Isolation and characterization of *Rhizobium* spp. and *Bradyrhizobium* spp. from legume nodules

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ARTICLE INFO

DOI:10.46223/HCMCOUJS.tech.en.12.2.2116.2022

Received: December 14th, 2021
Revised: February 18th, 2022
Accepted: March 02nd, 2022

Keywords:

Bradyrhizobium; germination; index nitrogen-fixing bacteria; legume nodule; *Rhizobium*

ABSTRACT

Rhizobia topic has been re-focused in recent years because of new findings on their traits not only as nitrogen-fixing bacteria but also as plant growth-promoting rhizobacteria. When combing rhizobial strains with novel biological carriers (e.g., biochar) for inoculant production, it brings great potential for improving soil health in long-term. Appreciating this trend, this study is designed to isolate and characterize local rhizobial strains from legume fields using the conventional method with some modifications to increase efficiency in rhizobial identification. As a result, 17 rhizobial strains were isolated and classified biochemically that genetic identification outcome confirmed 10 strains belong to 07 different *Rhizobium* species as *R. mayense, R. paknamense, R. pusense, R. miluonense, R. tropici, R. phaseoli,* and *R. multihospitium* while the rest belong to 06 various *Bradyrhizobium* species as *B. elkanii, B. centrosematis, B. guangxiense, B. liaoningense, B. yuanmingense*, and *B. arachidis*. Thermal and saline tolerant tests together with seed germination tests also performed on these rhizobial strains to gain data on their responses to abiotic stresses. By comparing rice and mung bean GI values, we can assess the effectiveness of each rhizobial strains to help seeds at their early germination.

1. Introduction

Soil is a critical resource that is responsible for food production, and it has been in great danger after intensively farming with chemical fertilizers and pesticides for a long time (Food and Agriculture Organization of the United Nations, 2018, 2021). Soil health improvement, therefore, requests urgent attention today from researchers, practitioners, and policymakers (Food and Agriculture Organization of the United Nations, 2015, 2016). Amongst 13 types of soil degradation, loss of nutrients or organic matter has been creating a severe impact on crop yield (Food and Agriculture Organization of the United Nations, 2015). Particularly, nitrogen is one of the major nutrients that are essential for plants via usable forms such as ammonium and nitrate. If

nitrogen does not supply adequately, it will result in limited plant growth (McClellan, Deenik, & Singleton, 2007). Thus, maintaining nitrogen availability in soil along with other nutrients will help the plant develop as best as it can (Galloway et al., 2004; Vitousek, Hättenschwiler, Olander, & Allison, 2002). Fortunately, there is a consensus that using the Biological Nitrogen Fixation (BNF) approach is more recommended than synthetic fertilizer because of sustainable agricultural demand (Mahmud, Makaju, Ibrahim, & Missaoui, 2020; Smil, 1999). On a global basis, natural biological nitrogen fixation on land was estimated to be approximately 200 Tg N per year which has played an important role in healthy ecosystem functioning (Gruber & Galloway, 2008; Mahmud et al., 2020). This is a huge contribution thanks to diazotrophs which include at least 60 genera of cyanobacteria, 15 genera of symbiotic actinomyces, and around 25 genera of free-living and symbiotic bacteria (generally called nitrogen-fixing bacteria or rhizobia) that have reduced atmospheric nitrogen to ammonia using their nitrogenase enzyme complex (Lindström & Mousavi, 2010; Smil, 1999). Far above potential nitrogen fixer candidates, the genus *Rhizobium* has been thoroughly researched because its symbiosis with legume plants creates a feasible solution to supply economically and effectively nitrogen for soil and plant through either applying crop rotation or rhizobial inoculant (De Bruijn, 2015; González-Andrés & James, 2016; Smil, 1999; Somasegaran & Hoben, 2012). Schematics of carbon and nitrogen metabolic pathways of *Rhizobium*-legume interaction were described in detail (Becana & Sprent, 1987; Lindström & Mousavi, 2020; Liu, Contador, Fan, & Lam, 2018) with notes that phosphorus (used for building ATP molecules) is one of important factor to help nitrogen fixation activity process properly. It is useful to recall that normally free-living rhizobia have fixed atmospheric nitrogen to ammonia for their cellular activities through ammonia assimilation pathway to produce amino acid (e.g., glutamine, glutamate) but there was no evidence for the operation of using ammonia inside bacterial cells (or bacteroid) instead of transferring ammonia to host plant (then quickly changed to ammonium) and receiving substantial building blocks due to symbiotic relationship (Becana & Sprent, 1987; Brown & Dilworth, 1975). Mode of infection and nodule types on roots and even on stems have been studied that enriched our understanding of rhizobia at the molecular level about the interaction between signaling inducers and *nod* genes regulation in the context of successfully co-living with plants (Boivin et al., 1997; Fisher & Long, 1992; Rao, 2014; Sharma et al., 2020). Nitrogen fixation rate of *Rhizobium* associated with seed legumes is also estimated from 3×10^2 to 3×10^4 mg N/m^2 as reported previously (Galloway et al., 2004; Hardarson & Danso, 1993; Smil, 1999). In addition, nitrogen fixation mechanism in legumes and non-legumes (cereals) was well-written in a number of notable articles (Mahmud et al., 2020; Yanni et al., 1997). The effectiveness of nitrogen fixing bacteria on plant growth in terms of reducing N-based fertilizer and increasing yield has been proved by extensive field trials on various peas and beans as well as rice, wheat, and maize since the 1980s (Kavimandan, 1985; Serraj, 2004; Yanni et al., 1997). Currently, more studies have been conducted to maximize the prospects of cereal nitrogen fixation to adapt the food security warning (Rao, 2014). Yet, the strategy is to select indigenous rhizobia and develop a better formula inoculant by mixing with other beneficial bacteria namely Phosphate Solubilizing Bacteria (PSB) or Arbuscular Mycorrhizal Fungi (AMF) that has resulted from a re-focus on BNF performance with open questions for synergistically optimum application (Dakora, Chimphango, Valentine, Elmerich, & Newton, 2008; González-Andrés & James, 2016; Lindström & Mousavi, 2010; Mendoza-Suárez, Andersen, Poole, & Sánchez-Cañizares, 2021; Rao, 2014). Recent advances in BNF topic emphasize on how rhizobia can adapt to different abiotic stresses like temperature, drought, salinity, nutrient and metal besides how to improve their survival by using a modified carrier such as biochar for providing better protection when applied to soil together with organic amendment (Hardy & Knight, 2021; Howieson & Dilworth, 2016; Rao, 2014). In Vietnam, research on rhizobia is now expended not only on traditional legumes (Nguyen & Ha,

2019) but also on other crops like cassava and rubber tree (Doan, Ha, Ngo, & Nguyen, 2018; Pham & Nguyen, 2021) by exploring a better combination of *Rhizobium* spp. with other soil bacteria in the aim to give the best nitrogen fixing performance for plant development. Taking all these mentioned into account, we conduct this study to first isolate and characterize local rhizobial strains from legume fields which thereby become the solid background for developing a mixed rhizobial inoculant that finally being able to enhance plant productivity through nurturing soil health sustainably.

2. Materials and methods

2.1. Legume nodule preparation

Root nodules were sampled from legume plants grown in Dong Thap (soybean), Long An (peanut), and Quang Ngai (peanut, mung bean) Provinces. Remove nodules from their roots and wash with Sterile Deionized Water (SDW) for 10s. Select the typical nodules (02 - 03mm in diameter) with cross sections that were bright or pink indicating active nitrogen-fixing bacterial community living inside (Somasegaran & Hoben, 1985, p. 33). Surface sterilization was performed by immersing nodules in 70% (v/v) ethanol for 30s then put them in another becher and placed in 3% (v/v) hydrogen peroxide solution for 03 mins. Later, nodules were washed with SDW 05 times to remove traces of hydrogen peroxide solution (if any). Nodules were subsequently crushed in 02mL SDW and stored in 1.5mL Eppendorf tubes according to sampling location (Chibeba, Kyei-Boahen, de Fátima Guimarães, Nogueira, & Hungria, 2017; Duangkhet et al., 2018; Palaniappan, Chauhan, Saravanan, Anandham, & Sa, 2010).

2.2. Rhizobial isolation procedure

From obtained nodule suspensions, they were individually diluted in a series up to 10^{-6} using SDW as diluent then spread onto YMA-CR agar plates with respect to their sampling origin. The composition (g/L) of the YMA-CR medium used in this study was yeast extract mannitol medium (denoted as YEM or YMA) that contained 0.5g yeast extract, 10g mannitol, 0.5g K_2HPO_4, 0.2g $MgSO_4.7H_2O$, 0.1g NaCl, 15g agar, adjust pH to 6.8, then add Congo Red (CR) solution to get the final concentration 25ppm (Somasegaran & Hoben, 1985, p. 386). These plates were then incubated at 28°C for 05 - 07 days in the dark. Continue to streak separate colonies until getting pure isolates having color ranging from white-opaque to faint pink and gummy texture. By screening through YMA-CR, rhizobia were separated from most other soil microbes (Kuykendall, Young, Martínez-Romero, Kerr, & Sawada, 2005; Vincent, 1981). For storing rhizobial isolates, YMA slants and ceramic bead method were applied (Somasegaran & Hoben, 1985, p. 47).

The obtained isolates continued to go through several presumptive media for classifying them into three common rhizobial genera as *Rhizobium*, *Bradyrhizobium*, and *Agrobacterium*. Because both *Rhizobium* spp. and *Bradyrhizobium* spp. are Gram negative, rod shape and nonsporeforming bacteria (Somasegaran & Hoben, 2012), the isolates with these characteristics were streaked on agar media with indicators to test for purity of cultures. Their interpretation is as follows:

i) YMA-BTB medium (g/L) using bromothymol blue (BTB) as pH indicator. Prepare BTB stock solution by diluting 0.5g in 100mL ethanol then add 05mL this stock solution to 01L of YMA to get final concentration of BTB is 25ppm. Fresh YMA-BTB plates at pH 6.8 are green. This medium helps separate if the fast-growing acid-producing isolates that changing agar color to yellow, so virtually the isolates of interest are *Rhizobium* spp.; otherwise they are *Bradyrhizobium* spp. because of slow-growing alkaline-producing property turning agar to blue (Somasegaran & Hoben, 1985, p. 386).

ii) GPA-BCP medium (g/L) containing 05g glucose, 10g peptone, and 15g agar; using bromocresol purple (BCP) as pH indicator. Prepare BCP stock solution by diluting 01g in 100mL ethanol then add 10Ml of this stock solution to 01L of GPA to get the final concentration of BCP is 100ppm. Commonly, rhizobia grow poorly on this medium and cause slight change in pH. Heavy growth is a sign of contamination so this highly nutritious medium is recommended for checking the purity level of isolates (Sharma, Srivastava, & Sharma, 2009; Singh, Kaur, & Singh, 2008; Somasegaran & Hoben, 1985, p. 70; Upadhayay, Pareek, & Mishra, 2015).

iii) Lactose agar medium (LAM) (g/L) containing 01g yeast extract, 10g lactose, and 15g agar. This medium is to differentiate *Rhizobium* sp. and *Agrobacterium* sp. based on the appearance of a yellow ring around the colonies after being flooded with a shallow layer of Benedict's reagent that confirms the presence of *Agrobacterium* sp. regarding its ability to convert lactose to 3-ketolactose. In some papers, this step is often called the keto-lactose test (Bernaerts & De Ley, 1963; Bouzar, Jones, Bishop, 1995; Kuykendall et al., 2005, p. 5).

iv) Hofer's alkaline medium (g/L) containing 01g yeast extract, 10g mannitol, 0.5g K_2HPO_4, 0.2g $MgSO_4.7H_2O$, 0.1g NaCl, and 15g agar; using thymol blue as pH indicator. Adjust pH to 10.8 - 11.2. This selective medium is for *Agrobacterium* isolation because of its ability to grow at higher pH while inhibiting *Rhizobium* spp. at such alkaline pH (Hofer, 1935).

v) Burk's N-free medium (g/L) containing 0.2g $MgSO_4.7H_2O$, 0.8g K_2HPO_4, 0.2g KH_2PO_4, 0.13g $CaSO_4$, 0.00145g $FeCl_3$, 0.000253g Na_2MoO_4, 20g sucrose, and 15g agar (HiMedia® M707 Burks Medium). This medium is for isolation free-living rhizobia which possess the ability to utilize atmospheric nitrogen gas for their cell protein synthesis. In this study, we used for checking isolates whether they can grow on a nitrogen-free medium or not as a part of rhizobial authentication (Newton, Wilson, & Burris, 1953; Stella & Suhaimi, 2010).

Biochemical tests like Gram staining, oxidase, catalase, Methyl Red (MR) and Voges-Proskauer (VP), starch hydrolysis, gelatin hydrolysis, urea hydrolysis, nitrate reduction, and citrate utilization were carried out following ASM protocol (American Society for Microbiology, 2021).

2.3. Genetic identification of strains

Rhizobial strains were grown in YMA plates for 04 - 07 days depending on their colony maturity. Select a typical single colony and put in tube 1.5mL holding 100µL SDW for each strain. Then extract thte DNA genome using Thermo Scientific™ GeneJET Genomic DNA Purification. The 16S rDNA genes of the strains were amplified by PCR method using the primer set 20F 5'-AGA GTT TGA TCM TGG CTC AG -3' and 1500R 5'- GGT TAC CTT GTT ACG ACT T -3'. The PCR product was purified using Thermo Scientific™ GeneJET PCR Purification Kit before sequencing (Ferreira, Bomfeti, Soares, & de Souza Moreira, 2012; Chibeba et al., 2017). Sequencing data were analyzed by MEGA software then compared based on the most similar sequence found in GenBank using BLAST technique with a threshold of identity greater than 98.7% (Stackebrandt & Ebers, 2006; Yarza et al., 2014).

2.4. Characterization of strains

Amongst abiotic stresses, we considered thermal and saline tolerant properties as important factors for screening rhizobial strains in the aim of introducing them into degraded soil. Besides, the effectiveness of rhizobial activities on seed germination of mung bean (as a candidate for legume) and rice (for non-legume) were observed for gaining new insights through comparison of the Germination Index (GI) (Barral & Paradelo, 2011; Gholamalizadeh, Khodakaramian, & Ebadi, 2017; Graham, 1992; Hossain, Gunri, Barman, Sabagh, & da Silva, 2019; Michiels, Verreth, & Vanderleyden, 1994).

2.4.1. Thermal tolerance

Rhizobial isolates were investigated for their thermal tolerance by streaking on YMA plates and placed in multi thermo incubator at temperature ranged from 14 - 16 - 20 - 24 - 28 - 32 - 36 - 40 - 44 - 48°C. Assays were performed in triplicate for each strain. Record the colony growth for one week (Datta, Singh, & Tabassum, 2015).

2.4.2. Saline tolerance

Similarly, rhizobial isolates were examined their saline tolerance by streaking on YMA plates containing 0 - 0.5 - 1 - 2 - 3 - 4% (w/v) NaCl and incubating at 28°C, triplicate and recording results after one week (Datta et al., 2015).

2.4.3. Impact on seed germination

Rhizobial isolates were accessed their impact on the germination of rice and mung bean seeds. These seeds were surface sterilized by NaCl 15% (w/v) for 5 mins then rinsed three times with SDW. Prepare bacterial suspensions of each strain on YMB previously, diluted to 1×10^7 cells/mL. Seeds were immersed in broth cultures and shaken at 150rpm, 2°C for 06 hours to help bacteria coating; for the control treatment, seeds were soaked in SDW instead (Gholamalizadeh et al., 2017). For enhancing seed inoculation, gum arabic solution could be used as a sticker if necessary (Somasegaran & Hoben, 1985, p. 478). Bioassay was conducted as Completely Randomized Design (CRD) with three replications by putting 20 seeds of rice on a sterile filter paper in a Petri dish which was considered a replication (Morrison & Morris, 2000; Solaiman, Murphy, & Abbott, 2012) for each rhizobial isolate respectively. Moisture was created by adding 05mL SDW. Apply the same procedure for thte mung bean test. After that, all Petri dishes were covered with lids and incubated in dark for 06 days at 28°C. Germination performance was measured at the final day (Solaiman et al., 2012).

• Final Germination Percentage (FGP) (%) = [Number of germinated seeds in the last day of germination] × 100 / [Number of total seeds in a seed lot (Petri dish)] (Kader, 2005);

• Total Root Length (TRL) (cm) were measured in fresh roots of germinated seeds and summed all for each Petri dish (Solaiman et al., 2012);

• Germination index (GI) (%) = [FGP × TRL of each strain] × 100 / [FGP × TRL of control treatment] (Emino & Warman, 2004; Gasco, Cely, Paz-Ferreiro, Plaza, & Mendez, 2016). This parameter is used broadly as a phytotoxic indicator for checking whether an agent (in this case a rhizobial strain) could yield a positive effect on seed or not in terms of delaying or promoting seed germination. According to Barral and Paraledo (2011), GI value < 50% indicating high phytotoxicity; between 50 - 80% indicates moderate phytotoxicity; > 80% indicating as nontoxic. Once GI value greater than 100%, the studied agent on seed germination can be considered as a phytonutrient, phytostimulant, or plant growth promoter concerning to which mechanisms they affected.

2.5. Statistical analysis

Raw data was analyzed and stored in MS Excel. Mean comparisons of GI (%) between two seed types regarding each rhizobial strain was calculated using an unpaired t-test, two tails, with p-value denoted as *, **, and *** for lesser than 0.05, 0.01, and 0.001 significance level correspondingly. An ANOVA test was carried out on SAS software for the set of rhizobial strains for each seed type; means were compared using the Tukey HSD test at a 5% significance level.

3. Results and discussion

3.1. Isolation and genetic identification of rhizobia

From legume nodules that were collected at different farming locations spreading from the South (Dong Thap, Long An Provinces) to the Middle (Quang Ngai) of Vietnam, we applied the conventional method for rhizobia isolation (Somasegaran & Hoben, 2012). Firstly, separation them from soil microbes thanks to YMA-CR for colony differentiation and Gram staining for checking the shape and cell wall properties of strains. The following presumptive test media is to check the rhizobia strain's responses that can be used for genus classification before the authentication step based on 16S rRNA genes. It is also advised that rhizobial isolates should go through a nodulation test against their host plants in growth pouches or Leonard jars for confirmation of their nitrogen-fixing ability in agreement with Kock's postulates (Somasegaran & Hoben, 1985).

Colony characterization of isolates were described in Table 1 with notes that all strains got negative results on LAM after being flooded in Benedict's reagent showing they may not be *Agrobacterium* sp.; hence the results on Hofer's alkaline medium were used as the main factor to arrange isolates to *Agrobacterium* sp. or *Rhizobium* sp. because of their ability to grow at high pH condition (Deshwal & Chaubey, 2014; Mir, Nagabhushanam, Quadriya, Kumar, & Hameeda, 2020). LA3-R3 and LA3-R4 strains had yellowish-opaque colonies on YMA-CR while others got white-opaque to a faint pink, however, their distinctive colors had been already mentioned (Somasegaran & Hoben, 1985, p. 68). Gram staining varied from young and old cells due to PHB accumulation that sometimes prevented staining reaction to whole cells (Somasegaran & Hoben, 1985, p. 25; Vincent, 1981, p. 829). For GPA-BCP test as a highly nutritious medium for checking culture purity, we observed that several rhizobial isolates can develop on this medium but not changed agar color, say, not caused pH changes, in contrast to other isolates had poor growth performance (Sharma et al., 2009). Instead of a nodulation test, we used Burk's N-free medium to check the ability to nitrogen-fixing of rhizobial isolates via their colony size. This result represents that not every *Rhizobium* sp. can grow well on N-free medium so their unique BNF should be at highest priority when working with these bacteria (Gauri et al., 2011; Park et al., 2005; Singh et al., 2008).

Starch hydrolysis, MR-VP test, and gelatin hydrolysis of 17 isolates were recorded negative results. This was consistent with Bergey manual for starch hydrolysis (Kuykendall, 2005; Kuykendall et al., 2005) but other studies showed different results (Panwar et al., 2012; Tyagi, Kumar, & Tomar, 2017). Although negative gelatin hydrolysis is a feature of *Rhizobium* spp. as proven by our studied isolates but there are some exceptions (Hunter, Kuykendall, & Manter, 2007; Mir et al., 2020; Sadowsky, Keyser, & Bohlool, 1983). For oxidase and catalase tests, there were both positive and negative results in rhizobial strains whereas these tests often got positive on *Rhizobium* spp. as reported (Deshwal & Chaubey, 2014; Gauri et al., 2011; Hossain et al., 2019; Panwar et al., 2012; Tyagi et al., 2017). The rest biochemical tests as urea hydrolysis, nitrate reduction, and citrate utilization varied in results depending on rhizobial species, as shown in Table 2, nevertheless, this illustrates diverse characteristics in rhizobia's metabolism even on the same *Rhizobium* species are highly affected by their living location (Becana & Sprent, 1987; Manhart & Wong, 1979; Panwar et al., 2012; Sadowsky et al., 1983; Streeter & DeVine, 1983; Thin et al., 2021).

Genetic identification of rhizobial isolates as given in Table 3; it showed results to be in accordance with the presumptive genus of *Rhizobium* and *Bradyrhizobium* species that encourages the strength of using simple selective media would help differentiate quickly rhizobial strains for further examination. Despite more *Rhizobium* species added as a result of rapid identification

methods based on molecular techniques that may change their taxonomic identities, a solid understanding of key distinguishing features of each typical rhizobial species would be worth to learn as for reducing unnecessary confusion when comparing with new ones to come (Chibeba et al., 2017; Hameed, Yasmin, Malik, Zafar, & Hafeez, 2004; Kuykendall, 2005; Kuykendall et al., 2005; Vincent, 1981).

Table 1

Presumptive test media for separating rhizobia from soil microbes

No.	Strain	YMA-CR	YMA-BTB	GPA-BCP	Hofer's alkaline medium	Burk's N-free medium	Presumptive genus
1	DT1-R2 Gram (-), short, rod shape	(+), colony gets white-opaque to faint pink, gummy; lens-shape with smooth margin	(+), agar changed yellow	(+)	(-)	(+)	*Rhizobium* sp.
2	DT1-R7 Gram (-), short, rod shape	(+), colony gets milky-translucent, gummy; lens-shape with smooth margin	(+), agar changed yellow	(-)	(-)	(-)	*Rhizobium* sp.
3	QN9-R2 Gram (-), short, rod shape	(+), colony gets watery-translucent with central red point pigmentation, gummy; domed-shape with smooth margin	(+), agar changed yellow	(+)	(+)	(+)	*Rhizobium* sp. or *Agrobacterium* sp.
4	QN9-R4 Gram (-), short, rod shape	(+), colony gets faint pink-opaque, small, gummy; domed-shape with smooth margin	(+), agar changed blue	(-)	(-)	(+)	*Bradyrhizobium* sp.
5	QN2-R2 Gram (-), short, rod shape	(+), colony gets faint pink with central red point pigmentation, gummy; domed-shape with smooth margin	(+), agar changed yellow	(+)	(-)	(+)	*Rhizobium* sp.
6	QN2-R8 Gram (-), short, rod shape	(+), colony gets faint pink with central red point pigmentation, gummy; domed-shape with smooth margin	(+), agar changed yellow	(+)	(-)	(+)	*Rhizobium* sp.
7	QN3-R3 Gram (-), short, rod shape	(+), colony gets watery-translucent with dark center, gummy; lens-shape with smooth margin	(+), agar changed yellow	(+)	(+)	(+)	*Rhizobium* sp. or *Agrobacterium* sp.
8	QN4-R1 Gram (-), short, rod shape	(+), colony gets faint pink, gummy; domed-shape with smooth margin	(+), agar changed yellow	(-)	(-)	(+), weak	*Rhizobium* sp.
9	QN5-R6 Gram (-), rod shape	(+), colony gets white-opaque with central red point pigmentation, gummy; domed-shape with smooth margin	(+), agar changed yellow	(+)	(-)	(+)	*Rhizobium* sp.

No.	Strain	YMA-CR	YMA-BTB	GPA-BCP	Hofer's alkaline medium	Burk's N-free medium	Presumptive genus
10	QN5-R9 Gram (-), short, rod shape	(+), colony gets white-opaque to, tend to be granular, small, gummy; lens-shape with smooth margin	(+), agar changed blue	(-)	(-)	(-)	*Bradyrhizobium* sp.
11	QN5-R11 Gram (-), rod shape	(+), colony gets white-opaque to faint pink, tend to be granular, small, gummy; lens-shape with smooth margin	(+), agar changed blue	(-)	(-)	(-)	*Bradyrhizobium* sp.
12	QN6-R2 Gram (-), short, rod shape	(+), colony gets white-opaque to faint pink, gummy; lens-shape with smooth margin	(+), agar changed yellow	(+)	(-)	(+)	*Rhizobium* sp.
13	QN6-R9 Gram (-), short, rod shape	(+), colony gets white-opaque with dark center, gummy; lens-shape with smooth margin	(+), agar changed yellow	(+)	(+)	(+)	*Rhizobium* sp. or *Agrobacterium* sp.
14	LA3-R1 Gram (-), rod shape	(+), colony gets faint pink-opaque, tend to be granular, small, gummy; convex shape with smooth margin	(+), agar changed blue	(-)	(-)	(+), weak	*Bradyrhizobium* sp.
15	LA3-R2 Gram (-), rod shape	(+), colony gets milk-opaque, tend to be granular, small, gummy; convex shape with smooth margin	(+), agar changed blue	(-)	(-)	(+), weak	*Bradyrhizobium* sp.
16	LA3-R3 Gram (-), short, rod shape	(+), colony gets yellowish-opaque, tend to be granular, small, gummy; convex shape with smooth margin	(+), agar changed blue	(-)	(-)	(+), weak	*Bradyrhizobium* sp.
17	LA3-R4 Gram (-), short, rod shape	(+), colony gets yellowish-opaque, tend to be granular, small, gummy; convex shape with smooth margin	(+), agar changed blue	(+)	(-)	(+), weak	*Bradyrhizobium* sp.

Source: The researcher's data analysis

Table 2

Biochemical characterization of rhizobia

No.	Strain	Oxidase	Catalase	Urea hydrolysis	Nitrate reduction	Citrate utilization
1	DT1-R2	(+)	(+)	(+), strong	(-)	(+)
2	DT1-R7	(-)	(-)	(+)	(-)	(+)
3	QN9-R2	(-)	(+)	(-)	(+)	(+)
4	QN9-R4	(+)	(+)	(-)	(+)	(-)
5	QN2-R2	(+)	(+)	(+), strong	(-)	(+), strong
6	QN2-R8	(-)	(-)	(+), strong	(-)	(+)

No.	Strain	Oxidase	Catalase	Urea hydrolysis	Nitrate reduction	Citrate utilization
7	QN3-R3	(-)	(-)	(+)	(-)	(+)
8	QN4-R1	(+)	(-)	(+)	(-)	(-)
9	QN5-R6	(+)	(-)	(+)	(+)	(+)
10	QN5-R9	(+)	(+)	(+), strong	(+)	(+)
11	QN5-R11	(+)	(+)	(-)	(+)	(+)
12	QN6-R2	(-)	(+)	(+), strong	(-)	(+)
13	QN6-R9	(-)	(+)	(+)	(-)	(+)
14	LA3-R1	(+)	(-)	(-)	(-)	(-)
15	LA3-R2	(+)	(-)	(-)	(-)	(-)
16	LA3-R3	(+)	(+)	(-)	(-)	(-)
17	LA3-R4	(-)	(-)	(-)	(-)	(-)

Source: The researcher's data analysis

Table 3

Identification of rhizobia isolated from legume nodules

No.	Strain	Origin	Host plant	Identification result		
				Accession number	Percent identity (%)	Species
1	DT1-R2	Dong Thap	Soybean	MN044788.1	1254/1254 (100%)	*Rhizobium mayense*
2	DT1-R7	Dong Thap	Soybean	NR114340.1	1333/1342 (99%)	*Rhizobium paknamense*
3	QN9-R2	Quang Ngai	Mung bean	MT573157.1 MT383661.1	1277/1277 (100%) 1277/1277 (100%)	*Rhizobium pusense* *Agrobacterium tumefaciens*
4	QN9-R4	Quang Ngai	Mung bean	MT501097.1	1277/1277 (100%)	*Bradyrhizobium elkanii*
5	QN2-R2	Quang Ngai	Peanut	MH236270.1	1314/1315 (99%)	*Rhizobium miluonense*
6	QN2-R8	Quang Ngai	Peanut	MN044788.1	1291/1291 (100%)	*Rhizobium mayense*
7	QN3-R3	Quang Ngai	Peanut	MK543082.1	1314/1314 (100%)	*Rhizobium tropici*
8	QN4-R1	Quang Ngai	Peanut	LC585447.1	1326/1329 (99%)	*Rhizobium phaseoli*
9	QN5-R6	Quang Ngai	Peanut	MH236279.1	1322/1325 (99%)	*Rhizobium miluonense*
10	QN5-R9	Quang Ngai	Peanut	MK519159.1	1263/1263 (100%)	*Bradyrhizobium centrosematis*
11	QN5-R11	Quang Ngai	Peanut	CP022219.1	1351/1353 (99%)	*Bradyrhizobium guangxiense*
12	QN6-R2	Quang Ngai	Peanut	MK872308.1	1343/1346 (99%)	*Rhizobium multihospitium*
13	QN6-R9	Quang Ngai	Peanut	MK543082.1	1322/1327 (99%)	*Rhizobium tropici*
14	LA3-R1	Long An	Peanut	KX230054.1	1351/1352 (99%)	*Bradyrhizobium liaoningense*
15	LA3-R2	Long An	Peanut	MT534129.1	1351/1352 (99%)	*Bradyrhizobium yuanmingense*
16	LA3-R3	Long An	Peanut	MN661168.1	1295/1296 (99%)	*Bradyrhizobium liaoningense*
17	LA3-R4	Long An	Peanut	MN661167.1	1347/1348 (99%)	*Bradyrhizobium arachidis*

Source: The researcher's data analysis

3.2. Characterization of strains

3.2.1. Thermal and saline tolerance

For most rhizobial strains, the optimum temperature for growth is 28 - 31°C; ambient temperature whether for cultivation in the laboratory or in field farming that being too hot or too cold, always creates negative impacts on their growth and nodulation activity, thus making BNF low and ineffective (Graham, 1992; Kuykendall et al., 2005; Rao, 2014; Zahran, 1999). In our study, DT1-R7, QN2-R8, and QN3-R3 owned thermal tolerance capability strongly that enabled them to grow from a wide range of temperatures 14 - 44°C, it could be explained by their sampling location is a place featuring hot and dry weather as Quang Ngai Province. Other rhizobial isolates can also deal with high-temperature stress at 40°C that not affecting too much to their growth as colonies on the agar surfaces.

Regarding saline tolerance that some studies have reported that their select rhizobia can be highly salt tolerant up to 3 - 4% (Datta et al., 2015; Gauri et al., 2011) or even to 5% (w/v) NaCl (Küçük, Kivanç, & Kinaci, 2006) but a medium containing NaCl with concentration below 1% (w/v) is preferred for most *Rhizobium* spp. (Kuykendall, 2005; Kuykendall et al., 2005; Vincent, 1981). In contrast with thermal tolerance, data given in Figure 1 showed a clear salt tolerance ability of each strain versus its origin; particularly, most strains from Dong Thap and Quang Ngai Provinces had better saline tolerance up to 04 - 06 times than strains from Long An Province which seems not to live well if NaCl concentration in the medium is greater than 0.5%. Abiotic stresses on BNF have been thoroughly studied and considered as main reasons for explaining why some commercial rhizobial strains can perform at best on their testing sites but often fail when applied in different geographical regions (Michiels et al., 1994; Rastetter, Vitousek, Field, Shaver, & Herbert, 2001; Vitousek & Field, 1999). This leaves a necessary space for local rhizobia isolation and screening promising strains for microbial inoculant which respect to the climate condition and soil types (Burton, 1984, p. 14; Lindström, Murwira, Willems, & Altier, 2010; Lindström & Mousavi, 2020).

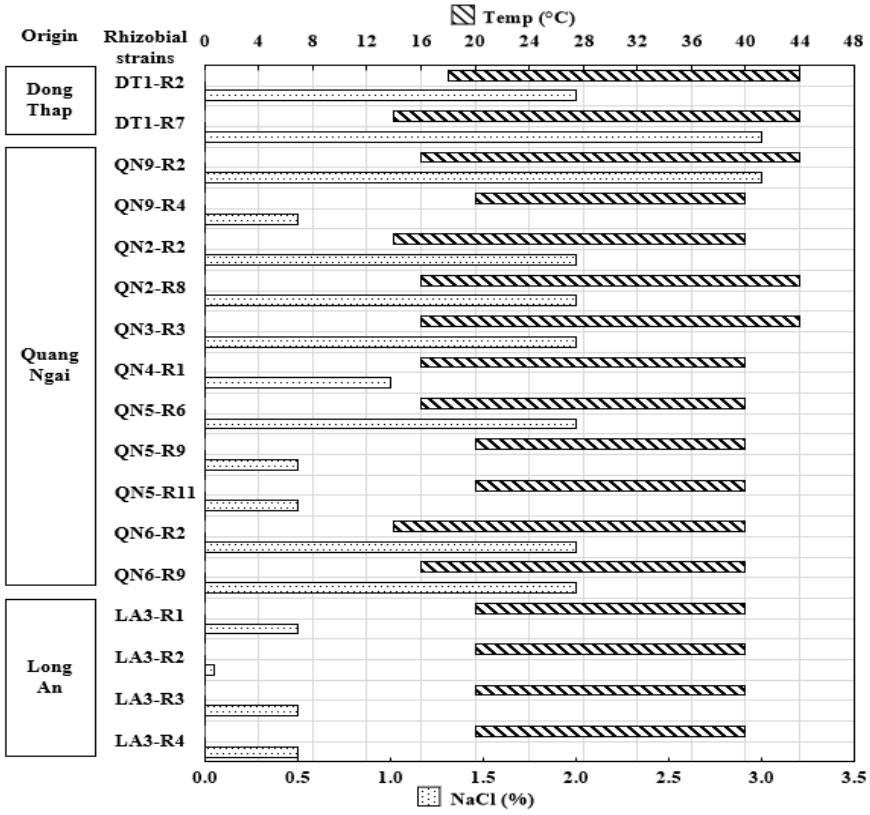

Figure 1. Thermal and saline tolerance of rhizobia regarding their origin

3.2.2. Impact on seed germination

Germination index is calculated based on the ratio of germination percentage multiplied total root length of seeds of the studied treatment over the control treatment (usually distilled water) in an isolated container. It is, therefore, a parameter representing both germination and growth status that seeds reflected on outside agents which inoculated at beginning day (Emino & Warman, 2004). Take advantage of this approach, the GI values of 17 rhizobial strains on each of their seed inoculated, as shown in Figure 2, have been plotted and compared their means for finding how much rhizobia can help seed germination if soaking seeds with bacterial suspension.

Interestingly, there was a pattern that rhizobia strains seemed to have better results on rice seed (non-legume) rather than on mung bean seed (legume) in the early seedling germination stage. Albeit large standard deviations of GI values on both two type seeds, DT1-R2, QN9-R4, QN3-R3, QN6-R9 had given positive effect on rice germination in terms of statistical significance while LA3-R1 helped mung bean seed to germinate effectively. Considering the GI values of rhizobia on mung bean germination, DT1-R2 gave the lowest result that raised a question about there is existing a chance that *Rhizobium* sp. would create a negative impact on the same group of legumes on their early germination while enhancing other non-legume seed germination as rice seed. In general, most of the rhizobial strains had helped the seed germinate strongly indicating through GI values surpass 100%. Thus, an inoculation step for coating seeds with rhizobia before sowing is often recommended in order to enhance plant productivity because of other valued compounds that *Rhizobium* spp. and *Bradyrhizobium* spp. released that turning their role not limited to as nitrogen-fixing bacteria but key players in plant growth-promoting rhizobacteria (Howieson & Dilworth, 2016; Mia, Shamsuddin, & Mahmood, 2012; Sistani, Kaul, Desalegn, & Wienkoop, 2017).

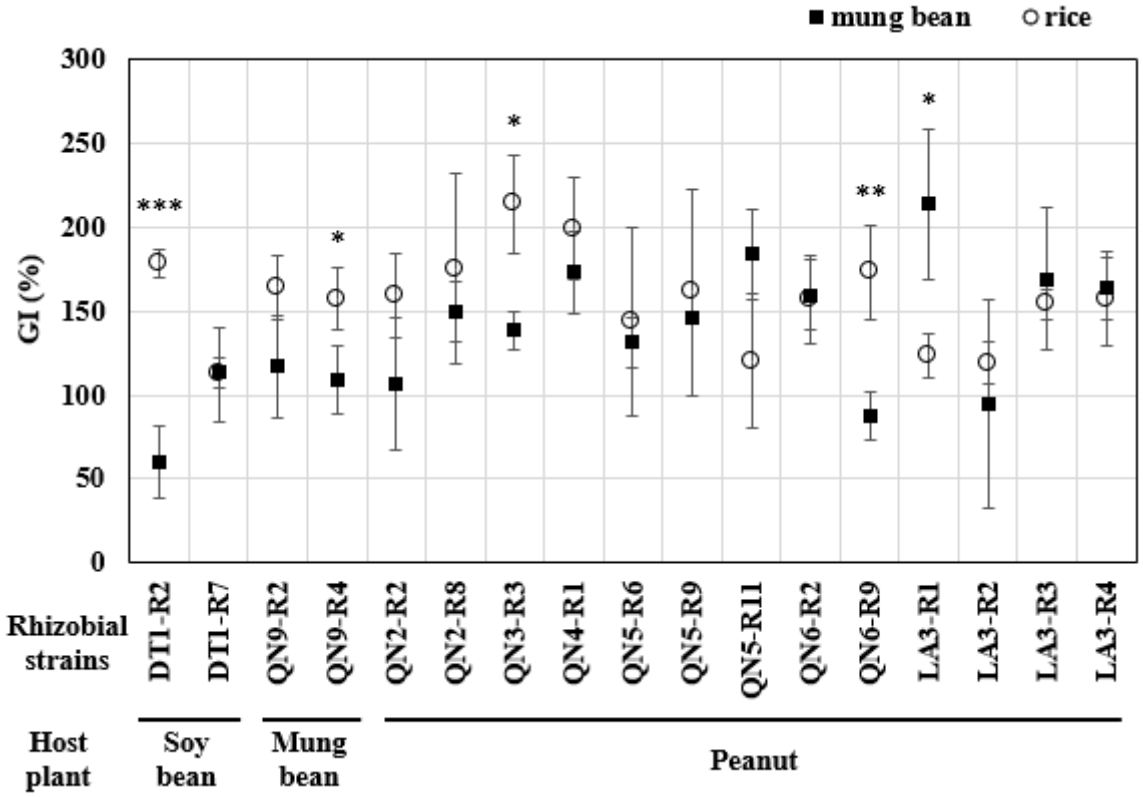

Figure 2. Effectiveness on seed germination of rhizobia regarding to their host plant

4. Conclusions & recommendations

By using a series of presumptive test media as a conventional rhizobial isolation procedure, there were 17 rhizobial strains isolated and classified biochemically that further genetic identification results confirmed that 10 strains belong to *Rhizobium* genus as DT1-R2, DT1-R7, QN9-R2, QN2-R2, QN2-R8, QN3-R3, QN4-R1, QN5-R6, QN6-R2, and QN6-R9 corresponding to their *Rhizobium* species as *R. mayense*, *R. paknamense, R. pusense, R. miluonense*, *R. tropici*, *R. phaseoli*, and *R. multihospitium*; the rest 07 strains belong to *Bradyrhizobium* genus as QN9-R4, QN5-R9, QN5-R11, LA3-R1, LA3-R2, LA3-R3, and LA3-R4 as *B. elkanii, B. centrosematis, B. guangxiense, B. liaoningense, B. yuanmingense*, and *B. arachidis* respectively.

Thermal and saline tolerant tests along with examining the impact on rice and mung bean seed germination of these rhizobial strains were helping to characterize them into groups that can be used for inoculant purposes. An understanding of *Rhizobium* spp. and *Bradyrhizobium* spp. in respect of their origin and host plant also achieved, that encourage us use widely these nature-gifted species at ease and confidence once we care enough for their optima living conditions.

ACKNOWLEDGEMENTS

The authors would like to express sincere appreciation to Biotechnology Center of Ho Chi Minh City for funding this research project coded MT01/19-21 as well as our hard-working students during their internship that both significantly helped us complete this article at the time being.

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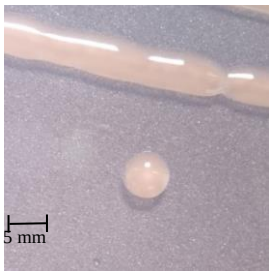
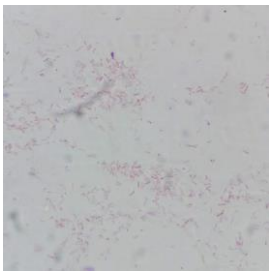
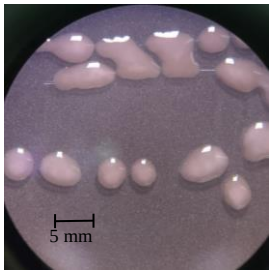
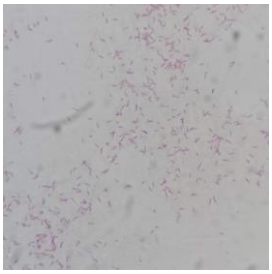
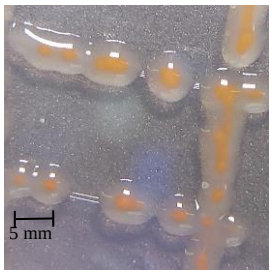
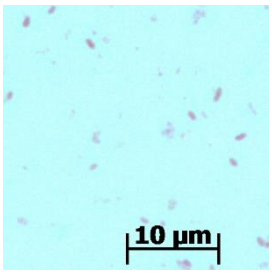
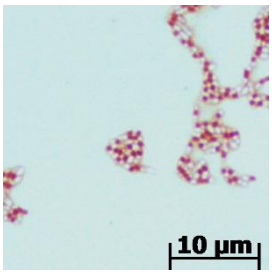
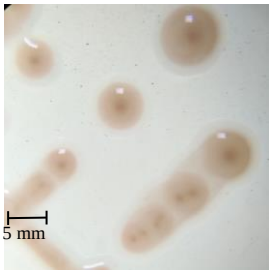
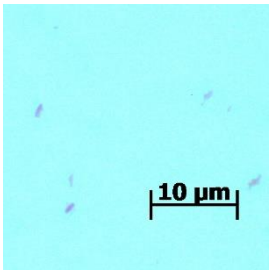
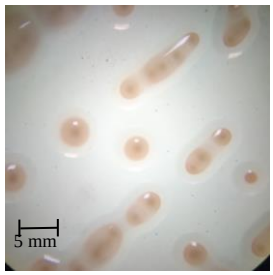
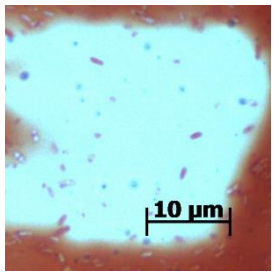
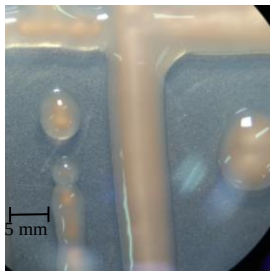
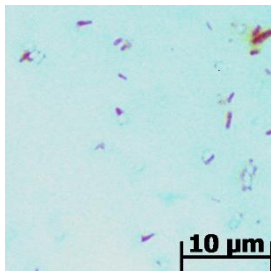
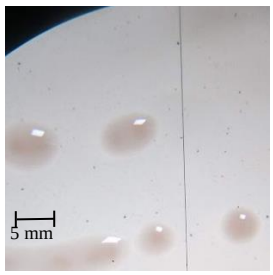
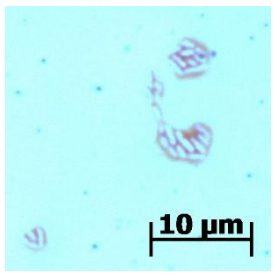
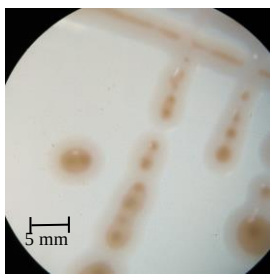
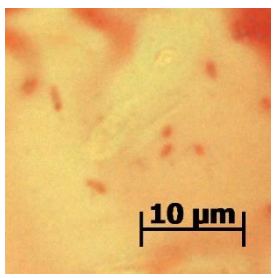
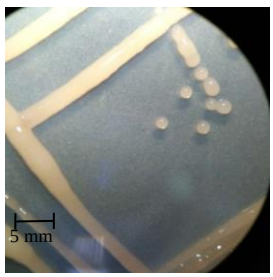
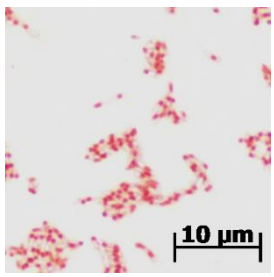
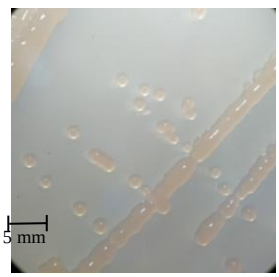
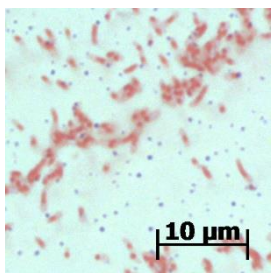
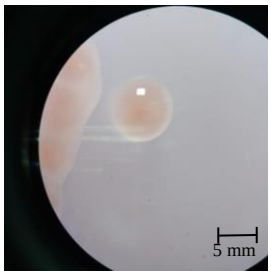
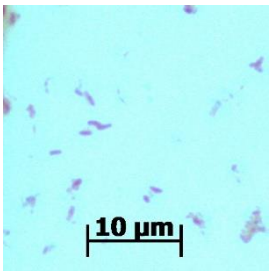
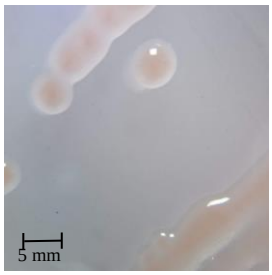
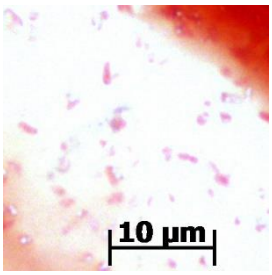
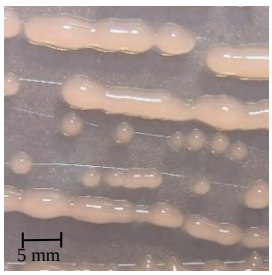
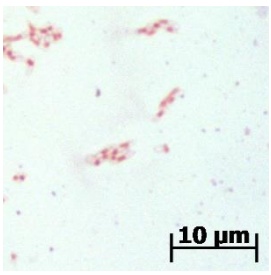
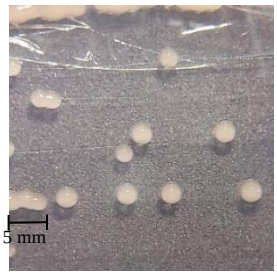
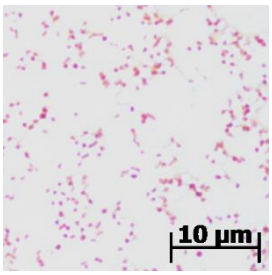
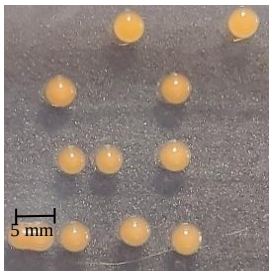
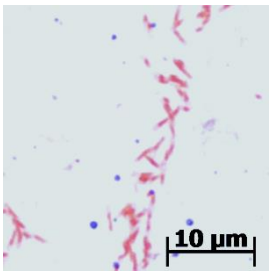
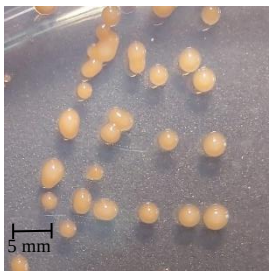
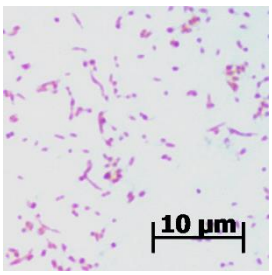
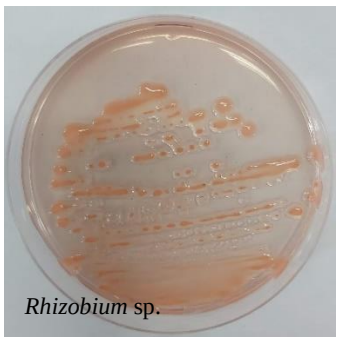
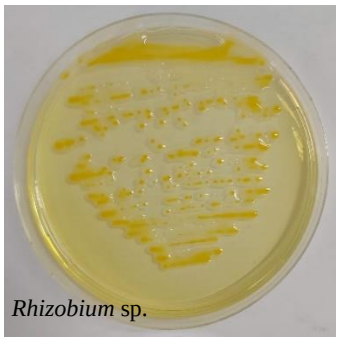
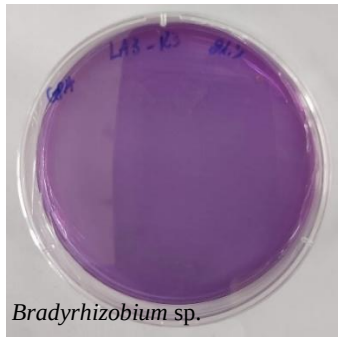
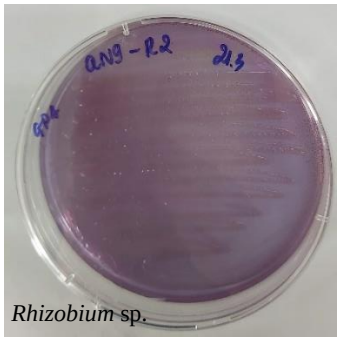
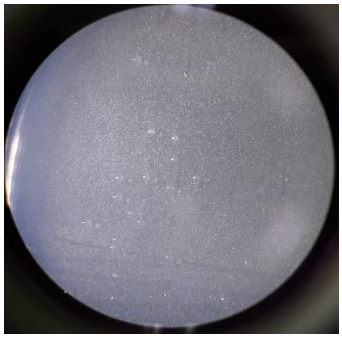
APPENDIX A
COLONY AND GRAM STAINING

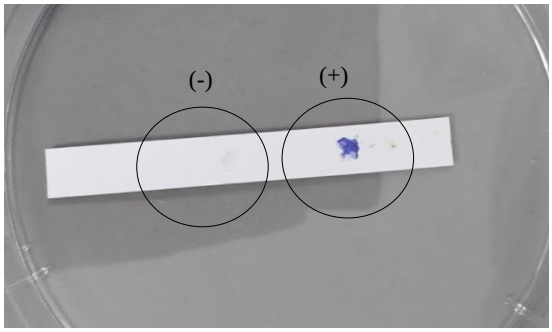
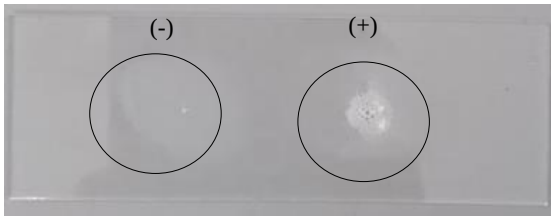
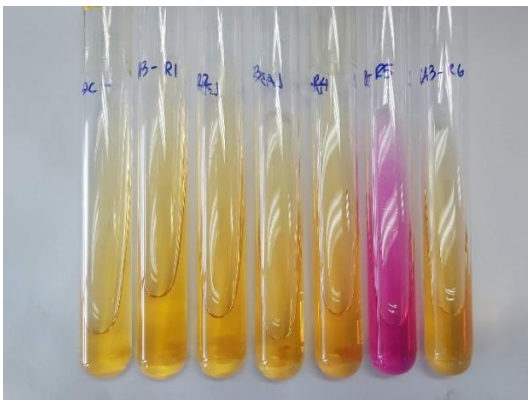
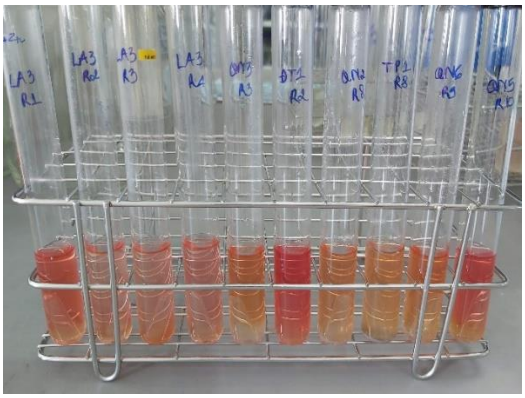
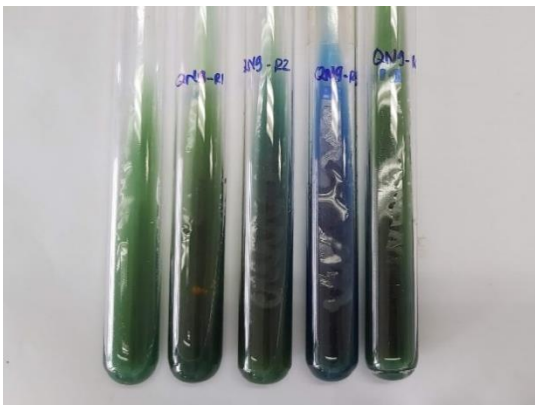
No.	**Strain**	**Colony** on YMA-CR agar plates	**Gram staining** 100×
1	DT1-R2		
2	DT1-R7		
3	QN9-R2		
4	QN9-R4		
5	QN2-R2		

No.	**Strain**	**Colony** on YMA-CR agar plates	**Gram staining** 100×
6	QN2-R8		
7	QN3-R3		
8	QN4-R1		
9	QN5-R6		
10	QN5-R9		
11	QN5-R11		

No.	**Strain**	**Colony** on YMA-CR agar plates	**Gram staining** 100×
12	QN6-R2		
13	QN6-R9		
14	LA3-R1		
15	LA3-R2		
16	LA3-R3		
17	LA3-R4		

CHEMICAL TEST

YMA-CR	 *Bradyrhizobium* sp.  *Rhizobium* sp.
YMA-BTB	 *Bradyrhizobium* sp.  *Rhizobium* sp.
GPA-BCP	 *Bradyrhizobium* sp.  *Rhizobium* sp.
Hofer's alkaline medium	 *Bradyrhizobium* sp.  *Rhizobium* sp.
Burk's N-free medium	

Oxidase	
Catalase	
Urea hydrolysis	
Nitrate reduction	
Citrate utilization	

APPENDIX B
SEQUENCING DATA

No.	Strain	Sequencing data
1	DT1-R2	GGGTAAAACCAACTCCCATGGTGTGACGGGCGGTGTGTACAAGGCCCGGGAACGTATTC ACCGCGGCATGCTGATCCGCGATTACTAGCGATTCCAACTTCATGCACTCGAGTTGCAGA GTGCAATCCGAACTGAGATGGCTTTTGGAGATTAGCTCACACTCGCGTGCTCGCTGCCCA CTGTCACCACCATTGTAGCACGTGTGTAGCCCAGCCCGTAAGGGCCATGAGGACTTGACG TCATCCCCACCTTCCTCTCGGCTTATCACCGGCAGTCCCCTTAGAGTGCCCAACTAAATG CTGGCAACTAAGGGCGAGGGTTGCGCTCGTTGCGGGACTTAACCCAACATCTCACGACA CGAGCTGACGACAGCCATGCAGCACCTGTCTCTGAGCCACCGAAGTGGAAAGTGCATCTC TGCACCGGTCCCAGGATGTCAAGGGCTGGTAAGGTTCTGCGCGTTGCTTCGAATTAAAC CACATGCTCCACCGCTTGTGCGGGCCCCCGTCAATTCCTTTGAGTTTTAATCTTGCGACC GTACTCCCCAGGCGGAATGTTTAATGCGTTAGCTGCGCCACCGAACAGTATACTGCCCGA CGGCTAACATTCATCGTTTACGGCGTGGACTACCAGGGTATCTAATCCTGTTTGCTCCCC ACGCTTTCGCACCTCAGCGTCAGTAATGGACCAGTGAGCCGCCTTCGCCACTGGTGTTCC TCCGAATATCTACGAATTTCACCTCTACACTCGGAATTCCACTCACCTCTTCCATACTCC AGATCGACAGTATCAAAGGCAGTTCCAGGGTTGAGCCCTGGGATTTCACCCCTGACTGA TCGATCCGCCTACGTGCGCTTTACGCCCAGTAATTCCGAACAACGCTAGCCCCCTTCGTA TTACCGCGGCTGCTGGCACGAAGTTAGCCGGGGCTTCTTCTCCGGATACCGTCATTATCT TCTCCGGTGAAAGAGCTTTACAACCCTAGGGCCTTCATCACTCACGCGGCATGGCTGGAT CAGGCTTGCGCCCATTGTCCAATATTCCCCACTGCTGCCTCCCGTAGGAGTTTGGGCCGT GTCTCAGTCCCAATGTGGCTGATCATCCTCTCAGACCAGCTATGGATCGTCGCCTTGGTA GGCCTTTACCCCACCAACTAGCTAATCCAACGCGGGCTCATCTCTTGCCGATAAATCTTT CTCCCGAAGGACACATACGGTATTAGCACAAGTTTCCCTGCGTTATTCCGTAGCAAAA
2	DT1-R7	TCGCCTCGGTCCTTGCGGTTAGCGCAGCGCCTTCGGGTAAAACCAACTCCCATGGTGTGA CGGGCGGTGTGTACAAGGCCCGGGAACGTATTCACCGCGGCATGCTGATCCGCGATTAC TAGCGATTCCAACTTCATGCACTCGAGGTGCAGAGTGCAATCCGAACTGAGATGGCTTT TGGAGATTAGCTCACACTCGCGTGCTCGCTGCCCACTGTCACCACCATTGTAGCACGTGT GTAGCCCAGCCCGTAAGGGCCATGAGGACTTGACGTCATCCCCACCTTCCTCTCGGCTTA TCACCGGCAGTCCCCTTAGAGTGCCCAACCTAATGCTGGCAACTAAGGGCGAGGGTTGC GCTCGTTGCGGGACTTAACCCAACATCTCACGACACGAGCTGACGACAGCCATGCAGCAC CTGTCTCCTTGCCCCCGAAGGGGAAACTGCATCTCTGCAGCGGTCAAGGGATGTCAAGA GCTGGTAAGGTTCTGCGCGTTGCTTCGAATTAAACCACATGCTCCACCGCTTGTGCGGGC CCCCGTCAATTCCTTTGAGTTTTAATCTTGCGACCGTACTCCCCAGGCGGAATGTTTAAT GCGTTAGCGGCGCCACCGAACAGTCAACTGCCCGACGGCTAACATTCATCGTTTACGGCG TGGACTACCAGGGTATCTAATCCTGTTTGCTCCCCACGCTTTCGCACCTCAGCGTCAGTA ATGGACCAGTAAGCCGCCTTCGCCACTGGTGTTCCTCCGAATATCTACGAATTTCACCTC TACACTCGGAATTCCACTTACCTCTTCCATACTCAAGACACCCAGTATCAAAGGCAGTTC CAGAGTTGAGCTCTGGGATTTCACCCCTGACTTAAGCGTCCGCCTACGTGCGCTTTACGC CCAGTAATTCCGAACAACGCTAGCCCCCTTCGTATTACCGCGGCTGCTGGCACGAAGTTA GCCGGGGCTTCTTCTCCGGATACCGTCATTATCTTCTCCGGTGAAAGAGCTTTACAACCC TAAGGCCTTCATCACTCACGCGGCATGGCTGGATCAGGCTTGCGCCCATTGTCCAATATT CCCCACTGCTGCCTCCCGTAGGAGTTTGGGCCGTGTCTCAGTCCCAATGTGGCTGATCAT CCTCTCAGACCAGCTATGGATCGTCGCCTTGGTAGGCCTTTACCCCACCAACTAGCTAAT CCAACGCGGGCCGATCCTTTGCCGATAAATCTTTCCCCCGTAGGGCTCATACGGTATTAA TTCCAGTTTCCCGGAGCTATTCCGTAGCAAAGGGCACGTTCCCACGCGTTACTCACCCGT CTGCCGCTCACCCCGAAGGATGCGCTCGACTGCA
3	QN9-R2	TAAAACCAACTCCCATGGTGTGACGGGCGGTGTGTACAAGGCCCGGGAACGTATTCACC GCAGCATGCTGATCTGCGATTACTAGCGATTCCAACTTCATGCACTCGAGTTGCAGAGT GCAATCCGAACTGAGATGGCTTTTGGAGATTAGCTCGACATCGCTGTCTCGCTGCCCACT GTCACCACCATTGTAGCACGTGTGTAGCCCAGCCCGTAAGGGCCATGAGGACTTGACGTC ATCCCCACCTTCCTCTCGGCTTATCACCGGCAGTCCCCTTAGAGTGCCCAACTAAATGCT GGCAACTAAGGGCGAGGGTTGCGCTCGTTGCGGGACTTAACCCAACATCTCACGACACG AGCTGACGACAGCCATGCAGCACCTGTTCTGGGGCCAGCCTAACTGAAGGACATCGTCTC CAATGCCCATACCCCGAATGTCAAGAGCTGGTAAGGTTCTGCGCGTTGCTTCGAATTAA ACCACATGCTCCACCGCTTGTGCGGGCCCCCGTCAATTCCTTTGAGTTTTAATCTTGCGA CCGTACTCCCCAGGCGGAATGTTTAATGCGTTAGCTGCGCCACCGAACAGTATACTGCCC

No.	Strain	Sequencing data
		GACGGCTAACATTCATCGTTTACGGCGTGGACTACCAGGGTATCTAATCCTGTTTGCTCC CCACGCTTTCGCACCTCAGCGTCAGTAATGGACCAGTAAGCCGCCTTCGCCACTGGTGTT CCTCCGAATATCTACGAATTTCACCTCTACACTCGGAATTCCACTTACCTCTTCCATACT CAAGATACCCAGTATCAAAGGCAGTTCCGCAGTTGAGCTGCGGGATTTCACCCCTGACT TAAATATCCGCCTACGTGCGCTTTACGCCCAGTAATTCCGAACAACGCTAGCCCCCTTCG TATTACCGCGGCTGCTGGCACGAAGTTAGCCGGGGCTTCTTCTCCGACTACCGTCATTAT CTTCATCGGTGAAAGAGCTTTACAACCCTAAGGCCTTCATCACTCACGCGGCATGGCTGG ATCAGGCTTGCGCCCATTGTCCAATATTCCCCACTGCTGCCTCCCGTAGGAGTTTGGGCC GTGTCTCAGTCCCAATGTGGCTGATCATCCTCTCAGACCAGCTATGGATCGTCGCCTTGG TAGGCCTTTACCCCACCAACTAGCTAATCCAACGCGGGCCAATCCTTCCCCGATAAATCT TTCCCCCGTAGGGCGTATGCGGTATTAATTCCAGTTTCCCGGAGCTATTCCGCAGGAAAG GGTATGTTCCCACGCGTTACTCCA
4	QN9-R4	AGCGCACCGTCTTCAGGTAAAACCAACTCCCATGGTGTGACGGGCGGTGTGTACAAGGC CCGGGAACGTATTCACCGTGGCGTGCTGATCCACGATTACTAGCGATTCCAACTTCATGG GCTCGAGTTGCAGAGCCCAATCCGAACTGAGACGGCTTTTTGAGATTTGCGAAGGGTCG CCCCTTAGCATCCCATTGTCACCGCCATTGTAGCACGTGTGTAGCCCAGCCCGTAAGGGC CATGAGGACTTGACGTCATCCCCACCTTCCTCGCGGCTTATCACCGGCAGTCTCCTTAGA GTGCTCAACTAAATGGTAGCAACTAAGGACGGGGGTTGCGCTCGTTGCGGGACTTAACC CAACATCTCACGACACGAGCTGACGACAGCCATGCAGCACCTGTCTCCGGTCCAGCCGAA CTGAAGAACTCCGTCTCTGGAGTCCGCGACCGGGATGTCAAGGGCTGGTAAGGTTCTGC GCGTTGCGTCGAATTAAACCACATGCTCCACCGCTTGTGCGGGCCCCCGTCAATTCCTTT GAGTTTTAATCTTGCGACCGTACTCCCCAGGCGGAATGCTTAAAGCGTTAGCTGCGCCAC TAGTGAGTAAACCCACTAACGGCTGGCATTCATCGTTTACGGCGTGGACTACCAGGGTA TCTAATCCTGTTTGCTCCCCACGCTTTCGTGCCTCAGCGTCAGTATCGGGCCAGTGAGCC GCCTTCGCCACTGGTGTTCTTGCGAATATCTACGAATTTCACCTCTACACTCGCAGTTCC ACTCACCTCTCCCGAACTCAAGATCTTCAGTATCAAAGGCAGTTCTGGAGTTGAGCTCCA GGATTTCACCCCTGACTTAAAGACCCGCCTACGCACCCTTTACGCCCAGTGATTCCGAGC AACGCTAGCCCCCTTCGTATTACCGCGGCTGCTGGCACGAAGTTAGCCGGGGCTTATTCT TGCGGTACCGTCATTATCTTCCCGCACAAAAGAGCTTTACAACCCTAGGGCCTTCATCAC TCACGCGGCATGGCTGGATCAGGCTTGCGCCCATTGTCCAATATTCCCCACTGCTGCCTC CCGTAGGAGTTTGGGCCGTGTCTCAGTCCCAATGTGGCTGATCATCCTCTCAGACCAGCT ACTGATCGTCGCCTTGGTGAGCCATTACCTCACCAACTAGCTAATCAGACGCGGGCCGAT CTTTCGGCGATAAATCTTTCCCCGTAAGGGCTTATCCGGTATTAGCTGAAGTTTCCCTCA GTTGTTCCGAACCAAAAGGTAC
5	QN2-R2	TCCTTGCGGTTAGCGCACTACCTTCGGGTAAAACCAACTCCCATGGTGTGACGGGCGGTG TGTACAAGGCCCGGGAACGTATTCACCGCGGCATGCTGATCCGCGATTACTAGCGATTCC AACTTCATGCACTCGAGTTGCAGAGTGCAATCCGAACTGAGATGGCTTTTGGAGATTAG CTCACACTCGCGTGCTCGCTGCCCACTGTCACCACCATTGTAGCACGTGTGTAGCCCAGC CCGTAAGGGCCATGAGGACTTGACGTCATCCCCACCTTCCTCTCGGCTTATCACCGGCAG TCCCCTTAGAGTGCCCAACTAAATGCTGGCAACTAAGGGCGAGGGTTGCGCTCGTTGCG GGACTTAACCCAACATCTCACGACACGAGCTGACGACAGCCATGCAGCACCTGTCTCTGA GCCACCGAAGTGGAAAGTGCATCTCTGCACCGGTCCCAGGATGTCAAGGGCTGGTAAGG TTCTGCGCGTTGCTTCGAATTAAACCACATGCTCCACCGCTTGTGCGGGCCCCCGTCAAT TCCTTTGAGTTTTAATCTTGCGACCGTACTCCCCAGGCGGAATGTTTAATGCGTTAGCTG CGCCACCGAACAGTATACTGCCCGACGGCTAACATTCATCGTTTACGGCGTGGACTACCA GGGTATCTAATCCTGTTTGCTCCCCACGCTTTCGCACCTCAGCGTCAGTAATGGACCAGT GAGCCGCCTTCGCCACTGGTGTTCCTCCGAATATCTACGAATTTCACCTCTACACTCGGA ATTCCACTCACCTCTTCCATACTCCAGATCGACAGTATCAAAGGCAGTTCCAGGGTTGAG CCCTGGGATTTCACCCCTGACTGATCGATCCGCCTACGTGCGCTTTACGCCCAGTAATTC CGAACAACGCTAGCCCCCTTCGTATTACCGCGGCTGCTGGCACGAAGTTAGCCGGGGCTT CTTCTCCGGATACCGTCATTATCTTCTCCGGTGAAAGAGCTTTACAACCCTAGGGCCTTC ATCACTCACGCGGCATGGCTGGATCAGGCTTGCGCCCATTGTCCAATATTCCCCACTGCT GCCTCCCGTAGGAGTTTGGGCCGTGTCTCAGTCCCAATGTGGCTGATCATCCTCTCAGAC CAGCTATGGATCGTCGCCTTGGTAGGCCTTTACCCCACCAACTAGCTAATCCAACGCGGG CTCATCTCTTGCCGATAAATCTTTCTCCCGAAGGACACATACGGTATTAGCAGAAGTTT CCCTGCGTTATTCCGTAGCAAAAGGTAGATTCCCACGCGTTACTCACCCGTCTGCCGCT

No.	Strain	Sequencing data
6	QN2-R8	CGGGTAAAACCAACTCCCATGGTGTGACGGGCGGTGTGTACAAGGCCCGGGAACGTATT CACCGCGGCATGCTGATCCGCGATTACTAGCGATTCCAACTTCATGCACTCGAGTTGCAG AGTGCAATCCGAACTGAGATGGCTTTTGGAGATTAGCTCACACTCGCGTGCTCGCTGCCC ACTGTCACCACCATTGTAGCACGTGTGTAGCCCAGCCCGTAAGGGCCATGAGGACTTGAC GTCATCCCCACCTTCCTCTCGGCTTATCACCGGCAGTCCCCTTAGAGTGCCCAACTAAAT GCTGGCAACTAAGGGCGAGGGTTGCGCTCGTTGCGGGACTTAACCCAACATCTCACGAC ACGAGCTGACGACAGCCATGCAGCACCTGTCTCTGAGCCACCGAAGTGGAAAGTGCATC TCTGCACCGGTCCCAGGATGTCAAGGGCTGGTAAGGTTCTGCGCGTTGCTTCGAATTAA ACCACATGCTCCACCGCTTGTGCGGGCCCCCGTCAATTCCTTTGAGTTTTAATCTTGCGA CCGTACTCCCCAGGCGGAATGTTTAATGCGTTAGCTGCGCCACCGAACAGTATACTGCCC GACGGCTAACATTCATCGTTTACGGCGTGGACTACCAGGGTATCTAATCCTGTTTGCTCC CCACGCTTTCGCACCTCAGCGTCAGTAATGGACCAGTGAGCCGCCTTCGCCACTGGTGTT CCTCCGAATATCTACGAATTTCACCTCTACACTCGGAATTCCACTCACCTCTTCCATACT CCAGATCGACAGTATCAAAGGCAGTTCCAGGGTTGAGCCCTGGGATTTCACCCCTGACT GATCGATCCGCCTACGTGCGCTTTACGCCCAGTAATTCCGAACAACGCTAGCCCCCTTCG TATTACCGCGGCTGCTGGCACGAAGTTAGCCGGGGCTTCTTCTCCGGATACCGTCATTAT CTTCTCCGGTGAAAGAGCTTTACAACCCTAGGGCCTTCATCACTCACGCGGCATGGCTGG ATCAGGCTTGCGCCCATTGTCCAATATTCCCCACTGCTGCCTCCCGTAGGAGTTTGGGCC GTGTCTCAGTCCCAATGTGGCTGATCATCCTCTCAGACCAGCTATGGATCGTCGCCTTGG TAGGCCTTTACCCCACCAACTAGCTAATCCAACGCGGGCTCATCTCTTGCCGATAAATCT TTCTCCCGAAGGACACATACGGTATTAGCACAAGTTTCCCTGCGTTATTCCGTAGCAAA AGGTAGATTCCCACGCGTTACTCACCCGTCTGCCGCT
7	QN3-R3	GCGGTTAGCGCACTACCTTCGGGTAAAACCAACTCCCATGGTGTGACGGGCGGTGTGTA CAAGGCCCGGGAACGTATTCACCGCGGCATGCTGATCCGCGATTACTAGCGATTCCAACT TCATGCACTCGAGTTGCAGAGTGCAATCCGAACTGAGATGGCTTTTGGAGATTAGCTCA CACTCGCGTGCTCGCTGCCCACTGTCACCACCATTGTAGCACGTGTGTAGCCCAGCCCGT AAGGGCCATGAGGACTTGACGTCATCCCCACCTTCCTCTCGGCTTATCACCGGCAGTCCC CTTAGAGTGCCCAACTTAATGCTGGCAACTAAGGGCGAGGGTTGCGCTCGTTGCGGGAC TTAACCCAACATCTCACGACACGAGCTGACGACAGCCATGCAGCACCTGTCTCTGAGCCA CCGAAGTGGAAAGTGCATCTCTGCACCGGTCCCAGGATGTCAAGGGCTGGTAAGGTTCT GCGCGTTGCTTCGAATTAAACCACATGCTCCACCGCTTGTGCGGGCCCCCGTCAATTCCT TTGAGTTTTAATCTTGCGACCGTACTCCCCAGGCGGAATGTTTAATGCGTTAGCTGCGCC ACCGAACAGTATACTGCCCGACGGCTAACATTCATCGTTTACGGCGTGGACTACCAGGG TATCTAATCCTGTTTGCTCCCCACGCTTTCGCACCTCAGCGTCAGTAATGGACCAGTGAG CCGCCTTCGCCACTGGTGTTCCTCCGAATATCTACGAATTTCACCTCTACACTCGGAATT CCACTCACCTCTTCCATACTCCAGATCGACAGTATCAAAGGCAGTTCCAGGGTTGAGCCC TGGGATTTCACCCCTGACTGATCGATCCGCCTACGTGCGCTTTACGCCCAGTAATTCCGA ACAACGCTAGCCCCCTTCGTATTACCGCGGCTGCTGGCACGAAGTTAGCCGGGGCTTCTT CTCCGGATACCGTCATTATCTTCTCCGGTGAAAGAGCTTTACAACCCTAGGGCCTTCATC ACTCACGCGGCATGGCTGGATCAGGCTTGCGCCCATTGTCCAATATTCCCCACTGCTGCC TCCCGTAGGAGTTTGGGCCGTGTCTCAGTCCCAATGTGGCTGATCATCCTCTCAGACCAG CTATGGATCGTCGCCTTGGTAGGCCTTTACCCCACCAACTAGCTAATCCAACGCGGGCTC ATCTCTTGCCGATAAATCTTTCTCCCGAAGGACACATACGGTATTAGCACAAGTTTCCCT GCGTTATTCCGTAGCAAAAGGTAGATTCCCACGCGTTACTCACCCGTCTGCCGCTCCCC
8	QN4-R1	AGCGCACTACCTTCGGGTAAAACCAACTCCCATGGTGTGACGGGCGGTGTGTACAAGGC CCGGGAACGTATTCACCGCGGCATGCTGATCCGCGATTACTAGCGATTCCAACTTCATGC ACTCGAGTTGCAGAGTGCAATCCGAACTGAGATGGCTTTTGGAGATTAGCTCACACTCG CGTGCTCGCTGCCCACTGTCACCACCATTGTAGCACGTGTGTAGCCCAGCCCGTAAGGGC CATGAGGACTTGACGTCATCCCCACCTTCCTCTCGGCTTATCACCGGCAGTCCCCTTAGA GTGCCCAACCAAATGCTGGCAACTAAGGGCGAGGGTTGCGCTCGTTGCGGGACTTAACC CAACATCTCACGACACGAGCTGACGACAGCCATGCAGCACCTGTGTCCCGGTCCCCGAAG GGAACCTTGCATCTCTGCAAGTAGCCGGGCATGTCAAGGGCTGGTAAGGTTCTGCGCGT TGCTTCGAATTAAACCACATGCTCCACCGCTTGTGCGGGCCCCCGTCAATTCCTTTGAGT TTTAATCTTGCGACCGTACTCCCCAGGCGGAATGTTTAATGCGTTAGCTGCGCCACCGAA CAGTATACTGCCCGACGGCTAACATTCATCGTTTACGGCGTGGACTACCAGGGTATCTA ATCCTGTTTGCTCCCCACGCTTTCGCACCTCAGCGTCAGTAATGGACCAGTGAGCCGCCT TCGCCACTGGTGTTCCTCCGAATATCTACGAATTTCACCTCTACACTCGGAATTCCACTC

No.	Strain	Sequencing data
		ACCTCTTCCATACTCCAGATCGACAGTATCAAAGGCAGTTCCAGGGTTGAGCCCTGGGA TTTCACCCCTGACTGATCGATCCGCCTACGTGCGCTTTACGCCCAGTAATTCCGAACAAC GCTAGCCCCCTTCGTATTACCGCGGCTGCTGGCACGAAGTTAGCCGGGGCTTCTTCTCCG GATACCGTCATTATCTTCTCCGGTGAAAGAGCTTTACAACCCTAGGGCCTTCATCACTCA CGCGGCATGGCTGGATCAGGCTTGCGCCCATTGTCCAATATTCCCCACTGCTGCCTCCCG TAGGAGTTTGGGCCGTGTCTCAGTCCCAATGTGGCTGATCATCCTCTCAGACCAGCTATG GATCGTCGCCTTGGTAGGCCTTTACCCCACCAACTAGGTAATCCAACGCGGGCCGATCCT TTACCGATAAATCTTTCCCCCAAAGGGCACATACGGTATTAGCACAAGTTTCCCTGCGTT ATTCCGTAGTAAAGGGTAGGTTCCCACGCGTTACTCACCCGTCTGCCGCTCCCCTTGCGG GGCGCTCGACTGCA
9	QN5-R6	GCTGCCTCCTTGCGGTTAGCGTCACTACCTTCGGGTAAAACCAACTCCCATGGTGTGACG GGCGGTGTGTACAAGGCCCGGGAACGTATTCACCGCGGCATGCTGATCCGCGATTACTA GGGATTCCAACTTCATGCACTCGAGTTGCAGAGTGCAATCCGAACTGAGATGGCTTTTG GAGATTAGCTCACACTCGCGTGCTCGCTGCCCACTGTCACCACCATTGTAGCACGTGTGT AGCCCAGCCCGTAAGGGCCATGAGGACTTGACGTCATCCCCACCTTCCTCTCGGCTTATC ACCGGCAGTCCCCTTAGAGTGCCCAACTTAATGCTGGCAACTAAGGGCGAGGGTTGCGC TCGTTGCGGGACTTAACCCAACATCTCACGACACGAGCTGACGACAGCCATGCAGCACCT GTCTCTGCGCCACCGAAGTGGACCCCATATCTCTACGGGTAACACAGGATGTCAAGGGCT GGTAAGGTTCTGCGCGTTGCTTCGAATTAAACCACATGCTCCACCGCTTGTGCGGGCCCC CGTCAATTCCTTTGAGTTTTAATCTTGCGACCGTACTCCCCAGGCGGAATGTTTAATGCG TTAGCTGCGCCACCGAACAGTATACTGCCCGACGGCTAACATTCATCGTTTACGGCGTGG ACTACCAGGGTATCTAATCCTGTTTGCTCCCCACGCTTTCGCACCTCAGCGTCAGTAATG GACCAGTGAGCCGCCTTCGCCACTGGTGTTCCTCCGAATATCTACGAATTTCACCTCTAC ACTCGGAATTCCACTCACCTCTTCCATACTCCAGATCGACAGTATCAAAGGCAGTTCCAG GGTTGAGCCCTGGGATTTCACCCCTGACTGATCGATCCGCCTACGTGCGCTTTACGCCCA GTAATTCCGAACAACGCTAGCCCCCTTCGTATTACCGCGGCTGCTGGCACGAAGTTAGCC GGGGCTTCTTCTCCGGATACCGTCATTATCTTCTCCGGTGAAAGAGCTTTACAACCCTAG GGCCTTCATCACTCACGCGGCATGGCTGGATCAGGCTTGCGCCCATTGTCCAATATTCCC CACTGCTGCCTCCCGTAGGAGTTTGGGCCGTGTCTCAGTCCCAATGTGGCTGATCATCCT CTCAGACCAGCTATGGATCGTCGCCTTGGTAGGCCTTTACCCCACCAACTAGCTAATCCA ACGCGGGCTCATCTCTTGCCGATAAATCTTTCTCCCGAAGGACACATACGGTATTAGCAC AAGTTTCCCTGCGTTATTCCGTAGCAAAAGGTAGATTCCCACGCGTTACTCACCCGTCTG CCGCTCCCTCGGTGCAGCGGGCGCTCGACTGCA
10	QN5-R9	CAGGTAAAACCAACTCCCATGGTGTGACGGGCGGTGTGTACAAGGCCCGGGAACGTATT CACCGTGGCGTGCTGATCCACGATTACTAGCGATTCCAACTTCATGGGCTCGAGTTGCAG AGCCCAATCCGAACTGAGACGGCTTTTTGAGATTTGCGAAGGGTCGCCCCTTAGCATCCC ATTGTCACCGCCATTGTAGCACGTGTGTAGCCCAGCCCGTAAGGGCCATGAGGACTTGA CGTCATCCCCACCTTCCTCGCGGCTTATCACCGGCAGTCTCCTTAGAGTGCTCAACTAAA TGGTAGCAACTAAGGACGGGGGTTGCGCTCGTTGCGGGACTTAACCCAACATCTCACGA CACGAGCTGACGACAGCCATGCAGCACCTGTGTTCCAGGCTCCGAAGAGAGGGTCACAT CTCTGCGACCGGTCCTGGACATGTCAAGGGCTGGTAAGGTTCTGCGCGTTGCGTCGAAT TAAACCACATGCTCCACCGCTTGTGCGGGCCCCCGTCAATTCCTTTGAGTTTTAATCTTG CGACCGTACTCCCCAGGCGGAATGCTTAAAGCGTTAGCTGCGCCACTAGTGAGTAAACCC ACTAACGGCTGGCATTCATCGTTTACGGCGTGGACTACCAGGGTATCTAATCCTGTTTG CTCCCCACGCTTTCGTGCCTCAGCGTCAGTATCGGGCCAGTGAGCCGCCTTCGCCACTGG TGTTCTTGCGAATATCTACGAATTTCACCTCTACACTCGCAGTTCCACTCACCTCTCCCG AACTCAAGATCTTCAGTATCAAAGGCAGTTCTGGAGTTGAGCTCCAGGATTTCACCCCT GACTTAAAGACCCGCCTACGCACCCTTTACGCCCAGTGATTCCGAGCAACGCTAGCCCCC TTCGTATTACCGCGGCTGCTGGCACGAAGTTAGCCGGGGCTTATTCTTGCGGTACCGTCA TTATCTTCCCGCACAAAAGAGCTTTACAACCCTAGGGCCTTCATCACTCACGCGGCATGG CTGGATCAGGGTTGCCCCCATTGTCCAATATTCCCCACTGCTGCCTCCCGTAGGAGTTTG GGCCGTGTCTCAGTCCCAATGTGGCTGATCATCCTCTCAGACCAGCTACTGATCGTCGCC TTGGTAGGCCATTACCCTACCAACTAGCTAATCAGACGCGGGCCGATCTTTCGGCGATA AATCTTTCCCCGTAAGGGCTTATCCGGTATTAGCACAAGTTTCCCTGTGTTGTTCCGAAC CAAAAGGTACG
11	QN5-R11	CTGCCTCCCTTGCGGGTTAGCGCACCGTCTTCAGGTAAAACCAACTCCCATGGTGTGACG

No.	Strain	Sequencing data
		GGCGGTGTGTACAAGGCCCGGGAACGTATTCACCGTGGCGTGCTGATCCACGATTACTA GCGATTCCAACTTCATGGGCTCGGGTTGCAGAGCCCAATCCGAACTGAGACGGCTTTTT GAGATTTGCGAAGGGTCGCCCCTTAGCATCCCATTGTCACCGCCATTGTAGCACGTGTGT AGCCCAGCCCGTAAGGGCCATGAGGACTTGACGTCATCCCCACCTTCCTCGCGGCTTATC ACCGGCAGTCTCCTTAGAGTGCTCAACTAAATGGTAGCAACTAAGGACGGGGGTTGCGC TCGTTGCGGGACTTAACCCAACATCTCACGACACGAGCTGACGACAGCCATGCAGCACCT GTGTTCCAGGCTCCGAAGAGAGGGTCACATCTCTGCGACCGGTCCTGGACATGTCAAGG GCTGGTAAGGTTCTGCGCGTTGCGTCGAATTAAACCACATGCTCCACCGCTTGTGCGGGC CCCCGTCAATTCCTTTGAGTTTTAATCTTGCGACCGTACTCCCCAGGCGGAATGCTTAAA GCGTTAGCTGCGCCACTAGTGAGTAAACCCACTAACGGCTGGCATTCATCGTTTACGGCG TGGACTACCAGGGTATCTAATCCTGTTTGCTCCCCACGCTTTCGTGCCTCAGCGTCAGTA TCGGGCCAGTGAGCCGCCTTCGCCACTGGTGTTCTTGCGAATATCTACGAATTTCACCTC TACACTCGCAGTTCCACTCACCTCTCCCGAACTCAAGATCTTCAGTATCAAAGGCAGTTC TGGAGTTGAGCTCCAGGATTTCACCCCTGACTTAAAGACCCGCCTACGCACCCTTTACGC CCAGTGATTCCGAGCAACGCTAGCCCCCTTCGTATTACCGCGGCTGCTGGCACGAAGTTA GCCGGGGCTTATTCTTGCGGTACCGTCATTATCTTCCCGCACAAAAGAGCTTTACAACCC TAGGGCCTTCATCACTCACGCGGCATGGCTGGATCAGGGTTGCCCCCATTGTCCAATATT CCCCACTGCTGCCTCCCGTAGGAGTTTGGGCCGTGTCTCAGTCCCAATGTGGCTGATCAT CCTCTCAGACCAGCTACTGATCGTCGCCTTGGTAGGCCATTACCCTACCAACTAGCTAAT CAGACGCGGGCCGATCTTTCGGCGATAAATCTTTCCCCGTAAGGGCTTATCCGGTATTAG CACAAGTTTCCCTGTGTTGTTCCGAACCAAAAGGTACGTTCCCACGCGTTACTCACCCGT CTGCCGCTGACGTATTGCTACGCCCGCTCGACTGCA
12	QN6-R2	AGCTGCCTCCTTGCGGTTAGCGCACTACCTTCGGGTAAAACCAACTCCCATGGTGTGACG GGCGGTGTGTACAAGGCCCGGGAACGTATTCACCGCGGCATGCTGATCCGCGATTACTA GCGATTCCAACTTCATGCACTCGAGTTGCAGAGTGCAATCCGAACTGAGATGGCTTTTG GAGATTAGCTCACACTCGCGTGCTCGCTGCCCACTGTCACCACCATTGTAGCACGTGTGT AGCCCAGCCCGTAAGGGCCATGAGGACTTGACGTCATCCCCACCTTCCTCTCGGCTTATC ACCGGCAGTCCCCTTAGAGTGCCCAACTTAATGCTGGCAACTAAGGGCGAGGGTTGCGC TCGTTGCGGGACTTAACCCAACATCTCACGACACGAGCTGACGACAGCCATGCAGCACCT GTCTCTGCGCCACCGAAGTGGACCCCCTATCTCTAGAGGTAACACAGGATGTCAAGGGCT GGTAAGGTTCTGCGCGTTGCTTCGAATTAAACCACATGCTCCACCGCTTGTGCGGGCCCC CGTCAATTCCTTTGAGTTTTAATCTTGCGACCGTACTCCCCAGGCGGAATGTTTAATGCG TTAGCTGCGCCACCGAACAGTATACTGCCCGACGGCTAACATTCATCGTTTACGGCGTGG ACTACCAGGGTATCTAATCCTGTTTGCTCCCCACGCTTTCGCACCTCAGCGTCAGTAATG GACCAGTGAGCCGCCTTCGCCACTGGTGTTCCTCCGAATATCTACGAATTTCACCTCTAC ACTCGGAATTCCACTCACCTCTTCCATACTCCAGATCGACAGTATCAAAGGCAGTTCCAG GGTTGAGCCCTGGGATTTCACCCCTGACTGATCGATCCGCCTACGTGCGCTTTACGCCCA GTAATTCCGAACAACGCTAGCCCCCTTCGTATTACCGCGGCTGCTGGCACGAAGTTAGCC GGGGCTTCTTCTCCGGATACCGTCATTATCTTCTCCGGTGAAAGAGCTTTACAACCCTAG GGCCTTCATCACTCACGCGGCATGGCTGGATCAGGCTTGCGCCCATTGTCCAATATTCCC CACTGCTGCCTCCCGTAGGAGTTTGGGCCGTGTCTCAGTCCCAATGTGGCTGATCATCCT CTCAGACCAGCTATGGATCGTCGCCTTGGTAGGCCTTTACCCCACCAACTAGCTAATCCA ACGCGGGCTCATCTCTTGCCGATAAATCTTTCTCCCGAAGGACACATACGGTATTAGCAC AAGTTTCCCTGCGTTATTCCGTAGCAAAAGGTAGATTCCCACGCGTTACTCACCCGTCTG CCGCTCCCTCTGCGGGGCGCTCGACTGCA
13	QN6-R9	AGCGCACTACCTTCGGGTAAAACCAACTCCCATGGTGTGACGGGCGGTGTGTACAAGGC CCGGGAACGTATTCACCGCGGCATGCTGATCCGCGATTACTAGCGATTCCAACTTCATGC ACTCGAGTTGCAGAGTGCAATCCGAACTGAGATGGCTTTTGGAGATTAGCTCACACTCG CGTGCTCGCTGCCCACTGTCACCACCATTGTAGCACGTGTGTAGCCCAGCCCGTAAGGGC CATGAGGACTTGACGTCATCCCCACCTTCCTCTCGGCTTATCACCGGCAGTCCCCTTAGA GTGCCCAACTTAATGCTGGCAACTAAGGGCGAGGGTTGCGCTCGTTGCGGGACTTAACC CAACATCTCACGACACGAGCTGACGACAGCCATGCAGCACCTGTCTCTGAGCCACCGAAG TGGAAAGTGCATCTCTGCACCGGTCCCAGGATGTCAAGGGCTGGTAAGGTTCTGCGCGT TGCTTCGAATTAAACCACATGCTCCACCGCTTGTGCGGGCCCCCGTCAATTCCTTTGAGT TTTAATCTTGCGACCGTACTCCCCAGGCGGAATGTTTAATGCGTTAGCTGCGCCACCGAA CAGTATACTGCCCGACGGCTAACATTCATCGTTTACGGCGTGGACTACCAGGGTATCTA ATCCTGTTTGCTCCCCACGCTTTCGCACCTCAGCGTCAGTAATGGACCAGTGAGCCGCCT

No.	Strain	Sequencing data
		TCGCCACTGGTGTTCCTCCGAATATCTACGAATTTCACCTCTACACTCGGAATTCCACTC ACCTCTTCCATACTCCAGATCGACAGTATCAAAGGCAGTTCCAGGGTTGAGCCCTGGGA TTTCACCCCTGACTGATCGATCCGCCTACGTGCGCTTTACGCCCAGTAATTCCGAACAAC GCTAGCCCCCTTCGTATTACCGCGGCTGCTGGCACGAAGTTAGCCGGGGCTTCTTCTCCG GATACCGTCATTATCTTCTCCGGTGAAAGAGCTTTACAACCCTAGGGCCTTCATCACTCA CGCGGCATGGCTGGATCAGGCTTGCGCCCATTGTCCAATATTCCCCACTGCTGCCTCCCG TAGGAGTTTGGGCCGTGTCTCAGTCCCAATGTGGCTGATCATCCTCTCAGACCAGCTATG GATCGTCGCCTTGGTAGGCCTTTACCCCACCAACTAGCTAATCCAACGCGGGCTCATCTC TTGCCAATAAATCTTTCTCCCGAAGGACACATACGGTATTATAACAAGTTTCCCTCCGT TATTCCGTAGCAAAAGGTAGATTCCCACGCGTTACTCACCCGTCTGCCGCTCCCCTTGCG GGGCGATCGACTGCA
14	LA3-R1	CTGCCTCCCTTGCGGGTTAGCGCACCGTCTTCAGGTAAAACCAACTCCCATGGTGTGACG GGCGGTGTGTACAAGGCCCGGGAACGTATTCACCGTGGCGTGCTGATCCACGATTACTA GCGATTCCAACTTCATGGGCTCGAGTTGCAGAGCCCAATCCGAACTGAGACGGCTTTTT GAGATTTGCGAAGGGTCGCCCCTTAGCATCCCATTGTCACCGCCATTGTAGCACGTGTGT AGCCCAGCCCGTAAGGGCCCTGAGGACTTGACGTCATCCCCACCTTCCTCGCGGCTTATC ACCGGCAGTCTCCTTAGAGTGCTCAACTAAATGGTAGCAACTAAGGACGGGGGTTGCGC TCGTTGCGGGACTTAACCCAACATCTCACGACACGAGCTGACGACAGCCATGCAGCACCT GTGCTCCAGGCTCCGAAGAGAGGGTCACATCTCTGCGACCGGTCCTGGACATGTCAAGG GCTGGTAAGGTTCTGCGCGTTGCGTCGAATTAAACCACATGCTCCACCGCTTGTGCGGGC CCCCGTCAATTCCTTTGAGTTTTAATCTTGCGACCGTACTCCCCAGGCGGAATGCTTAAA GCGTTAGCTGCGCCACTAGTGAGTAAACCCACTAACGGCTGGCATTCATCGTTTACGGCG TGGACTACCAGGGTATCTAATCCTGTTTGCTCCCCACGCTTTCGTGCCTCAGCGTCAGTA CCGGGCCAGTGAGCCGCCTTCGCCACTGGTGTTCTTGCGAATATCTACGAATTTCACCTC TACACTCGCAGTTCCACTCACCTCTCCCGGACTCAAGATCTTCAGTATCAAAGGCAGTTC TGGAGTTGAGCTCCAGGATTTCACCCCTGACTTAAAGACCCGCCTACGCACCCTTTACGC CCAGTGATTCCGAGCAACGCTAGCCCCCTTCGTATTACCGCGGCTGCTGGCACGAAGTTA GCCGGGGCTTATTCTTGCGGTACCGTCATTATCTTCCCGCACAAAAGAGCTTTACAACCC TAGGGCCTTCATCACTCACGCGGCATGGCTGGATCAGGGTTGCCCCCATTGTCCAATATT CCCCACTGCTGCCTCCCGTAGGAGTTTGGGCCGTGTCTCAGTCCCAATGTGGCTGATCAT CCTCTCAGACCAGCTACTGATCGTCGCCTTGGTGAGCCATTACCTCACCAACTAGCTAAT CAGACGCGGGCCGATCTTTCGGCGATAAATCTTTCCCCGTAAGGGCTTATCCGGTATTAG CACAAGTTTCCCTGTGTTGTTCCGAACCAAAAGGTACGTTCCCACGCGTTACTCACCCGT CTGCCGCTGACGTATTGCTACGCCCGCTCGACTGCA
15	LA3-R2	CTGCCTCCTTGCGGGTTAGCAGCACCGTCTTCAGGTAAAACCAACTCCCATGGTGTGACG GGCGGTGTGTACAAGGCCCGGGAACGTATTCACCGTGGCGTGCTGATCCACGATTACTA GCGATTCCAACTTCATGGGCTCGAGTTGCAGAGCCCAATCCGAACTGAGACGGCTTTTT GAGATTTGCGAAGGGTCGCCCCTTAGCATCCCATTGTCACCGCCATTGTAGCACGTGTGT AGCCCAGCCCGTAAGGGCCATGAGGACTTGACGTCATCCCCACCTTCCTCGCGGCTTATC ACCGGCAGTCTCCTTAGAGTGCTCAACTAAATGGTAGCAACTAAGGACGGGGGTTGCGC TCGTTGCGGGACTTAACCCAACATCTCACGACACGAGCTGACGACAGCCATGCAGCACCT GTGCTCCAGGCTCCGAAGAGAGGGTCACATCTCTGCGACCGGTCCTGGACATGTCAAGG GCTGGTAAGGTTCTGCGCGTTGCGTCGAATTAAACCACATGCTCCACCGCTTGTGCGGGC CCCCGTCAATTCCTTTGAGTTTTAATCTTGCGACCGTACTCCCCAGGCGGAATGCTTAAA GCGTTAGCTGCGCCACTAGTGAGTAAACCCACTAACGGCTGGCATTCATCGTTTACGGCG TGGACTACCAGGGTATCTAATCCTGTTTGCTCCCCACGCTTTCGTGCCTCAGCGTCAGTA CCGGGCCAGTGAGCCGCCTTCGCCACTGGTGTTCTTGCGAATATCTACGAATTTCACCTC TACACTCGCAGTTCCACTCACCTCTCCCGGACTCAAGATCTTCAGTATCAAAGGCAGTTC TGGAGTTGAGCTCCAGGATTTCACCCCTGACTTAAAGACCCGCCTACGCACCCTTTACGC CCAGTGATTCCGAGCAACGCTAGCCCCCTTCGTATTACCGCGGCTGCTGGCACGAAGTTA GCCGGGGCTTATTCTTGCGGTACCGTCATTATCTTCCCGCACAAAAGAGCTTTACAACCC TAGGGCCTTCATCACTCACGCGGCATGGCTGGATCAGGGTTGCCCCCATTGTCCAATATT CCCCACTGCTGCCTCCCGTAGGAGTTTGGGCCGTGTCTCAGTCCCAATGTGGCTGATCAT CCTCTCAGACCAGCTACTGATCGTCGCCTTGGTGAGCCATTACCTCACCAACTAGCTAAT CAGACGCGGGCCGATCTTTCGGCGATAAATCTTTCCCCGTAAGGGCTTATCCGGTATTAG CACAAGTTTCCCTGTGTTGTTCCGAACCAAAAGGTACGTTCCCACGCGTTACTCACCCGT CTGCCGCTGACGTATTGCTACGCCCGCTCGACTGCA

No.	Strain	Sequencing data
16	LA3-R3	CTGCCTCCCTTGCGGGTTAGCGCACCGTCTTCAGGTAAAACCAACTCCCATGGTGTGACG GGCGGTGTGTACAAGGCCCGGGAACGTATTCACCGTGGCGTGCTGATCCACGATTACTA GCGATTCCAACTTCATGGGCTCGAGTTGCAGAGCCCAATCCGAACTGAGACGGCTTTTT GAGATTTGCGAAGGGTCGCCCCTTAGCATCCCATTGTCACCGCCATTGTAGCACGTGTGT AGCCCAGCCCGTAAGGGCCATGAGGACTTGACGTCATCCCCACCTTCCTCGCGGCTTATC ACCGGCAGTCTCCTTAGAGTGCTCAACTAAATGGTAGCAACTAAGGACGGGGGTTGCGC TCGTTGCGGGACTTAACCCAACATCTCACGACACGAGCTGACGACAGCCATGCAGCACCT GTGTTCCAGGCTCCGAAGAGAGGGTCACATCTCTGCGACCGGTCCTGGACATGTCAAGG GCTGGTAAGGTTCTGCGCGTTGCGTCGAATTAAACCACATGCTCCACCGCTTGTGCGGGC CCCCGTCAATTCCTTTGAGTTTTAATCTTGCGACCGTACTCCCCAGGCGGAATGCTTAAA GCGTTAGCTGCGCCACTAGTGAGTAAACCCACTAACGGCTGGCATTCATCGTTTACGGCG TGGACTACCAGGGTATCTAATCCTGTTTGCTCCCCACGCTTTCGTGCCTCAGCGTCAGTA TCGGGCCAGTGAGCCGCCTTCGCCACTGGTGTTCTTGCGAATATCTACGAATTTCACCTC TACACTCGCAGTTCCACTCACCTCTCCCGAACTCAAGATCTTCAGTATCAAAGGCAGTTC TGGAGTTGAGCTCCAGGATTTCACCCCTGACTTAAAGACCCGCCTACGCACCCTTTACGC CCAGTGATTCCGAGCAACGCTAGCCCCCTTCGTATTACCGCGGCTGCTGGCACGAAGTTA GCCGGGGCTTATTCTTGCGGTACCGTCATTATCTTCCCGCACAAAAGAGCTTTACAACCC TAGGGCCTTCATCACTCACGCGGCATGGCTGGATCAGGGTTGCCCCCATTGTCCAATATT CCCCACTGCTGCCTCCCGTAGGAGTTTGGGCCGTGTCTTAGTCCCAATGTGGCTGATCAT CCTCTCAGACCAGCTACTGATCGTCGCCTTGGTGAGCCATTACCTCACCAACTAGCTAAT CAGACGCGGGCCGATCTTTCGGCGATAAATCTTTCCCCGTAAGGGCTTATCCGGTATTAG CACAAGTTTCCCTGTGTTGTTCCGAACCAAAAGGTACGTT
17	LA3-R4	TGCCTCCCTTGCGGCGTTAGCGCACCGTCTTCAGGTAAAACCAACTCCCATGGTGTGACG GGCGGTGTGTACAAGGCCCGGGAACGTATTCACCGTGGCGTGCTGATCCACGATTACTA GCGATTCCAACTTCATGGGCTCGAGTTGCAGAGCCCAATCCGAACTGAGACGGCTTTTT GAGATTTGCGAAGGGTCGCCCCTTAGCATCCCATTGTCACCGCCATTGTAGCACGTGTGT AGCCCAGCCCGTAAGGGCCATGAGGACTTGACGTCATCCCCACCTTCCTCGCGGCTTATC ACCGGCAGTCTCCTTAGAGTGCTCAACTAAATGGTAGCAACTAAGGACGGGGGTTGCGC TCGTTGCGGGACTTAACCCAACATCTCACGACACGAGCTGACGACAGCCATGCAGCACCT GTGTTCCAGGCTCCGAAGAGAGGGTCACATCTCTGCGACCGGTCCTGGACATGTCAAGG GCTGGTAAGGTTCTGCGCGTTGCGTCGAATTAAACCACATGCTCCACCGCTTGTGCGGGC CCCCGTCAATTCCTTTGAGTTTTAATCTTGCGACCGTACTCCCCAGGCGGAATGCTTAAA GCGTTAGCTGCGCCACTAGTGAGTAAACCCACTAACGGCTGGCATTCATCGTTTACGGCG TGGACTACCAGGGTATCTAATCCTGTTTGCTCCCCACGCTTTCGTGCCTCAGCGTCAGTA TCGGGCCAGTGAGCCGCCTTCGCCACTGGTGTTCTTGCGAATATCTACGAATTTCACCTC TACACTCGCAGTTCCACTCACCTCTCCCGAACTCAAGATCTTCAGTATCAAAGGCAGTTC TGGAGTTGAGCTCCAGGATTTCACCCCTGACTTAAAGACCCGCCTACGCACCCTTTACGC CCAGTGATTCCGAGCAACGCTAGCCCCCTTCGTATTACCGCGGCTGCTGGCACGAAGTTA GCCGGGGCTTATTCTTGCGGTACCGTCATTATCTTCCCGCACAAAAGAGCTTTACAACCC TAGGGCCTTCATCACTCACGCGGCATGGCTGGATCAGGGTTGCCCCCATTGTCCAATATT CCCCACTGCTGCCTCCCGTAGGAGTTTGGGCCGTGTCTCAGTCCCAATGTGGCTGATCAT CCTCTCAGACCAGCTACTGATCGTCGCCTTGGTGAGCCATTACCTCACCAACTAGCTAAT CAGACGCGGGCCGATCTTTCGGCGATAAATCTTTCCCCGTAAGGGCTTATCCGGTATTAG CACAAGTTTCCCTGTGTTGTTCCGAACCAAAAGGTACGTTCCCACGCGTTACTCACCCGT CTGCCGCTGACGTATTGCTACGCCCGCTCGACTGCA