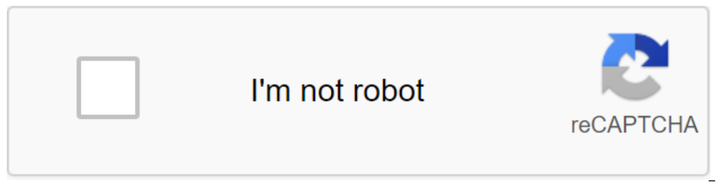
Isolation of root nodulating bacteria pdf



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Research article of Addis Ababa University, Ethiopia View: 07 March 2019; Published: June 11, 20199 Author: Asnake Beshah, Addis Ababa University, Addis Ababa, Ethiopia How to quote this article: Asnake Beshah, Fassil Assefa. Isolation, identification and feature An effective kind of risobium, butdulating Mung Bean (Vigna radiata) from some places of North Shiva. Int J Environ Sci Nat Res. 2019; 20(1): 5556026. DOI:10.19080/IJESNR.2019.20.556026 Nineteen root chickpea knots were collected from farm plots in the northern shoa area of Kevet and Ensaro worn. Three of the isolates were rejected in the process of isolation and alleged testing. The rest were re-inculcate in their host for authentication. Fifteen of the re-vaccinated isolates formed nodules in their host, but one isolate was unable to form a node after re-inoculation. Authenticated risobia was characterized by their morphological and eco-physiological features. From pre-screening 87% of isolates were slow-growing rhizobium. The symbiotic efficiency of isolates showed a significant difference in efficiency percentages (34-92%) At the owner factory. AAUMR2, AAUMR1, AAUMR3 and AAUMR7 were recognized as the most effective isolates with a percentage of efficiency, 86%, 84% and 81%. Most isolates were classified at an effective rate, which has a efficiency percentage of 50-80%. Isolates have also been tested for their tolerance of various eco-physiological features, such as pH, temperature, salt concentration, internal resistance to antibiotics and the use of various sources of nitrogen. These tests showed a wide physiological diversity between isolates. Plants, grafted with various isolates, displayed differences in the number of knots, knot dry weight and shot dry weight. Numerical analysis of isolates based on forty-six phenotypic features showed diversity among isolates, and statistical analysis indicates their diversity in the direction of symbiotic effectiveness. Some isolates, such as AAUMR1 (Kewet), AAUMR3 and AAUMR7 (Ensaro), which are highly effective and tolerant of a wide range of phenotypic features, are promising in the development of the inocultant. Keywords: Biological nitrogen fixation; Alleged tests; Inoculates; The phenotypic diversity of Mung bean (Vigna radiata) is the warm season of the annual legume legumes. The optimal temperature range for good production is 27-30 degrees Celsius (Imrie, 1998). Mung beans take 60-75 days to ripen. A useful crop in drier areas and has good potential for crop rotation and grain relay using residual moisture. Mung beans were also known as green gram or golden gram and were mainly cultivated on Indian Now the days; it is grown after harvesting rabbis (wheat, mustard, lentils, etc.). It can fit as a monetary culture between the main major Seasons. In a year it is grown three times and is 43,680 hectares with an average yield of 0.78t/ha. In addition to being one of the shortest long field crops in the world (can be harvested within two months), the rhizome bacteria around the Mung bean root area can symbioticly correct N2 gas from the air, making it one of the most popular components in pruning systems. However, seed yields in farm fields are still low, ranging from 0.3 to 2.1t/ha. Mung beans are a staple calorie (347-k cal. food energy) and protein (19.5% to 28.5%) sources in Asia, especially for the vegetarian population. High lysine content, which makes mung beans a good extra food for rice diets, is usually the first limiting amino acid (Chen et al.1987). It is a popular food among vegetarians because it contains a lot of protein and fiber and the main advantage of mung beans is that it helps in digestion and controls the amount of cholesterol in our body. Mung beans contain many minerals like calcium and potassium, which is essential for enhancing the strength of bones and teeth. The fat content in mung beans is very low, so it is highly recommended for people who want to minimize fat from their body. It is a warehouse of nutrients, and this food gives food, and they are rich in vitamin B, vitamin C, manganese and many other essential nutrients needed for effective human health functioning (AVRDC, 1988). In Ethiopia, these crops are also growing among small farmers in drier marginal conditions. Compared to other pulsed crops, its production is about 8 0000 per hectare (SARC, 2005). However, for the poor in the resources of farmers in drier marginal conditions, this has become an important legume. These farmers need diversity and fertilizers that produce the most and have a stable yield in their environment. Several papers have shown that much of the risobia of legumes is very effective in soil fertility and nitrogen fixation. However, working on mung beans is very limited about the isolation of riphobias that nod mung beans. Therefore, this study focuses on the isolation and characteristics of the root node bacteria of the Mung beans from one of the best pulses of the growing area of Amhara in the northern Shoa zones. The search for symbiotic effective isolates can contribute to the development of risobic inoculents to fully realize the potential of BPF in low-entry agriculture in the country. Selected sample sites of this study were covered in the main Mung bean growing areas in the northern Shoa areas of the Amhara region. In these areas Mung beans grow for a long time without any history of graft with risobia. The root nodules were collected in the period November 2013. A collection of Nodule node samples was randomly collected from the farmer's field and immediately stored in sealed vials containing desiccant (Silica gel), covered with 1 cm cotton wool to isolate the risobia. The insulation of root node bacteria, dehydrated or dried root nodules, was immersed in sterile distilled water during the night in labeled sets of cohesive water flasks. The soaked nodules were sterilized with 70% ethanol within 10 seconds and then up to 3% (v/v) sodium hypochlorite solution (NaHClO3) within 3 minutes according to Somasegaren and Hoben. Surface sterilized nodules are then rinsed in five changes of sterile distilled water to completely wash away and remove the sterilization chemicals. The superficially sterilized nodules were then transferred to sterile petri dishes and crushed with alcohol by an inflamed sterile glass rod in a drop of normal saline solution (0.85% NaCl) inside the laminar hood of the stream. The Red Congo Stock Decision (CR) was prepared to dissolve 0. 25g CR in 100ml sterile distilled water, and then 10 ml CR stock solution was added to one liter of YEMA before the autoclave. Finally, Loopful shredded nodules suspensions were striped on YEMA plates with CR and incubated at 28 ± 20C for 3-7 days. Single dome-shaped colonies were collected with sterile incubation loops and striped on sterile YEMA plates and incubated at 28'20C. The purity and uniformity of the types of colonies were carefully studied by re-strips and one isolated colony was selected and transferred to the YEMA tilt containing 0.3% (W/V) CaCO3 in the cultural tube and incubated at 28'20c. When there was enough growth, the culture was transferred to remain at 40c for future use. Each isolate has been investigated for pur supposed purity using the Peptone Glucose Test (PGT), coloring grams and growth reaction to the e-CR medium. Congo's red absorption Stock Solution Congo Red was prepared by dissolving 0.25 g congo red in 100 ml of sterile distilled water. From a spare solution, 10 ml was added to a liter of YEMA and autoclaved. The noose, full of test isolates, was pierced on the medium and covered with aluminum foil until dark incubation at 28-20C for 3-7 days to detect The absorption of Congo by red colonies. Peptone Glucose Test was prepared in accordance with lupwayi and Haque glucose procedure, dissolving 5 grams of glucose, 10 grams of peptone, 15 g of agar and 10 ml of crepo cresole (BCP) in a liter of distilled water and pH was adjusted to 7.0 with 1N NaoH and vinegar acid. Seven days old yeast extracts of mannitol broth culture containing an approximate number of cells (104 cells ml-1) was striped on Pepton Glucose Average to observe growth after incubation at 28 20C for 3 to 7 days. In order to check the final purity of all risobial isolates, a boiling test was conducted for each of the purified They were grafted into the receiving pot plant with up to 3kg capacity plastic pots containing sterilized and nitrogen nitrogen Sand. The sand was thoroughly rinsed with 1N sulphuric acid while the pots were surface sterilized with 95% ethanol. Six seeds were seeded in each pan and thinned to three after germination. Each isolate was grafted into the Erlenmeier flask and stay for 7 days and 1 ml of suspension culture was grafted into each seedling. Colony morphology morphological characteristics of isolates were identified according to Lupwayi and Haq (1994). A loop of old grown broth culture from each isolate was grafted onto the YEMA and incubated at 28±20C for 3-7 days. After 7 days, the diameter of the colony, morphology and texture of the colony were recorded. Acid-based production To determine the ability of risobial isolates to produce acid or alkaline in the environment, yeMA containing bromotamol blue (BTB) (0.025w/v) has been used. The loop, full of isolates from the five-day broth culture, was moved to the YEMA BTB environment and incubated for 3-7 days to record color changes Wednesday. For each biochemical and physiological test, the graft loop of the five-day broth culture was striped on the YEMA environment. The inculced YEMA plates were incubated at 28 ± 20C for 3-5 days. For each experiment, three replications and controls were used for each test, as indicated in Maatallah, etc. Amino acids using various types of amino acids including L-arginine, L-glutamate, L-leucine, L-phenylalanine, L-tryptophan, urea and L-Tyrosine were used in this experiment to determine the ability of isolates to use amino acids as a source of nitrogen. These amino acids were added at a concentration of 0.5 g/l to a basal media source that did not have ammonium sulfate and supplemented with 1 g/l of mannitol. The membrane filter of sterilized amino acids were added to autoclave and chilled (approximately 550C) basal media, as indicated in Amargar et al. Finally, five days of risobial suspensions were grafted into these basal media and incubated at 28±20 C within 3-5 days. The pH tolerance ability of each risobial isolate to grow on acidic and alkaline media has been determined by in zeroing each isolate on YEMA adjusted to pH 4.0, 4.5, 5.0, 6, 7, 8, 8.5, 9.0 using 1N NaOH and vinegar acid as described by Bernal and Graham. Salt tolerance The ability of isolates to grow at different levels of salt concentration was determined by inoculation of each isolate on YEMA products containing 1%, 4%, 5%, 6%, 7%, 8%, 8%, 9% and 10% NaCl, as indicated in Lupwayi and Haque. Temperature tolerance Growth of each isolate at different incubation temperatures was assessed by inoculation isolate on YEMA plates. The grafted plates were incubated at 40C, 100C, 150C, 200C, 200C, 300C, 350C, 400C, 450C and 480C, as indicated in Lupwayi and Haque. Internal antibiotic resistance to isolates in different concentrations was assessed by a strip of each isolate on YEMA containing freshly prepared filters of sterilized antibiotics using membrane filters measuring 0.22 m. Stock solution of each antibiotic was first prepared, as described in Lupwayi and Haque, and stored in the refrigerator until they were used in the test. Antibiotics were tetracycline, erythromycin, ampicillin, chloroffinic and penicillin. Each antibiotic has been tested in the following concentrations. Ampicilin at 10g/ml, Chloroamfinicol in 2. g/ml and 5g/ml, tetracycline 2.5 g/ml, erythromycin 2.5 g/ml, 5 g/ml and 10 g/ml) and penicillin 2.5 g/ml, 5 g/ml and 10 g/ml). Erythromycin dissolved in ethanol, and the other four dissolved in sterilized water. The stock solution of each antibiotic was prepared by dissolving 2 grams of each antibiotic into 100 ml of water. The required concentration was asepticly added to the media using one pipette for each antibiotic. The stock solution of each antibiotic was sterilized with a milli pore filter (0.22 m) and asepticly added to the autoclaved YEMA (preserved at 500C in a water bath) at a final concentration of 2.5, 5 and 10g/ml, which is 12.5, 25 and 50 liters of antibiotic solution per 100 ml of medium, respectively, and finally poured separately into the plates. After sixty days of planting after re-vaccination, the plants were uprooted to measure the number of nodules, knot dry weight and shoot dry weight. The efficiency of isolates in the accumulation of dry plants has been calculated, as described in Somasegaren and Hoben and Molungoy as follows: SE and plant vaccinations D.M. X 100, N-Fertilized Plant D.M Where, D.M. - Dry Substance, S.E. - S.E. - S.E. - Symbiotic Efficiency Rate of Nitrogen Fixation Efficiency Is Estimated as: Highly Effective zgt; 80%, Effective 50-80%, Low-effective 35-50% and Ineffective Symbiotic Strain Efficiency Has Been Measured in Terms of Node, Shoot Dry Weight and Dry Weight. Phenotypic variability was analyzed using computer cluster analysis using the unweighted pair group method with medium (UPGMA) statistical software PCORD ver. 5.0 hierarchical method of clustering. A single data discrepancy analysis was also carried out using the SPSS ver.17.0 statistical programme. The average division was calculated using Tukey values when the F test was significant at P-0.05 (12-15). A total of 19 nodules, 9 isolats from Ensaro and 10 isolates from Kewet werda were collected. Three of them were rejected in the alleged test, and one is discarded in authentication. All isolates grew up on the PGA and did not absorb the Congolese at YEMA-CR Media. Twelve of them changed the environment of YEMA-BTB YEMA-BTB blue, while three of them turned yellow. All but one isolate is formed by nodules and authenticated as root bacteria of the nodules after they have been reinstalled in the host plant (16-20). The morphological and growth characteristics of isolate isolate isolates were grown at the YEMA environment to determine the type of colony, colony diameter and texture of the colony. About the texture of the colony 46% showed large water colonies (LW) and 53% of isolates were described as a large texture of the mucous membrane (LM) on the YEMA mediums. The diameter of the colony of all isolates ranged from 2 mm to 4 mm, the largest diameter of the colony 4 was observed on the isolates AAUMR2 (Kewet) and AAUMR1 (Kewet), while the smallest diameter of 1 mm was recorded for AAUMR9 (Ensaro). Using carbon sources All isolates have been able to catabolize a wide variety of carbon sources. All isolates (100%) were found to catabolize dextrose, fructose, maltose and sucrose. However, lactose and pulp were used by 86%. Isolates AAUMR3 and, AAUMR9 ensaro, have not been able to grow on lactose for a while; everything else grew very well. Eleven isolates (73% of isolates) were found for catabolization and grew on a basal environment containing all 6 proven carbon sources while isolating AAUMR1, AAUMR2, AAUMR13 and AAUMR14 Kevet showed abundant growth on all proven carbon sources. On the other hand, isolating AAUMR3 Ensaro relatively used fewer carbon sources (66%) proven carbon sources (Figure 1). The use of amino acids Almost all isolates have been able to catabolize a large variety of nitrogen sources. All isolates (100%) used methanonine and arginine also, 80 and 86% of the rhizome isolates catabolized phenyl alanine and tryptophan, respectively. This is similar to the findings of Shraddha et al. (2013) about mungbean rhizobia, isolated from various parts of India. Seventy-three (73%) isolates, which include AAUMR (4, 2, 5, 6, 13, 12, 14 and 10) Kevet and isolates AAUMR (8, 11 and 15) of Ensaro used 100% proven amino acids while, isolates AAUMR3 Ensaro was found to be much more fastidious with the ability to catbolize 50% of proven amino acids (figure 2). pH tolerance All isolates are allowed pH levels 6-8 and 26% of isolates have been found to be tolerant of pH 4. The remaining 40% of isolates rose by pH 9. Kewet's AAUMR3 and AAUMR13 isolates showed growth at all proven pH levels of 4-9. This goes against the reports of Shraddha et al (2013), which showed that ming beans risobia rose on the YEMA environment with pH levels 5 - 8 It was also interesting to note that 13% of isolates from Kewet and Ensaro tolerate a wide range of pH 4-9 as opposed to the report Shraddha et al (2013), which reported that mungbean rhizobia showed a neutral-tolerant trend, as well as reported that from 90% to isolates from India grew by medium sour (p. 5)

and neutral pH (Figure 3). The salt tolerance of The Isolates reflected differences in growth on the YEMA environment, adjusted at different NaCl concentrations. All isolates were tolerant of salt concentrations of 1%, but showed a steady decline in growth when they were vaccinated in an environment containing 4 to 10% salt concentration. Consequently, 66% of isolates grew by 4% NaCl, while 20% of isolates were resistant to salt concentrations of 7%. AAUMR6 isolates from Kevet were found to be the most tolerant strains, which grew at a salt concentration of 9%. This is similar to the findings of Kucuk and Kivank that the tolerance to salt mungbean rhizobia from India. Isolates such as AAUMR6 (Kewet) were the most tolerant of all salt concentrations, with AAUMR2, AAUMR5 and AAUMR12 (Kewet) and AAUMR15 (Ensaro) being able to grow at a salt concentration of just 1% (Figure 4). Temperature tolerance All isolates were able to grow within the temperature range of 20 to 350c. Insulation AAUMR4, AAUMR2, AMRAU1, AAUMR10, and AAUMR10,14 (Kewet) and AAUMR3 AAUMR9, AAUMR8 and AAUMR15 (Ensaro) were grown at the lowest temperature of 40C. There has been a gradual decline in isolates from the temperature range from 400C to 450C. Only isolates AAUMR2, AAUMR10, and AAUMR14 (Kewet) and AAUMR9 and AAUMR15 (Ensaro) were grown at all experienced incubation temperatures (4-450C) (Figure 5). Internal Resistance to Antibiotics All Isolates (100%) were found to be resistant to all tested antibiotics at a concentration of 2.5 mg/ml (table 7). Isolating AAUMR9 (Ensaro) has been found to be the most resistant of isolates to various antibiotics followed by the isolate of AAUMR6 and AAUMR12 (Kewet). The most sensitive isolate was AAUMR6; does not grow on the YEMA environment, which contains antibiotics at 5 and 10 g/ml, and then isolates AAUMR4, AAUMR10 (Kewet) and AAUMR15 (Ensaro) (Figure 6). The result of this work indicates the wide variety in the risobiic isolates of Mungbean, collected from the various growing areas of the northern Shoah zone of the regional state of Amhara. A variety of isolates have been observed based on their morphological, physiological, host infection and symbiotic effectiveness. This study showed that risobial isolates showed diversity in regards to symbiotic infection and efficacy with their host. Similarly, numerical analysis also confirmed that these isolates were photypically diverse, indicating that their real diversity must also be confirmed by genetic analysis using molecular methods. Tolerance of isolates to different levels of pH, temperature, salinity and antibiotics is an important quality of risobium strains for testing and development of inoculates that are endowed with environmental competitiveness. If there are various environmental stresses isolates will survive, will occupy and fix nitrogen and provide the host to increase crop production. Accordingly, it has been found that the following isolates are effective in nitrogen fixation and are resistant to various environmental stresses. In most trials, the most effective of all isolates were AAUMR2, AAUM3, AAUM1 and AAUMR7, followed by AAUMR4, AAUMR5, AAUMR6, AAUMR13, AAUCR12, AAUMR10, AAUMR8 and AAUMR14. Herridge D, Rose I (2000) Breeding to enhance nitrogen fixation in legumes. Field Cultures res 65 (2-3): 229-248. Tesfaye G (2008) Symbiotic and phenotypic diversity of risobial isolate, not knowing Visia faba from Western Shoah and Hararga, Ethiopia. M.SC the dissertation, Addis Ababa University, Ethiopia. Belay (2006) Symbiotic and Phenotypic Diversity Rizobim leguminosarum var viceae Isolates (Vicia faba) from North Gondar, Ethiopia. M.sc thesis. University of Addis Ababa, Ethiopia. 1-73. 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