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STUDY ON SELECTION OF SALT-TOLERANT PLANT GROWTH-PROMOTING BACTERIA AND IMPACTING ON GENE EXPRESSION RELATED TO SALT TOLERANCE IN RICE

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SUMMARY OF BIOTECHNOLOGY DOCTORAL THESIS

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INTRODUCTION

1. The urgency of the thesis topic

Plant growth and yield are severely affected by biotic and abiotic stress factors. Biological stress factors such as insect pests or plant pathogens. Abiotic stress factors include drought, salinity, temperature, heavy metals and organic pollutants. Among the abiotic stress factors, salinity affects the most severely and is considered to be one of the most significant limiting factors for agricultural productivity and food security. Salinization is spreading globally at a rapid rate and increases the risk of food insecurity in some countries. The lowland regions of India, Myanmar and Bangladesh are major rice producing regions of the world facing a serious threat to food security due to saline coastal soils. In Vietnam, saline intrusion occurs irregularly in the coastal areas, especially in the Mekong Delta, causing great damage to agricultural production, especially rice. According to 2015 statistics, it was estimated that about 35.5% of the rice growing area in 8 coastal provinces have been affected. A record increase in saline intrusion in 2016 caused a loss of 139,000 hectares of rice in the Mekong Delta with an estimated yield reduction of 30-70%. In 2020, saltwater intrusion will cause damage to about 34,600 ha in 4 provinces of Ca Mau, Kien Giang, Ben Tre and Soc Trang with an estimated yield reduction of 30-70%.

There are many methods to overcome saltwater intrusion such as building a system to prevent salinity-sweet-drainage, creating salt-tolerant rice varieties and increasing coastal mangroves... These methods require a lot of time and money. Recently, there have been many studies on the application of salt-tolerant plant growth-promoting bacteria (PGPB) to minimize the harmful effects of various biotic and abiotic

stress factors in plants. The results showed that PGPB with the ability to produce indole-3-acetic acid (IAA), 1-aminocyclopropane-1-carboxylate deaminase (ACCD), nitrogen fixation, phosphate solution and siderophores supporting plants to withstand salt stress, which can be used to improve the productivity of agro-ecosystems in saline soils.

In Vietnam, the use of salt-tolerant PGPB to increase salt tolerance of rice has not yet been noticed. Studies have focused on isolating microbial strains with biological activities such as nitrogen fixation, phosphate degrading and IAA production… There have been no studies evaluating the effectiveness of IAA and ACC deaminase producing salt-tolerant bacteria supporting the growth and development of rice plants under saline conditions, and at the same there has not been research on the expression of genes related to salinity response in rice with the support of microorganisms. Therefore, the thesis: "Study on selection of salt-tolerant plant growth promoting bacteria and impacting on gene expression related to salt tolerance in rice".

2. Aims of the thesis

Isolatate and select salt-tolerant bacteria strains that produce plant growth stimulants (IAA, ACC deaminase, phosphate- solubilizing, cellulose-degrading, nitrogen fixation and siderophores) and study on gene expression related to salinity response of rice.

3. Contents of the thesis

1. Isolation and selection of salt-tolerant bacteria strains capable of producing plant growth stimulants such as IAA, ACC deaminase, phosphate-solubilizing, cellulose-degrading, nitrogen fixation and siderophores;

2. Evaluation of the ability to support salt-tolerant rice by selected bacteria;

3. Studying on the expression of genes involved in the response to salinity stress in rice with the help of selected bacteria;

4. Genome sequencing of selected strains and identification of genes related to salt tolerance and plant growth stimulants.

4. Scientific and practical significance of the thesis

The thesis have isolated salt-tolerant plant grownth promoting bacteria, thereby selecting bacteria capable of supporting rice growth and development in saline conditions. The results of the thesis are meaningful to serve the production of biological products, improve the efficiency of rice production in saline-affected growing areas. The results of the thesis also supplement and enrich useful microbial genetic resources and provide information on salt tolerance genes in rice that are stimulated by bacteria

CHAPTER I. OVERVIEW

1. 1. Introduction of plant growth-promoting bacteria

1.1.1. Concept of plant growth-promoting bacteria

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2.2.12. Evaluation of the ability of selected bacterial strains to support salt tolerance in rice

A total of 423 salt-tolerant bacteriawere isolated from 66 samples, of which 185 bacteria from 36 soil samples of Truong Sa archipelago, 21 strains of bacteria from 9 rice root samples, 25 strains from 9 mangrove root samples and 202 strains from 12 acres of land and sea water. The isolated bacteria were screened for producing IAA. The results obtained 65 strains of IAA-producing bacteria, of which 39 strains were obtained from Truong Sa soil samples, 7 strains from rice root samples, 9 strains from samples. mangrove roots and 10 strains from soil and seawater samples. In medium supplemented with tryptophan, strains isolated from different sources were able to produce IAA with relatively similar concentrations. Ten strains isolated from soil and seawater samples were able to produce IAA with concentrations of 19-44 µg/mL, a relatively higher concentration of 44.17 µg/mL for the isolate C7, followed by

isolates B9 and B7 with respective concentrations of 40.43 µg/mL and 36.53 µg/mL IAA. Nine strains isolated from mangrove roots have the ability to produce IAA with concentrations ranging from 14 to 38 µg/mL, including strain DM10 with the highest IAA concentration of 38.78 µg/mL. IAA-producing strains were isolated from soil samples on Truong Sa archipelago, with concentrations ranging from 11 to 38 µg/mL, of which some strains were able to produce IAA as high as STD2.1.3 (35.54 µg). /mL), S.T.T.1.1.2 (35.71 µg/mL), D1.2.2 (38.71 µg/mL), D3.2.3 (32.33 µg/mL), NY4.2.3 (30.17 µg/mL)), NY4.3.1(33.54 µg/mL) and STT3.2.3 (28.50 µg/Ml). Seven IAA strains from rice roots had concentrations ranging from 23 to 46 µg/mL, of which 3 strains RL5, RL6 and RL7 were able to produce high IAA concentration of 44.83; 35.95 and 46.50 µg/mL, respectively. From these 65 IAA seminarians, the thesis continues to screen for the ability to produce ACC deaminase, solute phosphate, fix nitrogen and degrade cellulose.

3.1.2. Screening for ACC deaminase-producing bacteria

The results of screening for ACC deaminase-producing bacteria showed that There are nine ACC deaminase-producing bacteria among 39 isolates from Truong Sa soil, of which three strains STT1.1.2, STT3.2.3 and NY4.3.1 have high activity of 128.70; 38.45 and 40.37 nmol α-ketobutyrate/mg/h, respectively. There are 5/9 isolates from mangrove roots capable of producing ACC deaminase, two high activity strains are DM10 (133.80 nmol α-ketobutyrate/mg/h) and DM20 (65.45 nmol α-ketobutyrate/mg/h). There are 4/7 strains from rice roots capable of producing ACC deaminase, of which six strains RL5, RL6 and RL7 have high activity of 44.83; 35.95 and 45.40 nmol α-ketobutyrate/mg/h, respectively.

Thus, at the end of the screening process of strains capable of producing ACC deaminase, six strains including DM10, RL5, RL6, RL7, STT1.1.2 and NY4.3.1 have been selected with both high ACC deaminase activity and high IAA production capacity.

3.1.3. Screening for nitrogen fixing endophytic bacteria

The results of screening for nitrogen-fixing strains showed that 25/65 strains were able to fix nitrogen, of which 22 were isolated from soil on Truong Sa archipelago and 3 were endogenous strains of rice roots. Soil and seawater isolates and mangrove root endophytes were not capable of nitrogen fixation. Three endogenous strains from rice roots RL9, RL10 and RL18 were able to fix nitrogen with ammonium concentration of 17.65, 12.63 and 15.32 mg/L, respectively. Strains isolated from soil on Truong Sa archipelago have relatively high nitrogen synthesis capacity with ammonium concetration ranging from 5.78 to 19.11 mg/L. In which, strain STT2.6.2 showed the highest ammonium concentration of 19.11 mg/L. The results of nitrogen fixation screening showed that all strains capable of nitrogen fixation were unable to produce ACC deaminase. Strains with high ACC deaminase and IAA production capacity such as DM10, RL5, RL6, RL7, STT 1.1.2 and NY 4.3.1 were also unable to fix nitrogen.

3.1.4. Screening for phosphate solubilizing bacteria

The results of screening showed that there were 29/65 strains able to grow and give CaCO3 resolution rings on NBRIP agar medium. Eighteen strains isolated from Truong Sa soil have the ability to degrade phosphate with PO_4^{3-} concentration in the range from 65 to 342 mg/L, especially strains with relatively high PO_4^{3-} concentration such as STT1.1.2 (191.16 mg/L STĐ2.1.3 (287,69 mg/L), STT3.5.2 (309,37 mg/L), D3.2.3 (342,06

mg/L) D1.2.2 (375,39 mg/L) and NY4.3.1 (286,45 mg/L). Six strains isolated from mangrove roots PO_4^{3-} concentration ranged from 147 to 338 mg/L, of which strain DM10 gave the highest PO_4^{3-} concentration of 338.71 mg/L. Five phosphate-degrading strains from rice roots had PO_4^{3-} concentration ranging from 116 to 312 mg/L, of which RL5, RL6 and RL7 had the highest PO_4^{3-}concentration of 304.53; 201.14 and 312.64 mg/L.

3.1.5. Screening for cellulose-degrading bacteria

The results showed that 20/65 strains were able to grow and form CMC degrading rings on CMC agar. Fifteen strains isolated from Truong Sa soil had cellulase activity ranging from 5 to 9 U/ml, of which strain STD2.1.3 had the highest cellulase activity of 9.25 U/ml. Five strains isolated from rice roots had high cellulase activity, in which the two highest RL5 and RL7 strains were 8.80 U/mL and 9.60 U/mL, respectively.

At the end of the process of isolation and screening salt-tolerant plant growth bacteria, the thesis has selected 12 strains of salt-tolerant bacteria with high ability to produce IAA, This is the priority criterion for strain selection, followed by ACC deaminase biosynthesis, nitrogen fixation, phosphate solubilization, cellulose degradation and siderophores generation.

Determination of the ability to produce siderophores of 12 selected strains showed that all 12 strains were capable of producing siderophores.

3.2. Studies on morphological and biochemical characteristics and 16S rRNA sequencing

3.2.1. Study on morphological characteristics of selected bacteria

3.2.2. Biochemical characteristics of selected bacteria

3.2.2. 16S rRNA sequencing and phylogenetic tree construction

The 16S rRNA sequencing results showed that strains RL5, RL7, STT3.2.3 and NY4.3.1 belong to the species of *B. aryabhattai*. Four strains including of D1.2.2, D3.2.3, NY4.2.3 and STD2.1.3 belong to the species B. *endophyticus*.

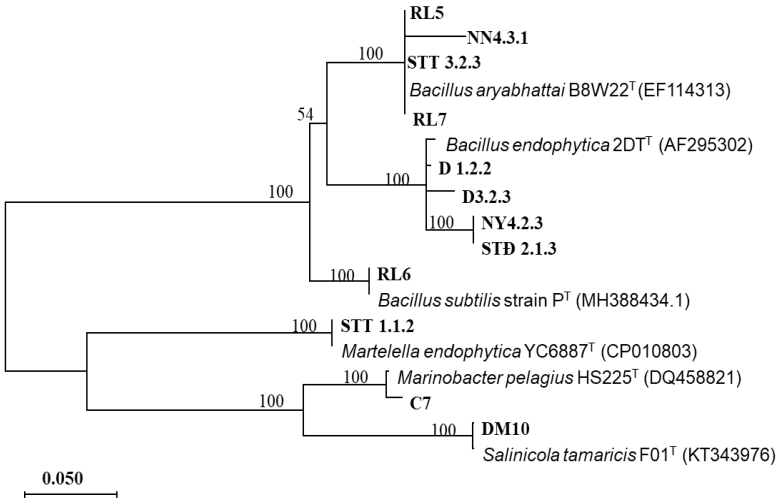

Figure 3.6. Phylogenetic tree of selected bacteria.

3.3. Study on factors affecting growth and synthesis of IAA of selected bacteria

3.3.1. Effect of temperature and pH on the growth of selected bacteria

The suitable temperature for growth and development of strains C7 and DM10 is 30-34 ^{0}C, of strains RL7, STT1.1.2 and RL5 is 34 ^{0}C, of strains D1.2.2 is 34-37 ^{0}C.

The suitable pH for the growth and development of strains RL7, D1.2.2, STT1.1.2 and DM10 was 7, of strain RL5 was 6.5-7.5, of strain C7 was 7.5.

3.3.2. Effect of nitrogen source on the growth and synthesis of IAA of selected bacteria

The organic nitrogen sources (especially yeast extract) was suitable for the growth and biosynthesis of IAA of all 6 studied strains. Therefore, yeast extract was selected as the nitrogen source for further experiments.

3.3.4. Effect of L-trytophan on the biosynthesis of IAA of selected bacteria

The appropriate tryptophan concentration was selected for the next experiment for 5 strains C7, RL7, STT1.1.2, D1.2.2 and RL5 was 2 g/L, for strain DM10 was 3 g/L.

3.3.5. Effect of carbon source on the growth and synthesis of IAA of selected bacteria

The suitable carbon source as the medium for IAA generation of RL5, RL7, D1.2.2 and STT1.1.2 was glucose and for DM10 was sucrose.

3.3.6. Effect of NaCl concentration on the growth and biosynthesis of IAA of selected bacteria

C7 (Figure 3.12 a): The growth of C7 increased gradually when the concentration of NaCl increased from 1 to 5%, the optimal NaCl concentration was 4%, and decreased gradually when the concentration of NaCl increased from 6% to 20%.

DM10 (Figure 3.12 b): NaCl concentration influenced significantly on the ability to produce IAA of strain DM10. The amount of IAA produced is inversely proportional to the salt concentration. The growth and development of strain DM10 increased gradually when the concentration of NaCl increased from 0 to 12.5% and then decreased gradually.

RL7 (Figure 3.12 c): Optimal growth when the salt concentration was from 0 to 1%, decreased gradually when the salt concentration increased from 1 to 10%, did not grow at NaCl concentration greater than 11%.

The effect of salt on growth and IAA synthesis of strain RL5 was similar to strain RL7. Optimum growth was observed at 0 to 1% salt concentration, decreased gradually with increasing salt concentration from 1 to 10%, no growth at NaCl concentrations greater than 11%.

D1.2.2 (Figure 3.12 e): The effect of salt on the growth and synthesis of IAA of strain D1.2.2 was similar to strains RL7 and RL5. Growth was optimal when the NaCl concentration was from 0 to 1%, then gradually decreased as the salt concentration increased from 1 to 10%, no growth at NaCl concentrations greater than 10%.

STT1.1.2 (Figure 3.12 f): Growth increased gradually when the salt concentration increased from 0 to 4%, suitable from 2 to 3%, then gradually decreased when the salt concentration increased to 10%, no growth. when the salt concentration is greater than 10%.

3.4. Evaluation of the ability of selected bacteria to reduce the effects of salt stress on rice

3.4.1. Selection of concentration to induce salinity stress in rice

The 200 mM NaCl concentration was intermediate between healthy discharge and growth stress.

3.4.2. Evaluation of the ability of selected bacteria to reduce the effects of salt stress on rice

Table 3.9. Effects of selected microbial strains on the growth of rice plants under salinization

Strains	**RL7**	**DM10**	**STT1.1.2**	**RL5**	**D1.2.2**	**Control**
Body length (cm)	$12,76^{d}$	$11,97^{c}$	$11,19^{b}$	$11,51^{bc}$	$10,94^{b}$	$9,96^{a}$
Root length (cm)	$4,03^{d}$	$3,76^{c}$	$3,53^{b}$	$3,70^{c}$	$3,46^{b}$	$3,23^{a}$
Dry weight (g)	$0,557^{d}$	$0,510^{c}$	$0,480^{b}$	$0,503^{c}$	$0,470^{b}$	$0,419^{a}$

Chlorophyll a (mg/g)	0,076^{d}	0,072^{c}	0,068^{bc}	0,074^{c}	0,066^{b}	0,058^{a}
Chlorophyll b (mg/g)	0,038^{c}	0,036^{c}	0,033^{b}	0,035^{b}	0,032^{a}	0,030^{a}
Chlorophyll total (mg/g)	0,114^{c}	0,108^{b}	0,101^{a}	0,109^{b}	0,098^{a}	0,088^{a}

(lowercase letters in the same row indicate the difference is not statistically significant with 95% confidence)

The results showed that the rice inoculated with strains RL7, DM10, STT1.1.2, RL5 and D1.2.2 increased stem length, root length, dry weight and chlorophyll content compared to the control samples. This difference is statistically significant at the 95% confidence level.

Strains RL7 and DM10 with the ability to support higher salt tolerance were selected to assess their ability to reduce the effects of salinity stress and study the expression of genes related to salt tolerance.

3.5. Study on the expression of genes related to salinity response in rice with the support of RL7 and DM10

3.5.1. Effects of RL7 and DM10 on the growth of rice under salinization

3.5.1.1. Effect of RL7

Table 3.11. Effect of RL7 on the growth of rice under salinization

	C	**C +NaCl**	**RL7**	**RL7+ NaCl**
Body length (cm)	18,40±0,76^{b}	15,23±0,42^{a}	23,26±0,41^{c}	20,63±0,57^{d}
Root length (cm)	5,93±0,22^{b}	5,33±0,31^{a}	7,33±0,34^{c}	7,1±0,24^{c}
Dry weight (g)	0,73±0,06^{b}	0,66±0,04^{a}	0,97±0,05^{c}	0,90±0,08^{d}
Chlorophyll a (mg/g)	0,082±0,005^{b}	0,071±0,004^{a}	0,096±0,006^{c}	0,089±0,005^{b}
Chlorophyll b (mg/g)	0,033±0,006^{b}	0,027±0,004^{a}	0,042±0,004^{b}	0,038±0,003^{b}
Chlorophyll (mg/g)	0,115±0,016^{b}	0,099±0,005^{a}	0,138±0,018^{d}	0,128±0,021^{c}

(Mean ± SD, lowercase letters in the same row indicate the difference is not statistically significant with 95% confidence)

Rice inoculated with RL7 had increased stem length, root length, dry weight and total chlorophyll content by 26.45%; 29.89%; 31.08% and

19.99% compared with control (C), respectively. Under saline stress, rice inoculated with strain RL7 had increased stem length, root length, dry weight and total chlorophyll content by 27.10%; 30.67%; 31.06% and 34.73% compared to the control (C+ NaCl), respectively. The differences in stem length, root length, dry weight, and chlorophyll content between treatments were statistically significant with 95% confidence.

3.5.1.2. Effect of DM10

Rice inoculated with DM10 had an increase in stem length, root length, dry weight and total chlorophyll content by 16.60%; 21.14%; 21.38% and 22.87% compared with control (C), respectively. When affected by salinity, rice plants infected with strain DM10 had an increase in stem length, root length, dry weight and total chlorophyll content increased by 19.55%; 29.6%; 21.71% and 25.73% compared to the control (C+NaCl) , respectively. The differences in stem length, root length, dry weight and chlorophyll content between treatments were statistically significant with 95% confidence.

Table 3.12. Effect of strain DM10 on the growth of rice under salinity

	C	C +NaCl	DM10	DM10 + NaCl
Body length (cm)	16,86±0,54^{b}	13,83±0,36^{a}	19,67±0,28^{c}	17,15±0,31^{d}
Root length (cm)	5,73±0,37^{b}	5,06±0,21^{a}	7,06±0,51^{c}	6,56±0,40^{d}
Dry weight (g)	0,65±0,05^{b}	0,55±0,04^{a}	0,85±0,03^{c}	0,71±0,02^{d}
Chlorophyll a (mg/g)	0,058±0,007^{b}	0,048±0,003^{a}	0,070±0,002^{c}	0,064±0,005^{b}
Chlorophyll b (mg/g)	0,032±0,005^{b}	0,029±0,003^{a}	0,040±0,004^{b}	0,035±0,002^{b}
Chlorophyll (mg/g)	0,083±0,012^{b}	0,074±0,008^{a}	0,110±0,013^{d}	0,100±0,017^{c}

(Mean ± SD, lowercase letters in the same row indicate the difference is not statistically significant with 95% confidence)

3.5.2. Study on the expression of genes related to salt response in rice under the influence of RL7 and DM10

3.5.2.1 Expression of genes related to salinity response of rice under the influence of RL7

The expression of genes including *SOS1*, *NHX1*, *MYC2*, *PR1*, *NADPMe2*, *CAT* and *SOD* in four treatments was determined by RT-PCR. The results are shown in Figure 3.22. Salinity increased the expression of *MYC2*, *NHX1*, *NADP-Me2*, *SOS1*, *SOD*, *PR1* and *CAT* genes by 1.34; 1.66;1.89; 2.16; 2.23; 2.57 and 3.07 times that of the control (C). Under non-saline conditions, strain RL7 did not change gene expression compared with control (C). When subjected to salinity, strain RL7 increased the expression of genes *MYC2*, *SOD*, *NADP-Me2*, *SOS1*, *PR1*, *CAT* and *NHX1 b*y 1.24; 1.29; 1.31; 1.35; 1.38; 1.46; 1.53; 1.55 and 1.59 times that of the sample (C+NaCl). The difference in expression levels of genes between treatments was statistically significant with 95% confidence.

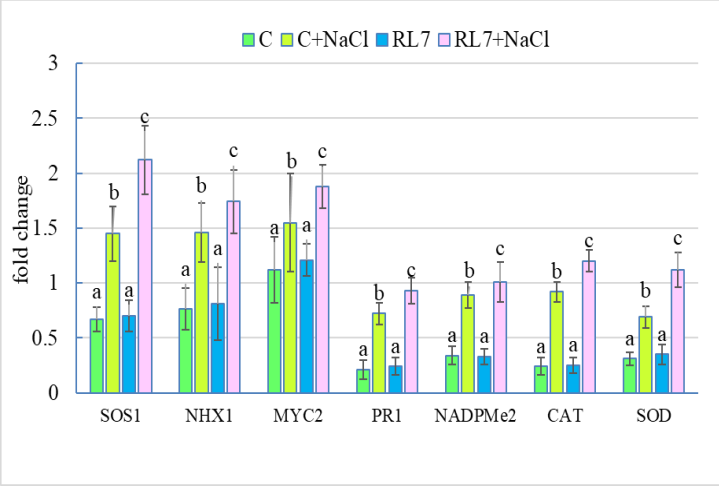

Figure 3.22. Expression of salt stress-related genes under the support of RL7

3.5.2.2 Expression of genes involved in salinity response of rice under the influence of DM10

The genes evaluated for expression include *MYC2, CAT, NADPMe2, SOD* and *PR1*, the results are shown in Figure 3.23.

The results showed that salt stress enhanced the expression of genes *MYC2, CAT, NADPMe2, SOD* and *PR1* from times 1 to 1.45; 1.82; 2.18; 2.42; 2.44 and 2.57 times compared with control (C). In the absence of salt stress, strain DM10 increased PR1 gene expression (1.24 times), and did not change the expression of the remaining genes compared with the control (C). In saline environment, strain DM10 increased the expression *of MYC2, SOD,, NADPMe2, CAT a*nd *PR1* genes by 1.19, 1.25, respectively; 1.38; 1.46; 1.47; 1.50 and 1.51 times the sample (C+NaCl). The difference in expression levels of genes between treatments was significant with 95% confidence

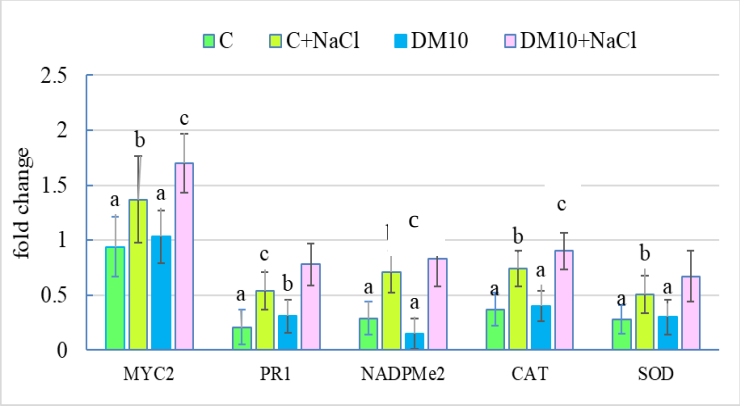

Figure 3.23. Expression of salt stress-related genes under the support of DM10

Through the above research results, the possible mechanisms to increase salt tolerance in rice mediated by strains RL7 and DM10 are summarized in Figure 2.24 below.

Rice tolerates salinity through two main mechanisms: ion homeostasis and osmotic pressure balance. When salinized, the genes involved in these two pathways were upregulated. Inoculation with RL7 and DM10 enhances the expression of genes involved in both mechanisms for salt tolerance in rice.

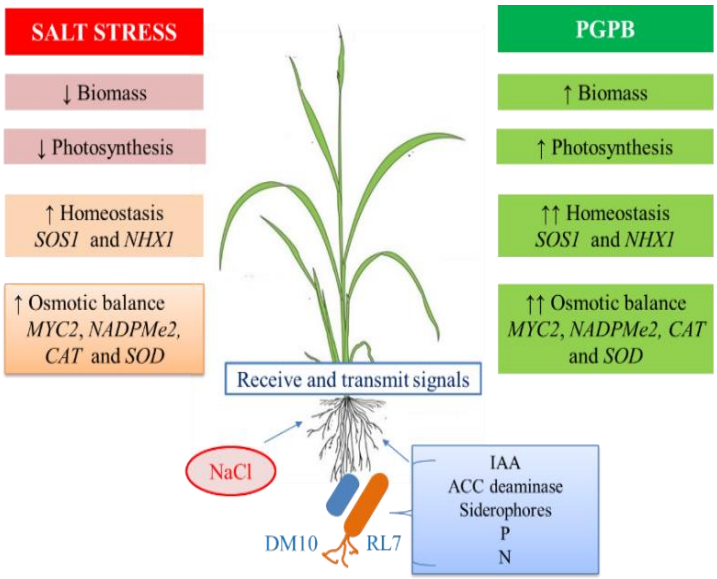

Figure 3.24. Summary of possible mechanisms that increase salt tolerance in rice with the support of RL7 and DM10

MYCs genes play important roles in responses to abiotic and biotic stresses. In rice, an increase in the expression of *MYC2*, *MYC4* and *MYC5* genes in high-salt media was observed [180]. Transcriptional and biochemical studies in Arabidopsis have shown that *MYC2* negatively

regulates proline biosynthesis. Proline is required for tolerance to salinity stress, however, its excessive accumulation is toxic to the plant. The *MYC2* gene is also involved in the jasmonic acid signaling pathway, a hormone that helps regulate a wide range of processes in plants, from growth and photosynthesis to reproductive development [181]. Under salt stress, infection with strains RL7 and DM10 increased the expression of the *MYC2* gene to support salt stress tolerance in rice.

Some endogenous or exogenous microorganisms enhance the expression of pathogenesis-related protein 1 (PR1) gene leading to increased immunity (particularly resistance to fungal diseases) [182], when subjected to disease. Rice inoculated with strains RL7 and DM10 increased the expression of PR1 gene by 2.2 and 2.75 times compared with the control, respectively. This suggests the role of endogenous microbial strains RL7 and DM10 in reducing the effects of salinity stress. The results of the study on the expression variation of *MYC2* and *PR1* genes in the thesis are the first demonstrations of the role of *MYC2* and *PR1* in salt stress conditions.

Salt stress induces a cascade of oxidants (ROS), namely H_2O_2, O^{2-} and OH^- that damage DNA, RNA and proteins. ROS compounds also cause chlorophyll destruction and disrupt root meristem activity. Antioxidant enzymes such as superoxide dismutase, catalase and ascorbate peroxidase, peroxidase, glutathione reductase and monohydroascorbate reductase are capable of removing ROS and maintaining them at low levels. Superoxide dismutase is a metalloenzyme that plays an important role in protecting cells from oxidative damage, by catalyzing the conversion of superoxide radicals to H_2O_2[183]. Catalase lowers ROS levels by catalyzing the breakdown of H_2O_2 into H_2O and

O_2. Under salt stress, infection with strains RL7 and DM10 enhanced the expression of SOD and CAT genes when compared with the corresponding control. Thus, by eliminating ROS through CAT and SOD activities, rice plants infected with RL7 and DM10 strains can adapt to salinity. Adaptation to salt stress through enhanced expression of ROS enzymes such as CAT and SOD has also been demonstrated in rice [41, 124], okra [184] and potato [185]. The enhanced expression of NHX1 and SOS1 genes under saline stress and infection with strains RL7, DM0 has been demonstrated in the thesis. Plants combat salinity stress by sequestering and accumulating salts into the vacuole, controlling salt concentrations as well as maintaining a high K+/Na+ ratio in the cytoplasm thereby reducing the harmful effects of salt. NHX1 and SOS1 participate in Na+/H+ exchange and decrease intracellular Na+ [56]. Thus, overexpression of SOS1 and NHX1 significantly supported plant growth and development at high salt concentrations.

NADP-malic enzyme 2 (NADP-ME 2) is one of the important enzymes involved in various metabolic processes in plants, present mainly in mitochondria, chloroplasts and cytoplasm, catalyzing decarboxylation of malate to produce pyruvate, CO_2 and NADPH in the form of metal ions (Mg^{2+}, Mn^{2+}...). Thus, this enzyme plays a role in enhancing plant tolerance to salt and osmotic stress [186]. Salt stress induces NADP-Me2 gene expression level 1.98 times higher, when infected with strains RL7 and DM10 the transcript level is 2.73 and 1.46 times higher, compared to control, respectively. This may suggest a supportive role of strains RL7 and DM10 in ameliorating salt stress. The expression of genes in the thesis is similar to the results of previous studies. Nautiyal et al. (2013) [124] determined the expression of genes

NHX1, SOS1, NADPMe2, rice under salt stress and infection with *B. amyloliquefaciens* strain NBRISN13. According to Sultana et al. (2020) [104] salinity tolerant *B. aryabhattai* MS3 isolated from rice roots increased the expression of *SOS1* and *NHX1* genes by 419% and 770% respectively in rice under salt stress. 200 mM NaCl. Streptomyces sp. GMKU36 also enhanced the expression of *NHX1, SOS1, CAT, SOD* genes in rice leaves under salt stress conditions [41].

3.6. Sequencing the genome of strain DM10 and C7

3.6.1. Results of assembling sequences and functional annotations

The results of genome sequencing showed that the genome size of strains DM10 and C7 was about 4.2 Mbp and 4.0 Mbp, respectively (Table 3.12). The number of scaffolds after assembly was 743 and 1,360, respectively. The GC content was 65.91% and 59.21%, respectively. The total number of predicted genes is 3635 genes and 3797 genes, respectively, the total number of genes predicted for function is 2270 genes (62.44%) and 2239 genes (accounting for 58.96%). Strain DM10 47 RNA genes includes 45 tRNA genes, 1 rRNA gene and 1 tmRNA gene. Strain C7 has 46 tRNA genes, 5 rRNA genes and 1 tmRNA gene. The genes in the COGs database of strains DM10 and C7 are 1786 and 1603, respectively

3.6.2 Classification based on whole-genome similarity index

The genome sequences of strains C7 and DM10 were compared with their closest relatives in the genera *Marinobacter* and *Salanicola*. The ANI index between strains C7 and *Marinobacter pelagius* was 86.33%, between strains DM10 and *Salanicola tamaricis* was 92.89%. Thus, strains C7 and DM10 with ANI index <95% should be classified as species of *Marinobacter* and *Salinicola* genera, respectively.

3.6.3. Classification of functional gene groups

3.6.4. Genes involved in plant growth stimulation

The results of genome sequencing of strain DM10 have identified genes related to plant growth stimulation such as IAA generation, phosphate solution (gcd), ACC deaminase production, siderophore generation

3.6.5. Genes associated with salt tolerance.

The K^+ transport system is encoded by *the trkAHI, ktrAB* and *kdpABC* genes. The genes *mnhC, mrpDEF* and *nhaP* encoding Na^+/H^+ antiporter and the gene *nhaP2* encoding K^+/H^+ antiporter to pump H^+ and pump Na^+ and K^+ out against osmotic stress were found in the genomes of strains DM10 and C7. The major genes *betA* and *betB/gbsA* for glycine-betaine synthesis encoding choline dehydrogenase and betaine aldehyde dehydrogenase, respectively, were found in strains DM10 and C7. In addition, strains DM10 and C7 include the genes *opuAA, opuAB, opuCA* and *opuE* encoding the betaine/proline/choline glycine transporters. The ectoine fusion gene was also found in the genomes of strains DM10 and C7.

3.6.6. Genes involved in the excretory system

The bacterial excretory system is the protein complex present on the cell membrane, which is also the system by which they enter the host cell. Strain DM10 has 6 potential protein secretory systems including types I, II, IV and VI, Tat and Sec..

3.6.7. Genes involved in endogenous processes

The DM10 genome contains genes involved in flagella and type IV pili movement, as well as genes involved in chemotaxis that support plant invasion.

CONCLUSIONS AND SUGGESTION

CONCLUSIONS

1. Sixty-five strains of IAA-producing salt-tolerant bacteria were selected from 423 isolates of 66 samples. Screened 18/65 ACC deaminase strains, 25/65 phosphate-degrading strains, 29/65 nitrogen-fixing strains and 20/65 cellulose-degrading strains. The morphological, biochemical and 16S rRNA sequences were determined to identify 12 selected bacterial strains. Based on the ability to produce plant growth stimulants, the origin of isolation and classification, 6 strains were selected including *M. pelagius* C7, *S. tamaricis* DM10, *B. aryabhattai* RL7, *M. endophytica* STT1.1.2, *B. endophyticus* D1.2.2 and *B. subtilis* RL5. The influence of some factors (temperature, pH, NaCl, carbon source, nitrogen and tryptophan) on the growth and biosynthesis of IAA of 6 selective strains C7, DM10, RL5, RL7, STT1.1.2 and D1.2.2 was investigated.

2. The ability to support rice plant to grow and develop under saline stress was evaluated by strains DM10, RL5, RL7, STT1.1.2, and D1.2.2. Selected RL7 and DM10 strains with the best ability to support salt-tolerant rice. RL7 and DM10 strains supported salt tolerance in rice and increased the expression of seven genes involved in salinity response, including *NADPME2, SOS1, PR1, MYC2, NHX1, CAT* and *SOD.*

3. Sequenced the entire genome of strain DM10 and C7 on which genes related to growth stimulation (ability to degrade phosphate, synthesis of ACC deaminase and generation of siderophores) have been identified salinity and endogenous ability.

SUGGESTION

Strains RL7 and DM10 are strains with many potential applications to produce bioproducts for use in rice-growing areas at risk of salinity. Therefore, it is necessary to develop inoculant production processes as well as field trials to evaluate the effectiveness of strains RL7 and DM10.

NEW CONTRIBUTIONS OF THE DISSERTATION

1. Sixty-five strains of salt-tolerant bacteria that produce IAA, ACC deaminase, and phosphate degradation have been isolated and screened from various sources such as soil and sea water, roots of rice and mangroves, and soil on the Truong Sa archipelago. Two strains of *S. tamaricis* DM10 and *B. aryabhattai* RL7 have been selected with many characteristics of plant growth-promoting bacteria, especially the ability to produce IAA and ACC deaminase. These are potential strains to make bioproducts used in agro-ecosystems at risk of salinity to limit the harmful effects of salinity on crops.

2. The expression changes of 7 genes related to salt tolerance in rice were evaluated with the help of two strains DM10 and RL7 in which two genes MYC2 and PR1 were studied for the first time.

3. Strains DM10 and C7 have been sequenced and analyzed for genes related to growth stimulation, salt tolerance, as well as endogenous ability.

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