The application of endophytic bacteria *Methylobacterium radiotolerans* to protect a strain of rice from potential fungal or bacterial infection

Shiyang Gong[1], Maximus Keller[1], and Jinhui Wang[1]

[1]Western Reserve Academy, Hudson, Ohio, United States;
[2]Northwestern University, Evanston, Illinois, United States

Reviewed on 7 May 2022; Accepted on 25 June 2022; Published on 15 October 2022

The world consumed over 504 million metric tons of rice in 2020. Rice is an essential part of many people's diet, and its large quantity and low price make it a necessity for those in developing countries. However, bacterial and fungal infections severely damage the production of this vital crop. Our team proposes the usage of endophytes, which are symbiotic, non-pathogenic bacteria in between the cell walls of plants, to fight diseases. We plan to insert various anti-microbial genes, such as antifungals, chitinase and beta-1 3-glucanase, and anti-bacterial gene anti-*Xoo* Xa4, into the specific endophyte *Methylobacterium radiotolerans*. By inserting one plasmid that encodes anti-fungal and anti-microbial genes into *M. radiotolerans*, an endophyte that transfers from the parent to the offspring via seeds, the endophyte would able be to combat various pathogens and thus protect the crop after transforming into the plant. In the first stage, we plan to practice a prototypical *in vivo* experiment with *Escherichia coli* and examine whether the endophyte produces the desired antibacterials and antifungals with SDS-PAGE. Then, we will transform the plasmid into *M. radiotolerans*, insert the endophyte into the rice plant, and expose the rice plant to bacterial blight and rice blast in another *in vivo* experiment. Lastly, we will grow the rice seeds and examine whether the endophyte and gene expression continues to exist in the offspring. Our project aims to increase crop yields by limiting disease and aiding in feeding the expanding population worldwide.

Keywords: *Oryza sativa*, *Methylobacterium radiotolerans*, vertical transmission, chitinase, Xa4

Mentors: Beth Pethel[1], and Kosuke Seki[2]

Direct correspondence to gongs23@wra.net; wangj23@wra.net; kellerm22@wra.net; pethelb@wra.net

Watch a video introduction by the authors at https://youtu.be/NhwN-JpTSwA

Background

Rice *(Oryza sativa)* is a staple food that grows globally and feeds more than half the world's population. According to projections, rice will remain a staple food source with a growing population (Fukagawa & Ziska, 2019). The importance of rice extends beyond providing a stable source of carbohydrates and fibers. Costing 71 cents per pound in 2019, rice is an affordable part of a meal for many families (Shahbandeh, 2021). Historically, changes in rice availability have led to social unrest, such as the food crisis of 2008, where rice cost tripled and pushed an estimated 100 million people below the poverty line, which is $1.90 a day. Therefore, rice is a fundamental and irreplaceable crop in many countries.

Various fungal, bacterial, or nematode infections threaten this vital crop (see table 1). Common rice diseases include rice blast caused by *Magnaporthe oryzae*, rice bacterial blight caused by *Xanthomonas oryzae* pv. *oryzae*, root-knot caused by the nematode *Meloidogyne graminicola*, rice white tip caused by the nematode *Aphelenchoides besseyi*, and ufra disease caused by *Ditylenchus angustus* (Liu & Wang, 2016). These pathogens cause significant crop loss every year. For instance, rice blast is responsible for over 30% of rice production loss worldwide (Liu & Wang, 2016; Nalley et al., 2016). *M. oryzae* is a fungus that infects the entire rice crop, resulting in the most significant damage when it infects the base of the stem, or the panicle, which is the cluster of flowers (Nalley et al., 2016). The damage caused by different pathogens varies. For example, rice bacterial blight caused by *Xanthomonas oryzae pv. oryzae* invade the xylem vessels, which are water-conducting vessels in plant, thus causing rots on rice leaves such like the ones shown in Figure 1 (Liu & Wang, 2016).

Figure 1: *Rice blight on the stem of a rice plant Donald Groth, Louisiana State University AgCenter, Bugwood.org*

The conventional measures used to prevent bacterial rice blight are ineffective and demanding. These treatments include: using a balanced amount of nutrients, especially nitrogen, ensuring good drainage of fields, and keeping fields clean, which includes removing weeds and plowing under rice stubble and more, which can serve as hosts for bacteria. Fallow fields are plowed but left unsown to restore soil fertility and left to dry in order to suppress disease agents in the soil and plant residues (*Bacterial Blight*, n.d.). The most common solution for bacterial blast is the use of fungicides. Unfortunately, fungicides are harmful to aquatic organisms—especially microorganisms and invertebrates—which are rudimentary components of the aquatic food chain (Zubrod et al., 2019). Since fungicide exposure in water is common, its malevolence towards water organisms

Table 1: *Summary of common rice diseases and pathogens*

Name	Pathogen Type	Disease	Infected Region
Magnaporthe grisea	Fungi	Blast	leaf, neck, nodal
Xanthomonas oryzae pv. oryzae	Bacteria	Bacterial blight	leaf
Ditylenchus angustus	Nematode	Ufra Disease	stem
Pseudomonas fuscovaginae	Bacteria	Brown Sheath rot	sheath
Burkholderia glumae	Bacteria	Grain rot	seed
Fusarium proliferatum	Fungi	Root rot	root
Aphelenchoides besseyi	Nematode	Rice White Tip	leaf
Gaeumannomyces graminis	Fungi	Crown Sheath Rot	sheath
Meloidogyne graminicola	Nematode	Rice root knot	root
Ceratobasidium oryzae-sativae	Fungi	Aggregate Sheath	sheath

makes it unfriendly to the environment (Zubrod et al., 2019). Furthermore, fungicides are expensive, costing over $70 per 10,000 square meters on average (Nalley et al., 2016). The intensive use of chemicals to control rice blast can result in blast pathogens gaining resistance to the antibiotic compounds and organophosphorus fungicides (Nalley et al., 2016). Another solution is genetically modified rice strains that are specifically immune to specific pathogens (Nalley et al., 2016). Although genetically modified seeds are commercially available worldwide, they are expensive to most consumers: genetically engineered strains cost over $237.12 per hectare (Nalley et al., 2016).

Our solution inexpensively combats the previously mentioned pathogens by genetically engineering vertically transmitted endophytes to produce antifungals and antibacterials. Endophytes are symbiotic microorganisms dwelling between plant cells (Gouda et al., 2016). These microbes release compounds with various enhancive abilities, including improving the plant's growth rate or combating foreign pathogens, and thus are beneficial to the plant's overall well-being (Gouda et al., 2016). Inhabiting various regions of a plant, endophytes can be fungi or bacteria (Gouda et al., 2016). Their wide range of habitats within the plant and their ability to release bioactive antimicrobial or antifungal compounds make them a prime candidate for use against plant pathogens (Gouda et al., 2016). The transmission of endophytes can be both horizontal and vertical. Horizontal transmission is between two nearby yet non-related plants via soil, and vertical transmission is the transmission between the parent plant and its offspring via seeds or pollen (Frank et al., 2017). Endophytes that vertically transmit do so naturally by inhibiting the seeds or pollen of their host plant (Frank et al., 2017). Due to this ability, our design does not involve a seed coating, as endophytes live within the host plant. In our design, we use the vertically transmitted bacterial endophyte *M. radiotolerans* because it inhabits the entire plant, including the seeds, which allows it to target pathogens invading different regions of the plant and pass down genes through generations (Kaga et al., 2009).

Our design proposes the insertion of antimicrobial-producing genes into *M. radiotolerans* through plasmids. This design would enable *M. radiotolerans* to combat pathogenic fungus and bacteria. The project incorporates a category of antifungals called lytic enzymes, which hydrolyze or break down a specific component of the microbial cell wall (Kobayashi et al., 2002; Tian et al., 2007). Chitinase binds with and hydrolyzes chitin, a polysaccharide and constituent of the cell wall of *M. oryzae*, resulting in cell death (Kobayashi et al., 2002). We retrieved the chitinase gene sequence from *Strenotropho maltophilia*, an endophytic bacterium that secretes chitinase to suppress rice blast fungus *M. oryzae* (Etesami & Alikhani, 2016; Kobayashi et al., 2002;). ß-1 3-glucanase has a similar antifungal mechanism as chitinase, except it targets ß-1 3-glucan of *M. oryzae* (Mouyna et al., 2013). Studies reveal that ß-1 3-glucanase can successfully regulate the growth of the pathogen *Fusarium*, including *Fusarium proliferatum*. This pathogen causes rice spikelet rot disease, a panicle disease that leads to rice grain rot (Lei et al. 2019; Ueki et al., 2020;). We retrieved the ß-1 3-glucanase gene from *Streptomyces sioyaensis*, an endophyte that regulates the growth of *Fusarium oxysporum* (Hong et al., 2002). The inclusion of two antifungals provides *M. radiotolerans* with the ability to combat most pathogenic fungi and thus increase the rice strain's resistance toward these fungus..

Our design includes the addition of the anti-*Xoo* gene Xa4 to enhance resistance to bacterial infections, such as rice blight caused by *X. oryzae* pv. *oryzae*, or *Xoo*. Xa4 gene commonly exists in rice crops in China, and it encodes a kinase that promotes cell wall synthesis of rice crops and thus enhances the cell wall's durability and renewability (Hu et al., 2017). This kinase prevents *Xoo* from creating white and milky lesions on rice leaves that lead to the leaf's death (Grant, 2021; Hu et al., 2017). Since *Xoo* infects the surface wounds of plants, enhancing cell wall durability and renewability reduces *Xoo* infection likelihood (Hu et al., 2017). Moreover, Xa4 improves the secretion and production of sakuranetin and momilactone A, antimicrobials that combat foreign infection (Hu et al., 2017). Our construct includes pairing the anti-fungal chitinase and Xa4 within the endophyte *M. radiotolerans* to provide immunity against fungal rice rot and bacterial blight. If the design is successful, the endophyte can become a sustainable possibility of securing rice production worldwide.

The insertion of multiple antimicrobials within *M. radiotolerans* would result in endophytes that can combat various pathogens and vertically transmit the antimicrobials through the rice strain to ensure herd immunity against these diseases. We plan to use promoters that only synthesize the anti-microbial genes after pathogens are introduced to the plant to prevent excessive production of antimicrobials that would unnecessarily harm the plant's microbiome. The promoters we selected also help reduce the effect on the environment as there should be no production of antimicrobials in the event *M. radiotolerans* migrates outside of the host plant and would act as it would naturally. Since our project utilizes endophytes to combat diseases and pass resistant genes into future generations of rice, it provides consumers with a sustainable and cheap method to defend rice and

similar crops. Moreover, as it does not involve the direct modification of the plant genome, it would not violate any GMO regulation, thus making it a usable and safe method.

Systems level

Our design, as described in Figure 2, involves the insertion of endophyte *M. radiotolerans* into rice plants. *M. radiotolerans* exists in most parts of the plant, including the seeds, which enables it to vertically transform from one generation to the next (Kaga et al., 2009). However, since *M. radiotolerans* has no records of successful transformation, we would first use *Escherichia coli* to examine the antimicrobial activity of our selected genes. We would insert chitin and OSSWEET14 as promoters into the plasmid. Chitin, a material found in most fungus's cell walls, would activate anti-fungal promoter *pChiIV3*, which then triggers the synthesis of chitinase and β-1 3-glucanase. We would transform the plasmid into *E. coli* via heat shock, and then examine the presence of the desired proteins, which includes chitinase, β-1 3-glucanase, and cell-wall associated kinase. Protein purification and SDS-PAGE are used to detect the presence of these antimicrobial proteins (see Figure 2).

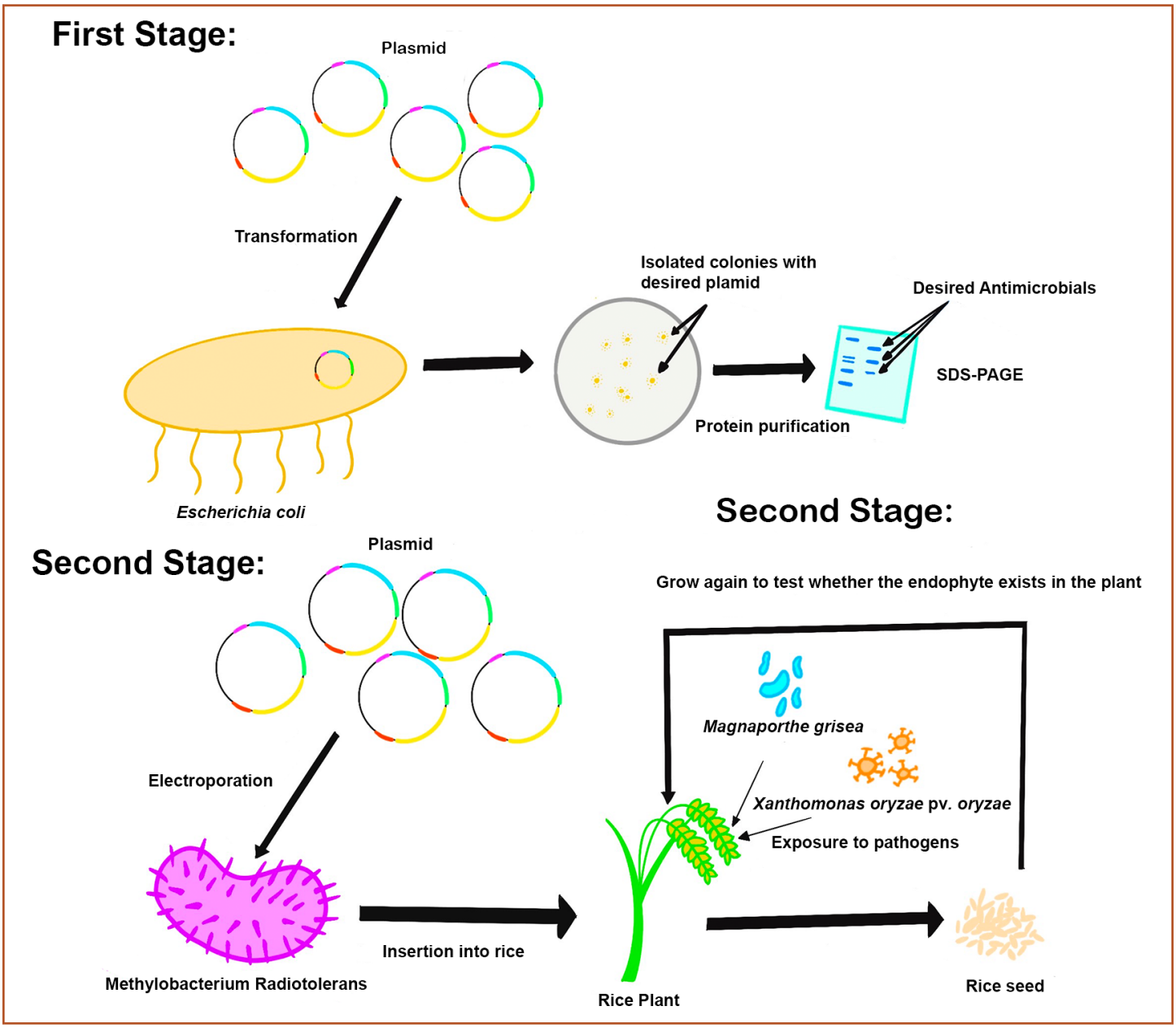

Figure 2: *Graphic description of the first stage and the second stage experiment*

Once this experiment proves the effectiveness of the plasmid, we will transform the plasmid into *M. radiotolerans* via electroporation. We will then transfer the *M. radiotolerans* into the rice plant. Due to endophytes' ability to transfer horizontally from the soil to the roots of the plant, inserting *M. radiotolerans* into the soil enables it to enter the rice roots, and thus transfers into the rest of the crop. Since *M. radiotolerans* naturally exist within the soil, it would not post any harm to the environment after the insertion. We would then practice an *in vivo* experiment where we infect the plant with rice blast fungi *Magnaporthe grisea* and rice blight bacteria *X. oryzae* pv. *oryzae*. We would then assess the antifungal activity of the inoculated *M. radiotolerans* with a modified dinitrosalicylate method, which measures the reduction in chitin and β-1 3-glucan in the infected region. A positive outcome of the *in vivo* experiment would prove that the inoculation of an endophyte containing a plasmid with anti-microbial genes would defend the rice plant from pathogenic infections (see Figure 2).

To further analyze the vertical transformation from the rice plant to the next generation, the seeds of the rice plant would be planted, and another *in vivo* experiment would be performed on the next generation to determine the presence of the plant in the offspring. If antimicrobial activity similar to that expressed in the previous generation is present in the infected region, then the inoculated *M. radiotolerans* has vertically transformed between generations. It also means that *M. radiotolerans* will remain present and active in the entire strain of rice, hence proving that this system would provide immunity against specific pathogens to the entire strain of rice.

Device level

The plasmid design contains the Chitinase A gene, ß-1 3-glucanase gene, anti-bacterial gene *XA4*, chitinase-driven promoter *pChiIV3* transcription activator like effector (TALE) or promoter, the green fluorescent protein, and an ampicillin resistance gene. The chitinase-driven promoter *pChiIV3* promotes the Chitinase A gene and ß-1 3-glucanase gene, while the transcription activator-like effector (TALE) or promoter promotes the anti-bacterial gene *XA4*. The green fluorescent protein acts as a marker protein for the Chitinase A gene, ß-1 3-glucanase gene, and the anti-bacterial gene *XA4*, which enables visual recognition when the three selected genes are expressed.

Parts level

Chitinase A gene, ß-1 3-glucanase gene, antibacterial gene *XA4*, chitinase-driven promoter *pChiIV3*, and transcription activator like (Tal) effector or promoter will be assembled onto expression vector backbone pTE100. As a derivative of IncP plasmid pDN19 in *Methylobacterium extorquens* AM1, pCM80 is an expression vector that exhibits high-level gene expression (Mark & Lidstrom, 2001). Due to its identity as an IncP plasmid found in *M. extorquens* AM1, a well-researched methylotroph that belongs to the same genus as *M. radiotolerans*, pCM80 demonstrates high-level gene expression in other methylotrophs, including *M. radiotolerans*, as well as other gram-negative bacteria such as *E. coli* (Mark & Lidstrom, 2001). This broad-host range and high-level gene expression makes pCM80 the most ideal backbone of our plasmid in the first experiment with *E. coli* and the further experiment with *M. radiotolerans*. However, pCM80 includes an existing promoter *mxaF* and lacks a multiple cloning site, which restricts us from freely inserting our parts into the vector (Mark & Lidstrom, 2001; Schada von borzyskowski et al., 2014). Thus, instead of directly utilizing pCM80, we will use the brick vector pTE100: a modified vector of pCM80 that removes the existing primers and inserts a multiple cloning site (Schada von borzyskowski et al., 2014). We retrieve the gene sequence of the backbone from Addgene with catalog number 59395 (Schada von borzyskowski et al., 2014). The pTE100 also includes a tetracycline resistance gene *tetA*, which makes it a selectable marker during transformation (Schada von borzyskowski et al., 2014). Bacteria, after the transformation, would grow on agar containing tetracycline, which would provide colonies with only the bacteria containing the plasmid after the transformation.

Both chitinase and ß-1 3-glucanase are glycosyl hydrolases that hydrolyze common carbohydrates constituting fungal cell walls (Tian et al., 2007; Kobayashi et al., 2002). Chitinase catalyzes the hydrolysis of chitin, and ß-1 3-glucanase catalyzes the hydrolysis of ß-1 3-glucan (Mouyna et al., 2013). These enzymes' hydrolytic activity inhibits the spore germination of various pathogenic fungi in plants and, therefore, efficiently reduces the proliferation of the pathogen within the plants (Chernin et al., 1997). In our design, we incorporated a specific chitinase gene *ChiA*. Kobayashi et al.(2002) discovered this gene in gram-negative bacteria *S. maltophilia*, an endophytic bacterium that displays chitinolytic activity on various fungal pathogens. We retrieved ß-1 3-glucanase from *S. sioyaensis*, another endophytic bacterium that represses the proliferation of fungal pathogen *Fusarium Oxyporum* through the hydrolysis of the fungal cell wall (Hong et al., 2002). We retrieve the *S. maltophilia* chitinase A gene *ChiA* from GenBank with access number AF014950, and *S. sioyaensis* endo ß-1 3-glucanase gene from GenBank access number AF217415 (Hong et al., 2002; Kobayashi et al., 2002).

To combat bacterial infections such as *X. oryzae* pv. *oryzae*, we included the anti-bacterial gene *XA4*. As a gene commonly found in mutated rice crops, *XA4* synthesizes a cell wall-associated kinase that enhances the production of cellulose and thus increases the rigidness of the plant cell wall (Hu et al., 2017). This mechanism improves the rice's resistance to various bacterial pathogens, including rice blight pathogen *X. oryzae* pv. *Oryzae* (Hu et al., 2017). Although the specific mechanism in which the cell wall-associated kinase enhances the cell wall rigidness remains unknown, researches show that the presence of *XA4* correlates with the induction of cellulose-producing gene *CesA* expression, thereby improving the overall cellulose level in the rice plants' cell walls (Hu et al., 2017). Moreover, *XA4* enhances the secretion and production of sakuranetin and momilactone A, which are both phytoalexins that have records of suppressing rice bacterial blight pathogen *X. oryzae* pv. *oryzae* (Hu et al., 2017). Therefore, inserting *XA4* into the endophyte enables *M. radiotolerans* to facilitate the resistance to bacterial pathogens. We obtain the gene sequence of *XA4* with GenBank access number KU761305 (Hu et al. 2017).

To prevent the overproduction of antimicrobials that potentially undermines the overall health of *M. radiotolerans* or the rice plant, the synthesis of antimicrobials should only start after the first exposure to the disease. To achieve this goal, we selected primers sensitive to the components of the pathogens. Chitin-driven promoter pChiIV3 is a promoter found in pepper plants that initiates the synthesis of pepper chitinase gene ChiIV after exposure to fungal pathogens (Liu et al., 2017). As formerly mentioned, chitin is a common constituent of fungal plant cells, therefore, the exposure of chitin to fungal pathogens would activate the synthesis of the anti-microbial gene (Liu et al., 2017). As shown in Figure 3, by inserting pCHiIV3 before all the antimicrobial genes, our device would only start the synthesis of the anti-microbial after the first exposure to the fungal pathogens.

Transcription activator-like effectors (TALEs) are effectors released by bacterial pathogens that bind

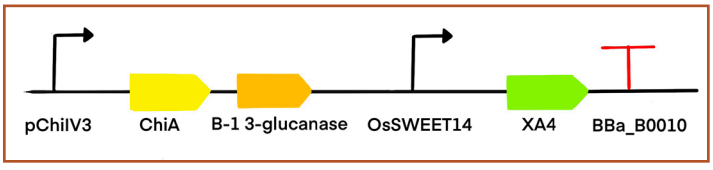

Figure 3: *Graphic description of the plasmid function and mechanism. pChiIV3 and OsSWEET14 would individually initiate the synthesis of the antimicrobials based on the type of pathogen that caused the infection. .*

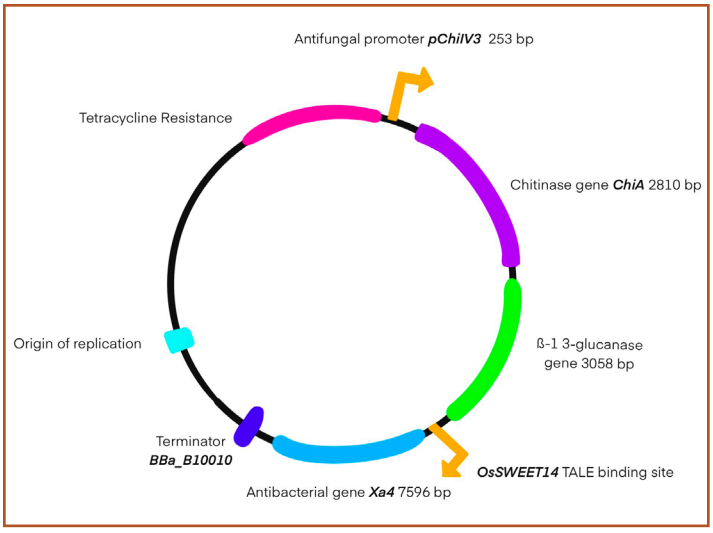

Figure 4: *An overview of the plasmid design. All of the described genes, promoters and terminators are inserted on the plasmid backbone pTE100. The tetracycline resistance, which is the selective marker already included in the plasmid.*

to specific regions of the host gene and lead to gene induction of the host gene sequence (Blanvillain-Baufumé et al., 2016). According to Blanvillain-Baufumé's study, OsSWEET14 is a common target of various TALEs of *X. oryzae* pv. *oryzae*, acting as a promoter of gene synthesis (2016). After the effectors bind with OsSWEET14, the effector would synthesize the following gene sequence (Blanvillain-Baufumé et al., 2016). Shown by Figure 3, inserting OsSWEEt14 into the vector and before the XA4 gene would allow the synthesis of the anti-microbial gene *XA4* to only initiate during the exposure of *X. oryzae* pv. *oryzae*.

We will insert *S. maltophilia*, chitinase A gene *chiA*, *S. sioyaensis* endo ß-1 3-glucanase gene, and anti-bacterial gene *XA4* into the pTE100 backbone to construct our plasmid. By inserting these specific gene sequences into the pTE100 brick vector, we will create a plasmid that accommodates *E. coli* and *M. radiotolerans*, thus usable in both the first and the second stages (see Figure 4).

Safety

There is a concern about the development of antibiotic resistance. When exposing any form of life to an antibiotic, antibiotic exposure places selective pressure on organisms to develop resistance. From a study done by Harvard, bacteria was able to travel from agar with 0 levels of antibiotics to agar with 1000x the original concentration in 11 days. This means that in 11 days, the bacteria were able to develop 1000x resistance (Pesheva, 2016). With our bacteria producing antibiotics,

there will always be selective pressure for resistance. For this reason, we designed our bacteria to only produce antibiotics when necessary. We will use our promoters, which only activate once they have sensed chemicals produced by pathogens to achieve this. Although adding a restriction to antibiotic production will not remove the issue of antibiotic resistance, it prevents the over saturation of antibiotics in the host plant giving bacteria and fungus a smaller chance to develop resistance.

Another concern is how the XA4 gene enhances cell walls. We are unaware of how this gene may affect the taste or texture of rice. It is possible that the added strength to the cell walls of the rice seed may require more time to cook to soften the rice. Another factor that we are unsure of is how the gene will affect rice production. A study on XA4's effects on plants concluded that plants under its effects would not grow as tall, but seed production would not be affected; hence, the likelihood of XA4 causing major environmental effects remains low. The plant's overall structure, however, will be changed because there will be less cellulose present, and the plant will be more brittle (Römer et al., 2009). A brittle plant may impact crop production in an unforeseen way.

Finally, with our antifungals targeting chitin, a common component of fungal cell walls, there is always the chance they may impact other fungi important to the rice plant's own microbiome. However, since the production is only activated when the plant is exposed to a major fungal infection, the possibility of which endophyte leads to the death of symbiotic fungus is low.

Discussions

We chose to use endophytes as a carrier to express the anti-bacterial and anti-fungal genes because, in their natural habitats, endophytes are nonpathogenic, symbiotic microorganisms dwelling between plant cells that are suited to improve plant growth and immunity (Gouda et al., 2016). We chose to use the bacterial endophyte *M. radiotolerans* to insert the plasmid-containing genes that prevent rice blight and rice blast. *M. radiotolerans* naturally exists in soil, on leaves, and in other parts of rice plants, allowing the plasmid it carries to target pathogens invading different regions of the rice plant. For these reasons, we selected *M. radiotolerans* (Kaga et al., 2019). By using bacterial endophyte *M. radiotolerans* in our design, we minimize the risks of the rice plant rejecting the insertion of foreign genes and maximize the effectiveness of the expression of the plasmids.

One major advantage of this design is the utilization of the unique properties of endophytes. Endophytes are harmless and can transmit vertically, enabling transmission between the parent plant and its offspring via seeds or pollen. By living and passing down the desired genes to their offspring, endophytes can provide immunity to future generations of rice plants with the designed insertion of specific disease-resistant genes. Compared to conventional treatments such as fungicides or genetically modified seeds, the endophyte design provides consumers with a more sustainable and less arduous method of protecting rice plants.

There are a few potential challenges, however, regarding the implementation and experimentation of the endophyte design. The foremost challenge is to control the rest of the variables to gather reliable data on the effects of the anti-bacterial and anti-fungal genes on the rice plant. Rice plants naturally have defense mechanisms and innate and adaptive immunity, and certain rice strains have different immunities to diseases than other strands. We must find a rice strain that hadn't previously been exposed to rice blast and rice blight, so the strand does not have any natural defenses against the two diseases, or else we cannot conclude whether it was the genes present in *M. radiotolerans* that fought off the diseases or just the rice strand's natural defenses. There is also concern regarding the anti-bacterial gene's effects on the *M. radiotolerans* bacteria's survival after being expressed as well. While the Xa4 gene only promotes the growth of cell walls to prevent diseases from invading the rice plants through incisions, the introduction of other bacteriocide such as penicillin may inhibit the survival of *M. radiotolerans* bacteria carrying the plasmid, which would limit the production of the anti-bacterial and anti-fungal genes and produce a less ideal result. While the safer approach can eliminate the anti-bacterial genes like penicillin from the bacterial plasmid, our design goal is to experiment with the endophytes' ability to act as a carrier of plasmids with foreign genes. Therefore, we included more than one anti-fungal gene. The final concern is that the Xa4 gene has an abnormally long length that may create issues for RNA Polymerase to read and express the entirety of the gene sequence correctly.

We plan to test and see whether or not our bacteria will naturally release our proteins. However, if this fails, we will insert the YepF gene into the plasmid. YepF is a carrier protein naturally produced by *E. coli* (Zhang et. al., 2006) Although its main function remains unknown, the *sec*-leader of this protein is a carrier protein that enables the protein to transfer between cell membranes (Zhang et al., 2006). With the YepF gene, the produced antimicrobials can move outside the cell membrane of the endophyte and combat the pathogens outside the plant.

Next steps

If the plasmid inside the endophyte *M. radiotolerans* successfully expresses its inserted genes and can provide a certain degree of immunity to the rice plant, then we can prove that endophytes are a plausible carrier of foreign genes to be used in the rice plant. Should the previous statement be true, there might be the possibility that endophytes yield similar results in other plants. Therefore, as rice and other plant pathogens evolve and develop a resistance to chemicals, we can insert novel disease-resistant genes through plasmids into *M. radiotolerans* to combat the new diseases. Then, the future applications of the endophyte design can range from any plant that needs prevention against diseases without using pesticides, herbicides, or gene modification. Another area of further study could be into other crops in the field of agriculture; since modern agriculture relies heavily on monoculture and high yield, there is increased susceptibility to diseases caused by the loss of biodiversity. Endophytes would act as a carrier of plasmids with genes that provide crops with immunity to specific pathogens.

Author contributions

M.K. is the founder of the Endophyte project. He recorded voice over for the video. He researched and discovered the most suitable endophyte, and designed the plasmid. J.G. is responsible for all the art and graphics of the websites and the graphs in the paper, and also the animation for the videos. He researched the anti-fungal genes that will be inserted into the plasmid. H.W. researched the anti-bacterial gene to be inserted into the plasmid. He is the organizer and editor of the entire project.

Acknowledgements

We like to acknowledge the continuous support from Beth Pethel[1] and Kosuke Seki[2] as our mentors that guided us through our project.

This project was accomplished through participation in the Synthetic Biology, a full-year course that immerses students in the cutting edge convergence of biology, engineering, and invention known as synthetic biology. Through an iterative process, students research issues, genes, and various organisms to create a novel genetically engineered machine to address a challenge of their choice.

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