates of island cotton; Secondly, the cotton plant silenced by VdxyL3 gene has been cultivated by HIGS technology, resulting in significantly more serious disease index after 14 and 21 days of inoculation (33.3% and 83.9% respectively) than that of the empty control vector (21.7% and 66.1% respectively). QRT-PCR analysis has shown that the quantitative expression of VdxyL3 gene in the stems of cotton plants silenced by the interference fragment of VdxyL3 gene is significantly lower than that of the control empty vector, with significant increase of the fungal biomass. According to the spectrophotometry results, the content of MDA in the leaves of the silent plant is higher than that of the empty control, whereas the activities of enzyme SOD and CAT are lower than that of the empty control, which would be related with the plant resistance against pathogen. These results reveals that the silencing of VdxyL3 gene increases the pathogenicity of *Verticillium dahliae* and makes the cotton more susceptible to the pathogens; Thirdly, the cotton plant silenced by VdITP1 gene has been also obtained by HIGS technology, leading to significantly lighter disease of the cotton after 14 and 21 days of inoculation, with disease index (25.6% and 53.4% respectively) significantly lower than that of the empty vector control (30.0% and 82.1% respectively). QRT-PCR analysis has shown that both the quantitative expression of VdITP1 gene and fungal biomass in the stems of cotton plants silenced by the interference fragment of VdITP1 gene are significantly lower than those of the empty vector control, so that the silencing of VdITP1 gene leads to the weaken pathogenicity of *Verticillium dahliae* and strengthened cotton resistance to disease, which would be the indicator for the molecular breeding of cotton disease resistance [6].

The Chinese research team have made significant progress in the research on the

pathogenicity of African swine fever virus, firstly revealing that the African swine fever virus with the MGF-110-9L gene knocked out has reduced its pathogenicity to pigs. In this study, an strain of African classical swine fever virus with MGF-110-9L gene deletion have been constructed. The blood and tissue carrying capacity of animals infected with the deletion strain is significantly lower than that of wild strains, and the tissues and organs of animals infected with the deletion strain are normal, indicating that the African classical swine fever virus MGF-110-9L gene is an important molecule for the weakened pathogenicity of the virus [24].

Xanthomonas oryzae pv.oryzae (Xoo), a pathogenic variant of *Xanthomonas oryzae*, can cause bacterial blight in rice crop, which is one of the most serious diseases in rice production, whose interaction with rice is a gene to gene relationship. The completion of Xoo genome sequencing has greatly promoted the study of Xoo gene function. Therefore, this article summarizes the identification of pathogenic genes of Xoo which has been conducted in the past, by investigating the interaction between rice crops infected and Xoo pathogens after inoculation of mutant strains of pathogenic genes on rice, and elaborates in detail the current research status of the identification of non-toxic genes of Xoo, providing a theoretical basis to breed rice for resistance against bacterial blight and to study pathogenic genes with its pathogenesis [25]. For example, the mutant strains of gene pilQ deletion lead to non-pathogenicity on rice [26], while the mutant strains of gene aroC results in weakened pathogenicity against rice [27].

Banana fusarium wilt is a kind of devastating disease caused by *Fusarium oxysporum f. sp. cubense* (FOC), which is widely distributed in banana growing regions worldwide. Studying the molecular mechanism of pathogenicity related genes deepens understanding of the pathogenesis and provide new methods and strategies for the prevention and control of this disease. The oste12 gene of banana fusarium wilt is a homologous gene of yeast ste12, which encodes specific transcription factor. In this study, PCR amplification has been used to obtain both upstream and downstream DNA fragments of the Foster12 gene of *Fusarium oxysporum*, which are cloned into pCT74 vector to obtain homologous recombinant DNA fragments, and then transformed into *Fusarium oxysporum* through protoplast transformation to obtain Foster2 gene knockout transformants. Using four pairs of specific primers for PCR detection and Southern Blot validation of the transformants, three Foster 12 deletion mutants with double markers of both gfp (green fluorescent protein) and hph (hygromycin phosphotransferase) have been obtained. By observing the phenotype of the mutant, it is found that the deletion of Foster 12 results in no effect on the growth rate of the colony, the morphology of spores and hyphae, but it would slow down the germination of spores and slightly increase the sporulation rate. Through inoculation experiments with pathogenic bacteria, it is reported that the Foster 12 deletion mutant significantly decreases its pathogenicity against bananas, almost becoming the strain without pathogenicity. It can be seen that the deletion of the Foster12 gene affects the infective growth ability of the pathogenic bacteria. Analyzing the changes in

extracellular enzyme activity, it is found that the cellulase and amylase activities secreted by the Foster 12 gene mutant decrease by 29.7% and 38.5% respectively, but the pectinase activity is almost not affected. Analyzing the expression changes of the genes encoding the above cell wall degrading enzymes, it is revealed that the expression of genes related to fibronectin synthesis in the Foster 12 mutant is down-regulated, while the expression of genes related to sporulation is up-regulated. Therefore, it is speculated that the Foster 12 gene may regulate the expression of genes related to cellulase synthesis and satiety production, affecting the pathogenicity and sporulation of FOC pathogens. In addition, a complementary deletion mutant of the Foster 12 gene from the wild type is found to have similar phenotypes and biological characteristics to the wild type strain. This study has found that Foster 12 gene is an important pathogenicity related gene of banana Fusarium wilt, which regulates the expression of other pathogenicity related genes and sporulation genes, affecting pathogenicity and sporulation [22].

Curvularia leaf spot disease is a severe leaf disease on maize, which has caused serious reduction in maize production. Therefore, studying its pathogenesis is the key issue to establish effective prevention and control techniques that need to be implemented in production. Previous studies in our laboratory have confirmed that the pathogen of Curvularia leaf spot of maize can secrete a series of cell wall degrading enzymes in both vitro leaves and callus, mainly including polygalacturonase (PG), pectin methylgalacturonase (PMG), laccase, cellulase (Cx), and β- Glucosidase. Particularly, during the process of infection against maize callus by *Curvularia spp*., the laccase activity produced is 1.097.3 times higher than that of other enzymes, revealing that the cell wall degrading enzymes induced by lignin have the strongest ability to leach the host tissue, which consequently becomes the study focus. Based on the previous study, a correlation analysis has been further conducted between the laccase activity and the pathogenicity of Curvularia leaf spot pathogen strains. The results have shown that the laccase activity produced by the strong pathogenicity strain LNC0907 of Curvularia leaf spot pathogen is significantly higher than that of the weak pathogenicity strain LNC1403. After inoculation with the inbred line Huangzao 4, there is also a significant difference in the disease index of Curvularia leaf spot disease in maize, with close correlation between the laccase activity and the disease index of Curvularia leaf spot disease in maize ($r = 0.958$), revealing that the weaker the resistance, the more significant the laccase activity of the induced pathogen is. Based on the conserved region of the amino acid sequence of the fungal laccase gene database registered on GeneBank, a merger primer has been designed and two laccase homologous gene fragments (593 bp) from the highly pathogenic strain LNC0907 of Curvularia maydis have been successfully cloned. The corresponding amino acid sequences are deduced and compared with those of *Verticillium dahliae* and *Fusarium oxysporum*, resulting in the sequence homology index of the laccase genes at 74% and 73% respectively. By qRT-PCR analysis, the instantaneous expression of melanin synthesis genes of both PKS and Brn1 in Curvularia maydis is closely correlated with the laccase activity of the pathogen [23].

4.Specificity in Both Gene Sequences and Enzyme Expression by Host

From 2019 to 2020, different corn varieties, including 30 core inbred lines of both Shanxi Group A and Shanxi Group B, as well as 17 excellent germplasm from both domestic and oversea origins, were selected for disease identification, which were planted in different places in Shanxi Province. The field inoculation of stem rot, ear rot, leaf blight and southern corn leaf blight, as well as the natural disease infection in field of gray spot have been carried out and identified. Randomized block design is adopted for the test, with two replicates for each test. Both leaf blight and southern corn leaf blight are inoculated with sorghum seeds at seedling stage. Gray spot is a natural disease, graded and identified at the late stage of milk ripening; *Fusarium graminearum* stem rot is inoculated by soil embedding method in maize tasseling stage, subsequently investigating stem splitting in late milking stage; *Fusarium graminearum/Fusarium verticillata* ear rot is inoculated twice by the means of filament channel and seed wound inoculation respectively, which is subsequently investigated after physiological maturity. The best linear unbiased prediction (BLUP) of disease index for each disease is calculated and correlated with disease resistance. Four disease-resistant genes that have been previously identified by clone are screened by molecular marker of gel PCR or sequencing in this experiment [7].

The eucalyptus leaf scorch caused by the fungus of the *genus Lycra* is one of the most threatening diseases against eucalyptus in China. In this study, two *Calonectriapentasepta* strains have been inoculated into eight eucalyptus genotypes widely planted in South China by spore suspension spray inoculation, including seven *eucalyptus urophylla×e.grandis* genotypes CEPT1848, CEPT1849, CEPT1850, CEPT1851, CEPT1852, CEPT1853 and CEPT1855, as well as one genotype of *E.urophylla×E.camaldulensis* CEPT1854. The disease resistance of eucalyptus genotypes has been evaluated by comparing the percentage of scorched leaves. The results has shown that there are significant differences in disease resistance among the seven genotypes of *eucalyptus urophylla×e.grandis*. The resistance of genotype CEPT1851 is expressed to be the strongest, whereas the resistance of genotype CEPT1849, CEPT1850 and CEPT1855 are relatively weak and the disease resistance of *E.urophylla×E.camaldulensis* genotype CEPT1854 falls in the middle level among 7 genotypes of *eucalyptus urophylla×e.grandis*. Analysis of variance shows that there is an interaction effect between *Ca.pentaseptata* strain and eucalyptus genotypes, provided guidance for the selection of pathogenic bacteria strains and eucalyptus genotypes in the process of eucalyptus breeding, which is screened for the trait of disease resistance [8].

In order to compare the gene variation in MHC II - DRB3 gene region between healthy goats and diseased goats, Ding.H has conducted experiment preliminarily

exploring the polymorphism of MHC II - DRB3 in the gene loci that may be associated with *paratuberculosis* infection in goats. The goats that are suspected of being infected by *Mycobacterium paratuberculosis* have been further diagnosed in the means of both pathological dissection and histopathological examination, and the full-sequences in genomic DNA of the bacteria in the feces of the infected sheep have been extracted, further confirmed by PCR amplification and sequencing. In total 122 samples of sheep feces, blood and serum have been collected for detection. Pathogens in blood and feces are detected by PCR, and antibodies in serum are measured by Enzyme-Linked Immuno Sorbnent Assay (ELISA). Samples are divided into healthy sheep in which negative pathogens and antibodies are determined, and infected sheep whose test results are diagnosed to be positive. Totally 50 bio-samples, including 25 samples identified to be positive by pathogen detection and antibody detection, and 25 samples that show negative results correspondingly, have been chosen to extract the whole genome DNA of blood and obtain the full sequences of DRB3 gene through PCR amplification, using MEGA 7.0 software to compare the difference of MHC II - DRB3 gene region between healthy sheep and infected sheep. The sequence variation in gene loci has been preliminarily screened between healthy sheep and infected sheep. The results have showed that the infected sheep are diagnosed as *paratuberculosis*, and the fecal pathogens of total 122 goats are positive in 43 cases, with a positive rate of 35.25%, while 24 of 122 blood pathogens are positive, with a positive rate of 19.67%. In total 55 positive samples are detected by pathogen detection leading to positive rate of 45.08%. 39 out of 122 sera have shown positive antibody, with positive rate of 31.97%, while 27 samples are positive by both pathogen detection and antibody detection resulting in a positive rate of 22.13%. A total of 32 nucleic acid polymorphic locus are found at MHCII-DRB3 gene sequence range through comparative statistics, resulting in the mutation rate of 13.22%. Among them, 29 polymorphic gene locus are unrelated to disease, accounting for 90.62% of all polymorphic locus, but 3 polymorphic locus are possibly related to disease, accounting for 9.38% of all polymorphic locus [19].

Wheat stripe rust has been classified into the first class disease list in crop production in China, due to its characteristics of rapid transmission, wide range, and severe host yield losses. With the development of molecular marker technology, it has become a routine methods to screen specific loci or gene sequences expressed as disease resistance. Through specific molecular markers closely linked to disease resistant genes or loci, seed materials carrying targeted genes or loci can be quickly screened, eliminating environmental and experimental errors, which improves the efficiency and accuracy of breeding work. This study has mainly chosen 188 wheat samples with multiple generations or varieties collected from Yangling Shanxi, Tianshui Gansu, to Jiangyou Sichuan across the country to participate in this regional trials, aiming to identify the resistance against stripe rust at the adult stage. At the same time, seedling stage identification tests have been conducted in artificial climate chambers and incubators. Based on the PCR results of mainly 13 specific molecular markers targeting resistance gene and field observation, it is showed that among the candidate

varieties by seedling stage race identification, 9 samples (5%) are immune against the physiological race CYR32, 22 samples (12%) are highly resistant, 49 samples (26%) are moderately resistant respectively, whereas 42 (22%) samples are moderately susceptible, and 66 (35%) samples are highly susceptible; Against the physiological race CYR34, there are 5 (3%) samples, 43 (23%) samples and 19 (10%) samples showing three resistant levels of immunity, high antibodies and moderate antibodies respectively, whereas 70 (37%) samples are moderate sensitivities, and 50 (27%) are high sensitivities; Among these seedling samples, 32 (17%) samples have shown resistance concurrently against both CYR32 and CYR34 races, while 46 (24%) samples have shown resistance to CYR32 only and 35 (19%) samples have shown resistance to CYR34 only, which may indicate that resistance breeding against physiological race CYR 32 has achieved advantages compared with CYR34; Against physiological race strip CYR 34, a total of 67 of samples have shown resistance (including three levels: immune, high resistance and moderate resistance), accounting for 35.6% of the total number of experimental materials, while the remaining 63.8% of the materials show susceptibility, which may indicate that physiological race strip 34 is still suffered under a high infection ability over the current candidate varieties, so that the resistance breeding against strip CYR 34 needs to be strengthened [21].

The results of both adult stage and seedling stage identification have revealed that 16 (9%) samples of the wheat materials express as stable adult stage resistance under different environments and microbial community structures, while 18 (10%) samples show stable resistance over the whole growth stage, but 14 (7%) of the materials show susceptibility under all experimental conditions and environment scenarios, and the remaining materials show different resistant (unstable resistance) and susceptible phenotypes under different environments and microbial community structures. Consequently, wheat samples that exhibit as stable resistance to stripe rust under different environments and bacterial community structures are the key materials for resistance breeding. By the molecular marker testing of resistance genes for candidate varieties, a total of 110 materials with multiple resistance gene aggregation form have been detected, accounting for 58.5% of the total materials; Among these materials, totally 54 materials have been detected in the form of polymerization of two disease resistance genes, while 13 materials are found in the aggregate form of three disease resistance genes and totally 18 materials are polymerized in the form of four disease resistance genes. Further more, the five resistant gene polymerized samples, Yr9+Yr10+Yr29+Yr30+Yr78, are susceptible to CYR34 at seedling stage, but show stable resistance at adult stage in all environment conditions designed in this experiment. In comparison, the materials polymerized by another five disease resistance genes Yr2+Yr7+Yr9+Yr29+Yr30 maintain resistance to the selected races at the seedling stage, and exhibit stable resistance throughout the entire growth period under five environment scenarios. It is also indicated that with the increase in the number and type of the aggregation of stripe rust resistance genes, the level of plant resistance is also significantly increased with a positive correlation between the two [21].

5.Bio-informatics Technology in Gene Sequence Database

Since the fundamental framework constructions of gene sequence database, such as Gene bank, in combination with the development in bio-informatics technology analyzing the gene sequence databases, it is convenient to screen the specific gene sequences according to the specific enzyme species involving in the pathogen-host interaction. Below the bio-informatics analysis methods of representative case study are chosen as study emphasis:

Leaf spot of blueberry (*Vaccinium corymbosum L.*) becomes one of the main diseases in blueberry monoculture production. In recent years, with the increasing of blueberry planting area, varieties and diversity of planting methods, the occurrence of blueberry leaf spot disease has also ascended significantly, which is of difficulty in prevention and has become a serious obstacle in blueberry production, leading to serious economic loss. β- Aminobutyric acid (BABA) is a kind of secondary metabolized non-protein amino acid that is isolated from tomato roots under exposure to the sun. It can induce plants to resist the infection of fungi or bacteria, which is a kind of phytochemical inducer with induced disease resistance activity at broad-spectrum. This study is based on β- Aminobutyric acid as an inducer, and the 'brilliant' variety of blueberry has been selected as the experimental material. The induced blueberry inoculated with leaf spot pathogen have been prepared as the experimental group, and the un-induced blueberry inoculated with leaf spot pathogen are used as the control group for comparison. The transcriptome sequencing library has been constructed, in combination with the investigation in the total anthocyanin content of different blueberry samples and the leaf spot disease rate. Through biological information analysis with regards to gene function annotation and differential analysis, the related genes of blueberry leaf spot resistance have been screened from the library, and the expression of 8 related genes in the process of disease resistance have been studied by fluorescence quantitative PCR technology, to explore how these genes change its expression on the resistance to leaf spot after β-aminobutyric acid is induced. Among 8 related genes, two disease-resistance related genes that are convinced to be highly expressed have been cloned, to preliminarily analyze and verify their functions. The main research results are as follows: Firstly, expression of blueberry resistance to leaf spot disease has been induced by β-aminobutyric acid, supported by the results that after the blueberry plants are treated with β- aminobutyric acid foliar spray, the resistance of blueberry to leaf spot disease is significantly improved. The activity of plant defense enzymes have shown that the activities of β- Polyphenol oxidase (PPO), peroxidase (POD), phenylalanine ammonia-lyase (PAL),β-1,3-glucanase and chitinase under induction of β-aminobutyric acid treatment are significantly higher than those of the control [20].

This study has built transcriptome sequencing library of β-Aminobutyric acid-induced blueberry, comprehensively analyzing the gene expression variation of blueberry

leaves after induction and inoculation, which explores the genes related to blueberry leaf spot disease resistance. In total 78507 transcripts with an average length of 957.92 bp have been obtained by assembling the sequencing data, of which the longest and shortest transcript is 15693 bp and 301 bp respectively. In KEGG metabolic pathway, there are 2122 genes involved in environmental information processing and signal transduction, 3108 genes corresponding to organic system and environmental adaptation, 7566 genes annotated to metabolism, 2694 genes involved in genetic information processing, 1717 genes annotated to cell process, and 3953 genes corresponding to disease respectively. Among the 3953 transcripts annotated to diseases, 1115 transcripts are involved in the interaction between plant and pathogens, accounting for 14.74% of the annotated metabolic genes, and 35 genes are involved in anthocyanin synthesis. Through comparative analysis of differential expression libraries, the up-regulated genes with resistance to blueberry leaf spot have been screened. The results of differential expression show that the amount of up-regulated differential genes contain 900, while the amount of down-regulated differential genes are 531, with total amount of differential genes of 1431. Among the 70 genes highly expressed in the library, there are 24 genes with no characteristic or hypothetical protein, and 11 genes are related with resistance protein against blueberry leaf spot pathogen, while 2 genes encode anthocyanin synthesis. The comparison results of Blast2PHI database have further shown that among the genes related to blueberry leaf spot disease, the transcription factor is the first major category with 727 genes; The disease resistance and defense category is the second largest category with 200 genes; There are 45 genes related to cell cycle, effector protein and pathogenicity in the third category; There are 7 genes controlling the biosynthesis of a - (1,3) - glucan. Through comparative analysis of differences, 11 genes with resistance to blueberry leaf spot disease are randomly selected from PHI database and DEG database of transcriptome library for Q-PCR detection. According to the results, 8 genes with good amplification specificity are up-regulated and highly expressively induced by β- Aminobutyric acid in blueberry leaves with resistance against disease, but are not found in the control samples, so these eight genes are the β-Aminobutyric acid-induced genes related to blueberry resistance to leaf spot, which is confirmed by further experiment results [20].

The functions of two induced resistance genes, including Comp126500_c0_seq1 and Comp126556_c0_seq2, have been selected and analyzed by three types of bio-informatics methods to study its expressed protein structure, including constructing a phylogenetic tree, GO enrichment analysis, NCBI sequence alignment. The results by constructing a phylogenetic tree have shown that the protein encoded by the gene Comp126500_c0_seq1 contains 1077 amino acids, which has the closest genetic relationship with the disease resistant protein in kiwifruit, and it is further estimated that there are 2 conservative domains in the protein Comp126500_c0_seq1. The estimated results of both secondary and tertiary protein structures has shown that the protein contains 38 α- Spiral, 32 β- Folding, 14 protein kinase phosphorylation sites, and 6 glycosylation sites. GO enrichment analysis has further revealed that

Comp126500_c0_seq1 protein is involved in cellular functions including DNA repair, regulating biological processes such as RNA splicing, and extracellular secretion. The results of NCBI sequence alignment of its amino acid sequences reveals that the Comp126500_c0_seq1 protein mainly aligns with the disease-resistant proteins of Chinese gooseberry, Populus euphratica, oak, and durian; For the gene Comp126556_c0_seq2, the phylogenetic tree constructed shows that the Comp126556_c0_seq2 protein encoding 1167 amino acids has the closest genetic relationship with the disease resistant proteins in wild mulberry silkworm and mountain jute. It is estimated that the Comp126556_c0_seq2 protein contains 3 conservative domains, and the secondary and tertiary structures of the protein indicate that the protein contains 45 α- Spiral, 16 β- Folding, 13 protein kinase phosphorylation sites, and 9 glycosylation sites. GO enrichment analysis shows that Comp126556_c0_seq2 is involved in biological processes such as DNA repair, receptor recovery, and mediated transportation. The NCBI sequence alignment of its amino acid sequence has further revealed that the Comp126556_c0_seq2 protein is mainly aligned with the disease resistance genes of citrus, wild silkworm, potato, pepper, as well as the leu-enriched protein of castor bean [20].

6.Specificity in Environmental Conditions for Disease Breaking Out

In above case studies, epidemic disease usually breaks out in the specific season through specific pathways located in specific geographic site. There are several examples with regards to the above case studies: bacterial leaf streak generally occurs from the transplanting stage to the heading stage in rice crops between July and August. Sliver disease spreads through wind and rain, and is prone to break out widely [30]; After invading cotton plants, the falling leaves of the diseased plants in the soil become an important pathway for the spread of verticillium wilt. Although the cotton seeds carry low rate of pathogens, it is another important way to expand the disease area. In the suitable temperature range of 25 to 28 ℃, excessive rainfall often leads to severe verticillium wilt disease in the field [31]; Banana wilt can be mainly spread through diseased seedlings, soil, running water, tools, wheels with mud, shoes, banana stumps, etc. The disease is severe when roots are damaged, plants are continuous cropping for several years, soil drainage is poor, fertility is poor, air permeability is poor, soil is acidic, soil temperature is high, or soil is barren [32]. The pathogens of maize curvularia leaf spot disease overwinter on the diseased residue of plant, becoming the important pathways spreading diseases. The disease is prone under high-temperature and high-humidity, which tends to break out during high-humidity or rainy seasons from July to August [33]; COVID 19 virus transmission pathways mainly include direct droplet transmission, aerosol transmission and contact transmission, which breaks out more severely during cold winter seasons reported by news [34].

7.Conclusion and Implication for Future Research

In the long-term evolutionary process, pathogens are evolved into specific gene sequences of pathogenicity, expressed as specific enzyme species against specific host cells (such as degradation of host cell wall), which are induced by specific external environmental conditions; correspondingly, host cells are also evolved into specific gene sequences of immunology/resistance, expressed as specific inhibitor enzyme species against specific invasive pathogen species, which are induced by the specific bio-signals released by specific pathogens. This is the overall philosophy of host-pathogen interaction at molecular biology study level. Based on the specificity in the interaction between pathogen invasion and host organism, this article designs a new table covering the systematic indicators to identify and classify the pathogen strains in Table 1. Both virus and bacterial classification would help to improve the accuracy in the identification of pathogen strains. This is especially for epidemic pathogens with broad infection rate (such as COVID 19), because if there is only one indicator (such as genotype) to analyze, the specificity in the interaction between pathogen invasion and host organism may not be apparent, but the specific virus strain would be much more accurately identified once there are comprehensive indicators selected to analyze below, targeting specific host population.

Table 1. The systematic indicators identifying and classifying the pathogen strains.

	Pathogen genotype	Pathogen phenotype	Host cell genotypes infected	Host cell phenotypes infected	Host cell genotypes and phenotypes with apparent immunology	Season to break out	Geography distribution (Is it origin?)	Pathogen transmission pathways
Pathogen Strain 1								
Pathogen Strain 2								
.....								

Both Table 2 and Table 3 below summarize the enzyme species studied and the representative experiment methods used in above case studies respectively. A novel matrix of Matrix Xsum is designed for the classification across different bio-samples of host cells [1]. However, This classification methods is also applicable on the identification of virus phenotype in the invasion-host experiment designed in my another article [2], and a combination of both genotype and phenotype classification method has been designed in my bacterial article previously [3].

Table 2. Summary of Protein/Enzyme studied in above case studies.

Species	Pathogen/Host	Protein/Enzyme Species	Function
Dogs	Host	Antibiotics [17]	CPV antigen
Infected patients	Host	S protein and N protein [28]	SARS-CoV-2 antigen
Human and animals	Host	RABV glycoprotein [29]	RABV antigen
Xoc	Pathogen	GRP4 protein [5]	Inhibiting plant resistance
Cotton	Host	CAT enzyme , SOD enzyme and MDA enzyme [6]	Resistance enzymes
Shrimp	Host	Antioxidant capacity (T-AOC); catalase (CAT); malondialdehyde (MDA) [9]	Immune Enzyme
Oysters	Host	Catalase (CAT), Malondialdehyde (MDA), superoxide dismutase (SOD) and total antioxidant capacity (T-AOC) [10]	Immune Enzyme
Shellfish	Host	Glutathione peroxidase (GSH-PX), Catalase (CAT), Peroxidase (POD) and acid phosphatase (ACP) [11]	Immune Enzyme
Large yellow croaker	Host	superoxide dismutase (SOD) catalase (CAT); acid phosphatase (ACP);alkaline phosphatase (ALP); lysozyme (LZM) [12]	Immune Enzyme
Shrimp	Host	Acid phosphatase, alkaline phosphatase and lysozyme; superoxide dismutase and total nitric oxide synthase [13]	Immune Enzyme
Blueberry	Host	β- Polyphenol oxidase (PPO), peroxidase (POD), phenylalanine ammonia-lyase (PAL), β-1, 3-glucanase and chitinase [20]	Plant defence enzymes
FOC	Pathogen	Cellulase and amylase Pectinase, fibronectin[22]	Cell wall degrading enzymes
Curvularia spp.	Pathogen	Polygalacturonase (PG), pectin methylgalacturonase (PMG), laccase, cellulase (Cx), and β-Glucosidase [23]	Cell wall degrading enzymes

Table 3. Summary of representative experiment methods chosen in above case studies.

Case studies	Experiment Methods Selected
[17]	Double antibody sandwich method, ELISA
[28]	Enzyme linked immunosorbent assay (ELISA) monoclonal antibody screening system
[29]	Enzyme linked immunosorbent assay (ELISA); monoclonal neutralizing antibodies
[5]	Clone Tech: inserting the targeted gene, obtaining the mutant strains of GD41
[6]	Screening DNA database by bioinformatics; qRT-PCR Technology to identify gene sequences and expression;gene silenced by HIGS technology; enzyme detected by spectrophotometry; Clone Tech: RNAi technology
[7]	Molecular marker of gel PCR or sequencing
[20]	Building transcriptome sequencing library;Randomly selecting from PHI database and DEG database of transcriptome library for Q-PCR detection;Gene functions analyzed by three types of bio-informatics methods to study its expressed protein structure: constructing a phylogenetic tree, GO enrichment analysis, NCBI sequence alignment
[24]	Clone Tech: Gene deletion, yielding mutant strain

As summarized in these articles [5][6][7][8], the majority of research with regards to the interactions between pathogen genotype and host genotype are from plant pathology studies, whereas pathological studies of mammals have seldom chosen this methodology to analyze, although limited studies have screened specific gene sequences on goat species infected by Paratuberculosis [19], which should become the research gaps to fill in. However, even for the plant pathology studies, they either emphasized on the pathogen genotype only such as [5][6] or focused on the host genotype only such as [7][8], without further discussing the interactions between both of them, which may become the shortage to exactly target the disease control methods. The representative case studies identifying the specific gene sequences between pathogen and host are summarized in Table 4 below. It is further noted that specific gene sequences that plays the key role in pathogen invasion physiology have been screened in the study [5][6]. In comparison, various gene mutation types of COVID-19 virus have been summarized by Banda et al.,(2023), followed by the public emotional responses on Twitter social media [15]. However, among these gene mutation sequences, much fewer studies have conducted the specificity research pointing out which specific gene sequence expressed as the invasive physiology like studies [5][6], as my another article argues that ‘only a small proportion of

occurrences in gene mutation may be directly related to the characteristics of virus invasive biology against host cells, which consequently should not overstate Big [16],' avoiding unnecessary public nervous or panic emotions during pandemic period.

Table 4. Representative case studies of gene sequences expressed as pathogenicity and host immunology.

Species	Gene Sequences	Gene Function
Xanthomonas oryzae pv.oryzicola (Xoc) [5]	TALIxoc gene sequences: mutant from TALE gene inserted by Tn5 Transposon	Pathogenicity against rice host
Verticillium dahliae [6]	VdxyL3 and VdITP1 genes	Pathogenicity against cotton host
Corn [7]	1.qRfg1; 2. Rcg1; 3. Htn1; 4.ZmCCoAOMT2	Host resistance against disease: 1.Stem rot disease caused by Fusarium graminearum; 2. Anthrax stem rot; 3. Leaf blight; 4. Grey speck disease
Goat [19]	MHC II - DRB3 gene region	Disease Resistance against *Mycobacterium paratuberculosis* infection

As discussed in these papers [9][10][11][12][13], a number of non-specific immunological enzyme families, mainly including acid phosphatase, alkaline phosphatase, total antioxidant capacity, superoxide dismutase, catalase and malondialdehyde, have been selected as indicators to analyze the effects of baits, high temperature, PH threat, low salts and marine red yeast on the physiology of aquatic species respectively. In these studies, the chosen enzyme species were classified into non-specific ones induced by abiotic environmental stress, but their corresponding isozyme families (enzyme species containing the same functional group) are believed to play important role in host immunology against invasive pathogens as well. As designed in my another metabolomics article [14], specific enzyme species may be identified at exact molecular weight level within an isozyme family which contains one of the above functional groups of immunological enzyme, which may become the improved methods to analyze the environmental effects on the aquatic physiology. The above functional groups of immunological enzyme becomes the indicator to synthesize the new isozyme primers used in isozyme electrophoresis experiment.

Illustrated in case studies [17][28][29], the specificity in host antibiotics has been utilized to diagnose specific virus species commonly by ELISA methods. However, these specific antibodies identified in case studies also become the ideal indicator for the development on the synthesis of primers to the new isozyme functional groups.

In the biological control paper that has been discussed previously [35], the evolution of insect resistance to bacteria *S.entomophila* displays as a spiral interaction, revealing that this DNA shaping evolution is determined by natural selection so that the sustainable diversity of genetics in *S.entomophila* population is essential for passing on the sets of gene expressed as pathogenicity to the next generations. Correspondingly, it is also reported that the increasing number and type of the aggregation of stripe rust resistance genes enhances the level of plant resistance [21], so this article implies that host gene diversity in disease resistance function plays the key role in the host defence system against multiple bacterial communities under various and changing environment scenarios. Consequently, the specificity in pathogen-host interaction is evolved into multiple gene diversity under the changing environment in nature.

Without adopting Clone Technology, my environmental study tries to design a more natural method to screen the gene traits for functional biology study by summarizing above case studies, taking epidemiology disease as an example below:

Step 1. Bio-samples of both host cells and pathogens are cultivated separately in Lab, and then simulation of pathogen invasion against host cells is conducted under Lab conditions. Then different phenotypes of both pathogen and host cells are identified and classified.

Step 2. To screen and identify the specific isozyme families involved in the invasion-immunology interactions through isozyme electrophoresis experiment, by comparing each phenotype of pathogen or host cell with the corresponding background bio-samples that are cultivated without implementing the simulation of pathogen invasion against host cells. It is believed that cell immunology function or pathogen pathogenicity is influenced by multiple enzyme species from different isozyme families, so the Principal Components Analysis (PCA) needs to be conducted to quantitatively analyze which isozyme families and which enzyme species explain the highest variation in the matrix of zymograms produced by isozyme electrophoresis experiment. Matrix Sn_{sum} and Matrix S are designed in my previous article [1] for this quantitative analysis to screen which isozyme families and which enzyme species respectively involving in the interaction between pathogen invasion and host cells. Then the specific enzyme species within an isozyme family can be further identified at exact molecular weight level, and the corresponding bio-materials of enzyme species identified in electrophoresis pipes can be collected for further analysis (such as to further characterize the structure of enzyme molecule by structural biology study for bio-medicine production);

Step 3. According to the functional groups of isozyme family screened in above experiment, preliminary screening of gene sequences can be implemented in Gene Bank by using bio-informatics technology such as case study [20], aiming to the design of primers for Polymerase Chain Reaction (PCR) experiment; After PCR

experiment testing each phenotype and background bio-sample, the polymorphism gene loci are screened by comparing different electrophoresis bands among various phenotypes and background sample.

Step 4. The gene materials identified in the electrophoresis bands can be collected for the purpose of further gene sequencing experiment, leading to the specific gene sequences identified in the polymorphism gene loci;

Step 5. The correlation between the specific gene sequences identified and the gene expression as the specific enzyme species are analyzed by comparison across different phenotype bio-samples and the background ones in the interaction between pathogen invasion and host defence. The specific enzyme species within an isozyme family can be identified and separated by Non-denaturing polyacrylamide gel electrophoresis firstly, and the correspondingly specific enzyme materials can be collected from the electrophoresis pipes for further enzyme activity analysis. The specific enzyme concentration can be still measured by spectrophotometry in water solution after Non-denaturing polyacrylamide gel electrophoresis, which becomes the gene expression indicator to analyze the correlation between the specific gene sequences and the gene expression as the specific enzyme species.

Step 6. Gene Silencing technology is selected to further identify the gene traits of specific gene sequences screened in above experiment, which have been applied over a broad range of biological kingdom, including plants, nematodes, fungi, insects, protozoa and mice [18].

Step 7. After the specific gene sequences with specific expression function is identified, the corresponding gene sequences can be used to design the DNA probes in FISH technology, which is applicable on the gene mapping technology. Then this specific gene sequences can be mapped on the specific genome.

In summary above, the full specificity research in the interaction between pathogen invasion and host organism should identify the specific gene sequences expressed as specific enzyme species at molecular weight level in the invasion - immunology physiology process, under the regulator of specific abiotic environmental conditions. This is the one of the principal natural Laws underlying evolutionary process in natural ecosystem.

The advantages of the new experiment methods designed above: firstly, the gene traits are screened by natural methods, other than the anti-nature method of Clone Technology, so that all the molecular biological functions are studied under the natural Law by evolutionary genetic analysis. My environmental study insists The Principle of Nature: only when the genetic resource is naturally evolved, the creatures can pass on it into their offspring through the reproduction process; Secondly, this experiment starts the screening process by screening the specific isozyme families involved in the

functional process at first, which should become more efficient and save time, because the total information of functional groups in isozyme families is much more limited than the DNA sequences database and the specific enzyme species playing the key role in the functional process can be easily identified and separated in the electrophoresis pipes for further analysis. In my experiment method, the specific enzyme is identified and separated at molecular weight level by using isozyme experiment firstly, based on which the enzyme activity is further analyzed by spectrophotometry in water solution, obviously improving the accuracy of analyzing method as compared with only using spectrophotometry to detect the enzyme activity in case studies; If other biological functions (such as drought tolerance, photosynthesis, reproduction traits or other genetic diseases) are chosen for study, the phenotype identification process in step 1 of above experiment method would be replaced accordingly, which requires to screen different isozyme families correspondingly.

My molecular structural biology article has discussed that: " However, within the whole biological organism, each biological function is correlated with the other functions, so each enzyme species expression would be correspondingly more or less correlated with the other enzyme species' expression as well. Consequently, it is necessary to screen the key enzyme species that plays the most important role in a specific biology function (such as antibody synthesis against specific pathogen) by using bio-informatics methods.[37]" This means that relying on the correlation test only may not successfully identify the key enzyme species regulating the specific biology function. My another article has critically discussed the deficiency in linear math: lack of time sequence among different enzyme species' gene expression [38]. To solve the limitations of bio-informatics, it is to next discuss the screening process for the key enzyme species: my article has proposed that "the memory of cells, in terms of identifying the bio-signals of a specific environmental factor (can be biotic or abiotic) triggering the gene expression for environmental adaptiveness or immunology, can be trained and strengthened by the biophysical simulation of other environmental factors, because multiple gene traits of environmental adaptiveness or immunology should be located in the same linkage group of genome [39]." This viewpoint is further supported by my another article demonstrating that the physiological resistance responses to different environment stress conditions may go through the same biochemistry pathways so that they strengthen each other, indicated by specific enzyme species, which have been proven by multiple case studies [40]. Consequently, in addition to the simulation of pathogen invasion against host cells in step 1 above, it is advised to simulate more environmental stress conditions (such us UV-B radiation) for the host cells, together with host-pathogen invasion simulation. Host cells' immunology against invasive pathogens may go through the same physiology pathway as the resistance under environmental stress, so the mutual strengthening of physiological pathway may help to identify the significance of specific enzyme species regulating immunology.

References:

[1]. Liu Huan. (2021). Metabolomics and Application on The Specificity of Host-Invasion Interaction. Journal of Environment and Health Science (ISSN 2314-1628). Feb. 2021. https://doi.org/10.58473/JBS0006
[2]. Liu Huan. (2021). The Identification of Pathogen Phenotype for Biophysical Simulation. Journal of Environment and Health Science (ISSN 2314-1628), 2021(02).https://doi.org/10.58473/JBS0010
[3]. Liu Huan. (2021). Classification of Bacteria by DNA Genetic Marker and Its Theory. Journal of Environment and Health Science (ISSN 2314-1628), 2021(02).https://doi.org/10.58473/JBS0001
[4]. Liu Huan (2022). Epidemiology------Comparison between indoor and outdoor air quality at three representative sites in Auckland Center. Journal of Environment and Health Science (ISSN 2314-1628), 2022(12).https://doi.org/10.58473/JEHS0010
[5]. 王稳占. 水稻细菌性条斑病菌效应子编码基因 TALIXoc 克隆及对水稻抗病性的抑制作用[D].南京农业大学,2019.DOI:10.27244/d.cnki.gnjnu.2019.000691.
[6]. 张小雪. 黄萎病菌 VdxyL3 和 VdITP1 基因对棉花的抗病性影响[D].石河子大学,2021.DOI:10.27332/d.cnki.gshzu.2021.000635.
[7]. 赵子麒,赵雅琪,林昌朋等. 重要玉米种质抗病性与抗病基因精准鉴定[C]//中国作物学会.第十九届中国作物学会学术年会论文摘要集.[出版者不详],2020:1.DOI:10.26914/c.cnkihy.2020.047937.
[8]. 汪全超,陈帅飞.华南地区 8 种桉树基因型对 Calonectria pentaseptata 的抗病性研究[J].桉树科技,2020,37(02):1-9.DOI:10.13987/j.cnki.askj.2020.02.01.
[9]. 李晨露,李京昊,成永旭等.不同饵料对克氏原螯虾仔虾养殖水质、生长、消化酶和非特异性免疫酶的影响[J].上海海洋大学学报,2022,31(04):883-892.
[10]. 岳绍波,王昭萍,张学开等.高温对长牡蛎与熊本牡蛎及其杂交子代的免疫酶活性及杂种优势的影响[J].中国海洋大学学报(自然科学版),2022,52(S1):116-122.DOI:10.16441/j.cnki.hdxb.20200295.
[11]. 丁瑞霞,黄星美,赵旺等.pH 急性胁迫对方斑东风螺行为及免疫酶活性的影响[J].渔业现代化,2022,49(06):84-90.
[12]. 何亮银,史晓丽,周逢芳等.低盐胁迫对大黄鱼非特异性免疫酶活力的影响[J].应用海洋学学报,2022,41(02):347-354.
[13]. 何玉,朱彦博,田相利等.海洋红酵母不同添加剂量对凡纳滨对虾生长、免疫酶活性及抗病力的影响[J].海洋湖沼通报,2022,44(04):33-41.DOI:10.13984/j.cnki.cn37-1141.2022.04.005.
[14]. Liu Huan. (2021). Metabolomics (1) --- The Systematic Chemistry Fingerprints Between Genotype and Phenotype and its Application on the Conservation Genetics. Journal of Environment and Health Science (ISSN 2314-1628), 2021(02).https://doi.org/10.58473/JEHS0005
[15]. Banda, Juan M., Tekumalla, Ramya, Wang, Guanyu, Yu, Jingyuan, Liu, Tuo, Ding, Yuning, Artemova, Katya, Tutubalina, Elena, & Chowell, Gerardo. (2023). A large-scale COVID-19 Twitter chatter dataset for open scientific research - an international collaboration [Data set]. In Epidemiologia (Version 154, Vol. 2, Number 3, pp. 315–324). Zenodo. https://doi.org/10.5281/zenodo.7655041.

[16]. Liu Huan. (2021). Discussion on Gene Sequencing and Classification of DNA Genetic Markers. Journal of Environment and Health Science (ISSN 2314-1628), 2021(05).https://doi.org/10.58473/JBS0019
[17]. 刘宏伟,田克恭,渠川玫等.犬细小病毒酶标试剂盒的研制[J].中国畜禽传染病,1994(06):10-13.
[18]. 基因沉寂。搜狗百科。
[19]. 丁涵. 山羊副结核病的检测和MHC-DRB3基因与副结核病感染相关抗病性的初步研究[D].内蒙古农业大学,2021.DOI:10.27229/d.cnki.gnmnu.2021.001066.
[20]. 金义兰. β-氨基丁酸诱导蓝莓对叶斑病的诱导抗病性及其基因表达研究[D].贵州大学,2019.DOI:10.27047/d.cnki.ggudu.2019.000061.
[21]. 张一博. 中国冬小麦主产区候选品种条锈病抗病性评价与基因检测[D].西北农林科技大学,2022.DOI:10.27409/d.cnki.gxbnu.2022.000214.
[22]. 周端咏. 香蕉枯萎病菌 Fostel2 基因的功能研究[D].海南大学,2012.
[23]. 吕宾,夏淑春,张茹琴等. 玉米弯孢叶斑病菌(Curvlaria lunata)漆酶的致病性、基因克隆与黑色素合成相关性分析[C]//中国植物病理学会青年委员会.植物病理学研究进展——中国植物病理学会第十二届青年学术研讨会论文选编.中国农业大学出版社（CHINA AGRICULTURAL UNIVERSITY PRESS）,2015:1.
[24]. 我国首次发现降低非洲猪瘟病毒致病性基因[J]. 致富天地,2021,No.269(05):54.
[25]. 刘文平,吴宪,郭东梅等.水稻白叶枯病致病性基因研究进展[J].黑龙江农业科学,2014,No.240(06):140-144.
[26]. Functional analysis of pilQ gene in Xanthomanas oryzae pv. oryzae , bacterial blight pathogen of rice[J]. Seon-Hwa Lim;;Byoung-Ho So;;Ji-Chun Wang;;Eun-Seong Song;;Young-Jin Park;;Byoung-Moo Lee;;Hee-Wan Kang.The Journal of Microbiology, 2008(2).
[27]. Functional analysis of the aroC gene encoding chorismate synthase from Xanthomonas oryzae pathovar oryzae[J]. Eun-Sung Song;;Young-Jin Park;;Tae-Hwan Noh;;Yeong-Tae Kim;;Jeong-Gu Kim;;Heejung Cho;;Byoung-Moo Lee.Microbiological Research,2011(6).
[28]. 李妮. 抗新型冠状病毒特异性抗体制备及应用研究[D].北京协和医学院,2022.DOI:10.27648/d.cnki.gzxhu.2022.000505.
[29]. 田玉玲. 基于竞争 ELISA 快速检测狂犬病病毒中和抗体的研究[D].华中农业大学,2022.DOI:10.27158/d.cnki.ghznu.2022.000261.
[30]. 水稻细菌性条斑发生在什么时期？农业种植网。
[31]. 棉花枯萎病 黄萎病. 科普中国。
[32]. 香蕉枯萎病的发生与防控技术。中国农垦（热作）网。
[33]. 玉米弯孢霉菌叶斑病防治方法。沈阳市科学技术协会“科普惠农 专家送科技下乡”项目而建立的云端信息发布及交流平台。
[34]. 新冠病毒。搜狗百科。
[35]. Liu Huan (2021). Biodiversity Conservation & Management Studies in New Zealand. 2021 (02). Journal of Environment and Health Science (ISSN 2314-1628).https://doi.org/10.58473/JEHS0008
[36]. Liu Huan. (2021). The specificity of host-pathogen interactions with emphasis on the specific inhibitor enzyme synthesized in the invasion-defense process. Journal

of Environment and Health Science (ISSN 2314-1628), 2021(2). https://doi.org/10.58473/JBS0018
[37]. Liu Huan. (2022). Essay: Structural Molecular Biology. Journal of Environment and Health Science (ISSN 2314-1628), 2022(08). https://doi.org/10.58473/JBS0021
[38]. Liu Huan (2024). Original Review of Biodiversity Conservation Progress in China: The Theories and Cases in Practice. Journal of Environment and Health Science (ISSN 2314-1628). 2024(04). https://doi.org/10.58473/JEHS0012
[39]. Liu Huan. (2021). Metabolomics and Immunology Cultivation. Journal of Environment and Health Science (ISSN 2314-1628), 2021(02). https://doi.org/10.58473/JBS0008
[40]. Liu Huan (2024). Original Review of Environmental Physiology: Photosynthesis and Systematic Metabolomics. Journal of Biological Sciences (ISSN 2958-4035).2024(07). https://doi.org/10.58473/JBS0024