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DIVERSITY OF RHIZOBIA NODULATING *Lablab purpureus* AND THE EFFECT OF THE ACIDITY, ALUMINUM AND FERRIC DEFICIT ON THEIR SYMBIOSIS.

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ABSTRACT:

A collection of 20 isolates from fresh Nodules of the legume plant *Lablab purpureus* was isolated. These isolates have been authenticated by seedling inoculation grown in jars containing sand. The results obtained after two months of culture have revealed that the 20 isolates (100% of the isolates) are able to nodulate their host plants. The results obtained were analyzed statistically by ANOVA using the software statistica and had shown that the effect of the inoculation has significantly improved all the growth parameters (the height of the plant and the dry weight of the aerial parts and roots, and the number of nodules). We have evaluated the tolerance of all strains of the collection to the major stress factors as the salinity, pH and extreme temperature. The osmotolerance reached a concentration up to 1710mm of NaCl. The strains were also able to grow on a wide range of pH, ranging from 4.5 to 9.5, and temperature, between 4°C and 40°c. Also, we tested the effect of the acidity, aluminum and ferric deficit on the *Lablab-rhizobia* symbiosis. *Lablab purpureus* has not been affected by the presence of high concentrations of aluminum. On the other hand, iron deficiency has caused a net decrease in the dry biomass of the aerial part. The results of all the phenotypic characters have been treated by the statistical Minitab software, the numerical analysis had shown that these bacterial strains are divided into two distinct groups at a level of similarity of 86 %. The SDS-PAGE was carried out to determine the profile of the total protein of the strains. The coefficients of similarity of polypeptide bands between the isolates and strains reference (*Bradyrhizobium, Mesorizobium sp.*) confirm that our strain belongs to the groups of rhizobia.

Key words: SDS-PAGE, *Rhizobia*, symbiosis, phenotypic characterization, *Lablab purpureus.*

[I] INTRODUCTION

Lablab purpureus (Linn.) *Sweet* (synonym Dolichos lablab L. ex Sweet), is an herbaceous legume, perennial, climbing or bushy plant but often grown as an annual plant. It is originated from India [38] and is widely distributed in the world [24]. It grows in various types of soils, ranging from deep sands to heavy clay soils, and from acid to alkaline soils (pH 4.4 - 7.8). This legume is used for human food as well as animal feed, as an annual or perennial short cycle forage crop [37]. It is

also much cultivated to improve the fertility of the soil [16], although very little taxonomic work has been done on the symbiotic bacteria associated with this plant. In China, the genetic diversity of rhizobia associated with *Lablab purpureus* has been revealed in different geographical regions [16], the bacterial lineages associated to this legume were relatives of *Mesorhizobium*, *rhizobium* and *unravel* after analysis of the RFLP profile of the ARNr 16S. In addition to the molecular techniques, the characterization of rhizobia can be carried out by several taxonomic tools as well as the protein profile [40]. In general, salinity and temperature are major constraints limiting considerably crop productivity on an area of approximately 40 per cent of the earth's surface, especially in Mediterranean regions; it is for this reason that the research of legumes and symbiotic bacteria tolerant to these factors is an invaluable asset [46]. In addition, several factors such as mineral nutrients and the physico-chemical composition of the soil affect the symbiosis between the BNL (bacteria Nodulating legumes) and their host plants. The acidity represents a limiting factor the symbiotic fixation of nitrogen by limitation of the survival of the rhizobia and its persistence in the soil, and thus, the reduction of the nodulation [5]. It is well known that the iron and aluminum are two essential elements for the growth and development of plants [13], their deficiencies can cause disturbances on the growth of the plant , nevertheless their excesses also can be responsible of the phenomenon of toxicity [33].

In this context, a collection of strains associated to *Lablab purpureus* has been isolated from nodules sampled *in natura* in three different regions of Algeria in the purpose of phenotypic characterization, to study their tolerance to various abiotic constraints as well as to reveal their diversity through the protein profile. We also tested the effect of the acidity and aluminum as well as the deficit ferric iron on the symbiosis between the two partners.

[II] MATERIALS AND METHODS

2.1. Sampling

A collection of root nodules from of different plants of *Lablab purpureus* is carried out at three sampling sites: Mostaganem, Bouira and Bejaia **(Figure-1)**, these areas are located in the bioclimatic floor with semi-arid or rainfall varying between 200-400 mm, the temperature is surrounded of 5°C in Winter and varies from 32°C to 35°C in summer [3]. The nodules harvested are preserved by drying in the CaCl2. The soil from each region has undergone physical-chemical analysis: (granulometry, total carbon, organic matter, pH, and electrical conductivity, determination of available phosphorus and determination of total nitrogen).

2.2. Condition of culture

2.2.1 Isolation of bacteria from nodules

The nodules are disinfected by immersion in ethanol at 95% for 5 to 10 seconds and then in the sodium hypochlorite at 12° for 3 minutes and rinsed 10 times extensively in sterile distilled water. They are then cut in Petri dishes containing Yeast Extract Agar medium: YEMA. After 4 to 5 days of incubation at 28°C, the colonies obtained are purified by successive sub culturing, examined by photon microscop to check their purity, and then isolates were stored at -80°C with glycerol (1:1 v/v) in eppendorf.

2.2.2. Phenotypic characterization of the strains

The strains were cultivated on YEMA medium supplemented by Congo red to be able to determine their time of growth, their colors and appearance of colonies.

The acidifying nature or alkali capacity of strains was determined by the addition of the bromothymol blue (BTB) in the YEMA medium [19]. Also, the production of 3-ceto-lactose [8] has been studied, the strains were placed in a culture at 28°C for three days on a first medium containing 10g of yeast extract, 20 g of glucose, 20 g of calcium carbonate, 18g of agar, 1000 ml of distilled water; then are transferred to a second solid medium containing 10g lactose, 1g yeast extract, 18 g agar, 1000 ml distilled water. The plates are incubated 48 hours at 28°. The revelation of 3-keto-lactose production was highlighted by flooding of the Petri Dishes with Benedict's reagent in room temperature.

2.3. Symbiotic characterization (test of nodulation)

The seeds of *Lablab purpureus* are disinfected with absolute ethanol during 5 to 10 seconds and then in the sodium hypochlorite at 12° for 3 minutes, rinsed thoroughly with sterile distilled water and putting to germinate on Petrie Dishes containing water agar (0.8 %) in the dark at 28°C.

After 4 to 5 days, the root lets obtained are transferred into disinfected plastic pots containing 150g of sterile sand.

The seedlings were inoculated with 1ml of bacterial culture containing (10^8 bacteria/ ml). All plants were watered 3 times per week in alternation with a free nitrogen nutrient solution [41]. The test of nodulation has been conducted in triplicate forms. The treated plants are stored in a house of culture with 16/8h of light/darkness and 28 / 20°C day/night.

2.4. Statistical Analyzes

The infectiveness and effectiveness of the different strains were determined after two months of growth. The number and the dry weight of the nodules as well as the dry weight of the aerial parts are analyzed statistically by (ANOVA) with the software Statistica.

2.5. Effect of the acidity (pH 4.5), aluminum (AlCl3) and the ferric iron deficit on the symbiosis of *Lablab purpureus*-rhizobias

The inoculated seedlings by all the strains isolated have undergone three treatments:

The first treatment has been divided into two lots; the first was doused with a nutrient solution for plant at pH 6.8, which has served as a control and the second to pH 4.5. The second batch is sprayed with a nutrient solution at neutral pH in the presence of $AlCl_3$ at concentrations of 0.30 µm and 100 µm, and for the third batch, the plants were watered with a free iron citrate nutrient solution. The inoculated plants with 200 µl of a bacterial culture without stress were considered as control. After two months of growth in the glasshouse, the plants were harvested in order to measure their aerial parts biomasses and orthophosphate. The results were analyzed statistically by (ANOVA) using the software Statistica.

2.6. Test the bacterial tolerance to pH, salinity, temperature and antibiotics

For each strain, two series of test tubes containing 8 ml of culture medium Yeast Mannitol Broth (YMB) were prepared, the first for studying the tolerance of strains to the pH and the second for the tolerance of strains to salinity. The pH of the medium of the first series has been adjusted by the addition of HCL or NAOH in order to obtain a range of pH from 2 to 11.

The second series contains increasing concentrations of NaCl (1 %, 3 %, 4 %, 6 %,8 %, 10 %,13 %,15%). Each tube is inoculated with 0.1 ml of a fresh pre-culture of 10^8 bacteria/ml, and then incubated at 28°C in a water bath with agitation. Each treatment is repeated 3 times. After a week of incubation, the bacterial population is estimated by the measurement of the optical density at a wavelength of 620 nm, by comparing with a negative witness at pH optimum.

The tolerance of these strains to different temperatures has been performed on solid medium YEMA, incubated at 4, 10, 15, 20, 30, 35, 38 and 42°C.

Four antibiotics (Ampicillin, Nalidixicacid, Kanamycin and Streptomycin) were applied at different concentrations. To perform this test, the method of dilution in agar medium recommended by Vincent [1970] was applied by using stocks of solutions of antibiotics added to the middle YEMA. The isolates were grown on middle YEMA to different concentrations of antibiotics, the plates are incubated at 28Â°C.

2.7. Digital analysis of the phenotypic characterization

The phenotypic characterization of 20 bacterial strains was processed by the statistical software using Minitab based on **55** phenotypic characters.

2.8. **Determination of the protein profile by SDS-PAGE**

The characterization of strains by this technique based on the degree of similarity between the polypeptides bands of the isolates and two reference strains *(Bradyrhizobium, Mesorizobium* sp. which belong to the collection of national center for culture of cereals of Algeria.

A bacterial culture is incubated on TY medium at 28°C for 18h. In the exponential growth phase, 100 µl of each bacterial culture is centrifuged at 7000 rpm for 15 minutes. The lys is of the cells is carried out after treatment of the bacterial obtained by the addition of 50 µl of lys is buffer and heating the samples in a water bath for 3 min at 100°C.

From des protein extracts of strains thus obtained, 25 µl of each protein sample is deposited in then containing the solution of the electrophoresis buffer, on a polyacrylamide gel to 12 % (p/v).

After migration, the gel is placed in a staining solution containing the blue of Coumassie and the trichloracétique acid (60 %) during a night under low agitation. Finally the gel is discolored in distilled water under low agitation until the emergence of polypeptide bands. In order to determine the molecular weight of the polypeptide bands obtained, we used a Kit (Low Molecular Weight "LMW") consisting of a mixture of proteins of known molecular weight.

III- Results:

3.1. Physico-chemical characteristics of soils

The results of the soils analyzes of the three studied sites [**Table -1**] showed that the soils of Mostaganem and Bejaia contain clay elements in a very great quantity which gives them a clay-loam texture. Concerning the soil of Bouira, it contains coarse elements in a very small quantities and an important content of fine elements, which gives it a muddy-sandy texture. These 3 types of soils are classified among the balanced textures [17]. The soil samples from the site of Mostaganem and Bouira have a neutral pH and an important content in organic matter. The soil of Bejaia has an acid pH and having a low content in organic matter and a low percentage in available phosphorus. The three samples are rich in calcium carbonate (CaCo3) which shows good calcium content and total assets with the exception of the soil of Bejaia which has a low content in active lime. Depending on the scale of salinity of Hermann (1980) the three samples are considered as non-saline soils.

Nitrogen is the more variable and deficient element and the more in the soil, its content depends on the Percentage of organic matter, of amendments of fertilizer but also bacterial species of nitrogen fixation. In our case, the majority of the soil analyzes showed an average rate in nitrogen N2. The values obtained for phosphors are greater than 0.030 and 0.01 g.kg-1soil respectively [10]; this means that the soils are very rich in phosphorus.

3.2. Phenotypic Characteristics On YEMA, Twelve isolates were fast growing (appearance

of colonies 4 to 5 days after incubation), the colonies are circular, convex, translucent. By contrast, the other 8 isolates showed a slow growth on YEMA (appearance of colonies after 5 days of incubation). All isolates are Gram negative.

The strains do not absorb the Congo red as it has been described by Jordan [1984] for the rhizobia. On the other hand 60% of the isolates have acidified the YMA + bromothymol blue after 24 h incubation (the indicator turned yellow). The strains showed a negative result after the addition of the Benedict's reagent, no halo was observed. Because Benedict's reagent does not oxidise the carbon from the glycosyl of lactose and do not produce 3-cetoglucosidase.

3.3. Symbiotic Characters

The 20 strains were able nodulate their host plant *Lablab purpureus*. While no nodule was observed on the not inoculated roots seedlings. **(Figure-2)** showed that the inoculation of plants by different strains reported an average number of nodules by plant varies from 4to 16. The more infective strains are F141 and 243 with 19 nodules formed by plant **(Figure-2)**. The strains F228 and F207 are the less infective.

The efficiencies relating obtained varies from 50% to 90%. The most important values were obtained with the strains F141 and 243 with 77% and 72% of efficiencies **(Figure-3)**. In general, the strains were very efficient, 78% of strains present a relative efficiency greater than 60%

3.4. Statistical Analyzes

The results of statistical analyzes showed that the effect of inoculation has significantly improved all growth parameters (height and the dry weight of the aerial parts and orthophosphate). The analyzes of variance showed highly significant effects on the dry weight of the aerial part of inoculated plants. The T-test showed that the report dry weight of the aerial part of inoculated plants on the dry weight of the aerial parts of seedlings controls varies from 4 to 10. This shows that the inoculated plants are significantly more developed than the controls with a standard deviation difference of 12.45.

The effect of inoculation on the plants heights is highly significant $P<0.005$, which means that the inoculated plants have a better growth than not inoculated plants, the T-test indicates that the ratio of height of the seedlings inoculated / height of seedlings witnesses varies from 2.65 to 5.95 which explains the significant growth of seedlings inoculated regarding to the control with a standard deviation difference of 27.45.

A highly significant effect on the dry weight of the root part to $P<0.005$, meaning that the dry weight of the root part of inoculated plants is more important compared to plants not inoculated. The T-test show wed that the report dry weight of the root part of inoculated plants on the dry weight of the aerial parts of control seedlings varies from 4 to 13, thus, the inoculated plants are significantly more developed than the controls.

No significant difference between the inoculated strains in all growth parameters (height and the dry weight of the aerial parts and orthophosphate). This lack of significant difference in the nodulation is due to the significant variability observed between inoculated plants within the same treatment strain and therefore to the genetic variability of the plant material.

3.5. Effect of the acidity (pH4.5) and aluminum on the symbiosis *Lablab purpureus-rhizobium*

The acidity does not inhibit the growth of the inoculated plant, there is no difference between the heights of the plants and the biomasses of the aerial parts and orthophosphate produced at pH 4.5 and 6.8 [**Table-2**], the statistical analyzes showed no significant difference between plants growing at pH 4.5 and pH 6, 8.

With regard to the nodulation, the pH 6.8 promotes the nodulation since the number and

biomass of nodules by plants at pH 6.8 are higher than at pH 4,5 .

The aluminum does not inhibit the growth and the nodulation of *Lablab purpureus*. All the tested strains induce nodules as well as in the presence of 30 that of 100 µm of aluminum that in absence of this latter, which confirms the perfect adaptation of the symbiosis *Lablab*-rhizobia to high concentrations of aluminum.

The results presented in [**Table-3**], shows that the iron deficiency decreases the growth (biomass of the aerial part) and the nodulation of the plant.

Through our results, it seems that the symbiosis *Lablab*- rhirobia is demanding in iron, which means that *Lablab purpureus* has difficulties to establish efficient symbiotic nitrogen fixation with the rhizobia in soils where the free iron is in insufficient quantities.

The statistical analysis shows a highly significant difference on the height and biomass of the aerial part between plants watered by the nutrient solution equipped with iron and free iron irrigated plants, with a standard deviation difference of 25,781.

3.6. The effect of the salinity, pH, temperature and antibiotics on the growth of strains

The majority of the strains are capable of growing on a pH between 4 and 11 [**Table-4**] with an optimum growth of all the strains tested between a pH of 6.5 and 7.5.

The results of the test of salinity [**Table-4**] obtained shows that the growth of strains is not affected by concentration up to 513mm NaCl. While at a concentration of 1710mm, the growth is moderately affected.

Most of the strains were able to grow at temperatures between 20°C and 42°C [**Table-3**], and showed an optimal growth in the interval of 20°C to 37°C. Concerning the intrinsic resistance to antibiotics, most of the strains showed a strong resistance to ampicillin and nalidixic acid while lower resistances, to 10mcg/ml, were noticed with kanamycin and streptomycin.

3.7. Numerical Analysis of the phenotypic characterization

The results of numerical analysis of the studied phenotypic characters are represented in a phenodendrogramme [**Figure-6**] and shows 72 per cent of level of similarity. Thus, the strains are divided into three groups:

The group1 is formed by the strains isolated essentially of the two sample sites of Bouira and Bejaia. These strains have common morphological characteristics. Concerning the physiological criteria, the strains of this group are characterized by a pH tolerance mostly high at 10, 5 and a level of tolerance to salt stress (NaCl) ranging from 150 to 700 mM.

Group 2: contains ten strains which are originating from the region of Mostaganem. These strains formed translucent colonies, viscous and do not absorb the Congo red. These isolates are particularly tolerant to salt stress (700 mm) and to the high pH 10, 5.

The group 3 contains two strains which exhibited high sensitivity to all the antibiotics tested.

3.8. Determination of protein profile by SDS-PAGE

The SDS-PAGE was realized in order to determine the total protein profile of all the strains [**Figure-2**]. The protein profile dendrogramme [**Figure-5**] shows that the strains are clustered into two electrophoretic groups with a coefficient of similarity of 0.95, value at which the species of reference differ. Two groups are distinguished:

Group 1 :contains the 14 fast growing strains , ten with an index of similarity between 75-85% and two with a coefficient of similarity very high 95-100% regarding to the reference strain " *Mesorizobium* sp. **".**

Group2: is constituted of only 6 slow-growing strains with an index of similarity 0.85 compared to the reference strain of *Bradyrhizobium.*

[IV] DISCUSSION

Firstly, this study provided the phenotypic characterization of rhizobia nodulating *Lablab*

purpureus in Algeria. We can notice that these results are in agreement with those of Jordan [1982] for the rhizobia. The results obtained from the test of nodulation showed that all strains have been able to nodule their host plant , several authors have studied the symbiotic parameters of different strains nodulating the *Lablab* for the selection of couples rhizobia – *Lablab* of high symbiotic performance [20] . The selection of performant symbiotic couples of *Lablab* and their microsymbiotes can increase the yield of the symbiosis [45].

Most of the isolates have been able to tolerate temperatures between 20 and 37°C, with an optimal growth at 30°C (100 %), but some of them were also able to develop to 4 °C and 42° C. The results of this test are in concordance with previous studies [46,18] which have shown that the rhizobia were mesophile bacteria, and could grow at temperatures between 10 and 37° C with an optimum temperature for growth at28 °C. However several studies have reported that the rhizobia of leguminous trees have the ability to tolerate a temperature of 40°C [14] Our results are in agreement with those of Abde-wahab *et al.* [15] who reported a tolerance of a few strains associated with *Lablab purpureus* to pH from 4 to 10, according to [18], the rhizobia can tolerate pH ranging from 4.5 to 9.

[3] have even shown that twenty strains isolated from eight different legume species have been able to tolerate high pH values of the order of 10 under conditions of non-saline. In effect, the tolerant strains at low values of pH were found in fast-growing species such as *Mesorhizobium loti and Rhizobium tropici* [33].

We have noted that the majority of strains have a good tolerance to salinity. However, in the saline environments, the symbiosis *rhizobia* - *Lablab* depends not only of the microorganism but also from the host plant. It has been reported that the species *purpureus* may develop to a level of salinity of soil very high [45]. Abdellah *et al.* [3] have isolated strains of Lablab that can tolerate up to 1190 mm NaCl. In parallel to our results, [46] reported levels of tolerance among strains of *lupin* isolated from Egypt up to 1700 mm NaCl. However, growth development in saline concentration of 1700 mm and even more has been reported for different strains nodulating of annual plants grown as fodder in Morocco [2]. Maâtallah *et al.*, [28] were able to identify strains nodulating *Cicer arietinum* which tolerate 850 mMof NaCl. The rhizobia nodulating the fenugreek are able to grow to a concentration in NaCl as high as 2380 mm (14 %) [2].

As regards to the intrinsic resistance to antibiotics, [18] reported that the inhibitory effect of an antibiotic depends on its nature and its concentration in the medium and the degree of inhibition is variable from a species to another and of a strain to the other. In parallel to our results, the Kanamycin has proved also the antibiotic the more inhibitor of the growth of certain strains of *Bradyrhizobium* nodulant the lupin in Poland [29].

It has been reported that the aluminum is at the level of the DNA of the strains of Rhizobium [19] . The exposure of these bacteria to high concentrations of aluminum also affects their mobility in the soil and their infective ability [36]. Aluminum does not inhibit the growth and the nodulation of *Lablab purpureus*. Contrary to our results, [47] have shown that the fast growing strains associated to *Acacia* were more sensitive to aluminum. Joining our results, [33] reported that iron deficiency, adversely, affects the growth of plants. An excess of iron exhibit an inhibitory effect not only on the growth of rhizobia but also on their efficiency through the loss of their plasmids.

It is clear from the numerical analysis of the total protein profiles obtained by the SDS-page a high similarity between the isolated strains and the reference strains, these results are consistent with the those of [26,42], the strains studied on *Lablab purpureus* belonged to the genre: *R. leguminosarum,*

Rhizobium sp., *Ensifer (unravel)* sp. and *Mesorhizobium* sp .

[V] CONCLUSION

In this work, we were interested in the rhizobia nodulating *Lablab purpureus* grown in different regions of Algeria. A collection of 20 strains isolated from fresh Nodules has been established. These isolates have been authenticated by seedling inoculation of *Lablab purpureus* growing in jars containing autoclaved sand.

As well, we have assessed the tolerance of all strains to the main stress factors; in this case the salinity and mainly the NaCl, pH and temperature extremes. THE osmotolerance was very importan,t up to 1190 mM and 1700 mM NaCl. The strains have been able also to growon a fairly wide range of pH ranging from 4.5 to 9.5 and a temperature varies between 4°C to 42°C.

The numerical analyzes by the statistical software using Minitab has allowed to indicate the existence of a diversity of strains nodulating *L. purpureus*. The determination of the protein profile by SDS-PAGE has allowed us to give an indication on the taxonomic position of some strains of them. The strains isolated could be affiliated to the genus *Mesorizobium* sp *and* *Bradyrhyzobium* sp. To verify this suggestion, we must accomplish this study by using molecular techniques such as REP/PCR or RFLP/PCR, the gene sequencing of the RDNA 16S and the hybridization DNA/DNA which may clarify whether these strains constitute a new species among the rhizobia or among any other kind of a proteobacteria.

REFERENCES

1. Angel, J.N, *et al.,* (1993). Inoculation effect on legumes grown in soil previously contaminated with sewage sludge. Soil Biology. 25, 575-580.
2. Abdelmmoumen, B.F, *et al*., (1999). Nodule invasion and symbiosome differentiation during Rhizobium etli-Phaseolus vulgaris symbiosis. Molecular Plant-Microbe Interaction. 13, 733-41.
3. Abdellah, Y.R, *et al.,* (2012). Numerical taxonomic study of fast growing soybean rhizobia and a proposal that Rhizobium fredii be assigned to Sinorhizobium gen. International Journal of Systematic Bacteriology. 38, 392-397.
4. Abdewahab, T.K, *et al.,(*1993). Nitrogen-fixing bacteria that efficiently nodulate Neptunia natans in Senegal. International Journal of Systematic Bacteriology. 48, 1277-1290.
5. Appunn,F.j, *et al.,* (2006) . Rhizobium hainanense sp isolated from tropical legumes. International Journal of Systematic Bacteriology. 47, 870-873.
6. Aurag, B.F . *et al.,* (1995). Phenotypic characterization of rhizobia from legumes and its application as a bioinoculant. Microbiology. 215, 79-82.
7. Bai, F.S, *et al* ., (2002). Diversity of rhizobia associated with Amorpha fruticosa isolated from Chinese soils. Microbiology. 48,230–238.
8. Bernaerts, A.L, *et al.,* (1963). Interactions between non-pathogenic soil microorganisms and plants. Ecology. 4, 335-372.
9. Brenière, S.X, *et al*., (1991). Molecular diversity of rhizobia occurring on native shrubby legumes in south astern Australia. Applied and Environmental Microbiology. 312,449-454.
10. Boyer, Z.V, *et al.,* (1983). Evaluation of the diversity of rhizobia in Brazilian agricultural soils cultivated with soybeans. Science and nature. 293, 668-672.
11. Burnie, G. H, *et al* ., (2005). Genetic diversity of rhizobia associated with common bean. Applied Soil Ecology.12, 329-331.
12. Chang, Y.L, *et al* ., (2011). Evaluation of the diversity of rhizobia in Brazilian agricultural soils cultivated with soybeans. Systematic and Applied Microbiology. 34, 276–284.
13. Connoly, T.D, *et al.,* (2003). Rhizobium Nod Factor Perception and Signalling. Plant Cell. 14, 239-249.
14. De Lajudie, P .G, *et al.,* (2004).Genetic diversity of rhizobia of Acacia saligna. International Journal of Systematic and Evolutionary Microbiology. 54, 2269-2273.
15. Driana, A.C, *et al* ., (2000). Genetic diversity of rhizobia from natural populations varies with the soil dilution sampled. World Journal of Microbiology & Biotechnology. 16, 475-480.
16. Gao, X.U, *et al.,* (2001). Symbiotic Nitrogen Fixation and Chicken Manure Fertilization in Soybean. Ecology and the Environment.14, 78-96.

17. Gadrat, J.L, *et al*.,(1997). Genetics of phosphate solubilization and its potential applications for improving plant growth-promoting bacteria. Plant Soil. 287, 15-21.
18. Graham, B.D, et al ., (1992). Statistical Procedures for Agriculture Research. Nature .45, 14-547
19. Johnsom, X.T, *et al.,* (1990). Characterization of rhizobia that nodulate legume species of the genus Lespedeza and description of Bradyrhizobium yuanmingense. Nature. 387, 394-401.
20. Kurlovick, B.S, *et al.,* (1997). Stress tolerance in Rhizobium and bradyrhizobium, and nodulation under adverse soil conditions. Canadian Journal of Microbiology. 38, 475-484.
21. Han, L.T, *et al .,* (2008) . Molecular diversity and phylogeny of rhizobia associated with wild legumes native to Xinjiang. Microbiology. 31, 287–301.
22. Hirsch, A.W, *et al .,* (2001). Characterization of Rhizobia Isolated from Different Divergence Groups of Tropical Leguminosae by Comparative Polyacrylamide Gel Electrophoresis of their Total Proteins. Soils. 127, 1484–1492.
23. Konduri, V.U, *et al.,* (2000). Genetic mapping of the Lablab purpureus genome suggests the presence of 'cuckoo' gene(s) in this species, International Journal of Microbiology. 100, 866–871.
24. Landaver, X.T, *et al.,* (2002). Maximum temperature for nitrogen fixation in common bean. Crop Science. 29, 1260-1265.
25. Li chang, H.G, *et al.,* (2011). Phosphate solubilizing bacteria and their role in plant gro promotion. Biotechnology Advances. 17, 319-339.
26. Liu, C.W, *et al.,* (1996).Genetic diversity and relationships among Lablab purpureus genotypes evaluated using RAPD markers. Nature. 91, 115–119.
27. Maatalah, F.K, et al., (2002). Nitrogen Fixation Research in Sudan. . Australian Journal Experimental Agriculture. 43, 553-577.
28. Madzak, S.K, et al., (1995). A modified single solution method for determination of phosphate in natural. Analytic chimica. 27, 31-36.
29. Makembe, N.T, *et al.,* (1996). Genetic diversity of rhizobia associated with indigenous legumes in different regions of Zimbabwe. Nature. 27 (2), 335-341.
30. Morris, J. E, *et al.,* (2009). Morphological and reproductive characterization in hyacinth bean. International Journal Systimatical Bacteriology. 62 (5), 263–279.
31. Mohan, N.P, *et al.,* (2006). Collection and evaluation of Dolichos bean Germplasm in Tamil Nadu. International Journal Systimatical Bacteriology.77 (8), 55-58.
32. Mohamed, S. D, *et al.,* (2000). Symbiotic Nitrogen Fixation and Chicken Manure Fertilization in
33. Soybean/Sorghum Intercropping System. Ecology and the Environment.76,24-26.
34. Mukhtar, N. V, *et al .,* (1994). Genetic diversity of rhizobia associated with Acacia. Agronomie . 44, 85-101.
35. Saadallah, D.T, *et al .,* (2001). Genetic diversity of rhizobia isolated from medicinal legumes growing in the sub-Himalayan region of Uttaranchal. Agronomie. 21, 627-634.
36. Octive, H.K, *et al.,*(1994). Effect of combined application of *Rhizobium* phosphate solubiling bacterium and *Trichoderma spp* on growth nutrient uptake and yield of chickpea (*Cicer aritenium* L.). Applied Soil Ecology. 28,139-146.
37. Omokanye, E.L, *et al.,* (2001) . Rhizobium loti a new species of legume root nodule bacteria. International Journal of Systematic Bacteriology. 32, 378-380.
38. Ogundip, Z .G, *et al.,* (2003). Rhizobium tropici a novel species nodulating Phaseolus vulgaris beans and Leucaena sp. trees. International Journal of Systematic Microbiology. 41, 417-426.
39. Rivas, M. P, *et al .,* (2009). Multilocus sequence analysis of the genus Bradyrhizobium. International Journal of Systematic Microbiology. 32, 101–110.
40. Vandemmr, B.S, *et al .,* (1997). Model legumes get the nod, Plant Cell. 9, 275–280.
41. Vadez, N.Q, et al ., (1973). Effet of Salt and pH tolerance of rhizobia. International Journal of Microbiology. 18, 242-247.
42. Xing, Y.Z, et al.,(2011). Interactions of Arbuscular Mycorrhiza and Nitrogen Fixing Simbiosis in Sustainable . Agriculture.54,78-96.
43. Yanagi, M.J, *et al .,* (1993). Phylogenetic analysis of the family Rhizobiaceae and related bacteria by sequencing of 16S rRNA gene using PCR and DNA sequence. International Journal of Microbiology. 107, 115-120.
44. Yuan, J.N, *et al.,* (2009), Phenotypic characteristics and composition of rhizobia associated with Lablab purpureus growing in diverse conditions. International Journal of Microbiology. 8,167–175.
45. Younes, R.E, *et al.,* (2005). Identification of Rhizobium-specific intergenic mosaic elements within an essential two-component regulatory system of Rhizobium species. Journal of Bacteriology. 177, 5485- 5494.

46. Zahran, H. *et al.*, (1991). Conditions for successful Rhizobium-legume symbiosis in saline environments.Nature. 12, 73-80.
47. Zehrari, H.L, et al., (2000). Interaction between barley and mixed cultures of nitrogen fixing and phosphate solubilizing bacteria. Plant and Soil, 173, 29-37.
48. Zhang, F.M, *et al.*, (1996). Effects of Inoculation by Bradyrhizobium japonicum Strains on Nodulation, Nitrogen Fixation and Yield of *Cicer arietinum*),Nature. 179, 233-241.
49. Zhang, F.M, *et al.*, (1996). Biodiversity and inhibition of the expression of Bradyrhizobium japonicum at low temperatures. Soil Biology. 28, 1579-1583.

Tables and Figures :

Site	PH	Limestone Total (%)	Electrical conductivity Ms/cm	N%	P (Mg\Kg)	Limestone Active (%)	Carbon (%)	Regards Organic (%)	Granulometry			
									Sand (%)	Limon Coarse (%)	Limon End (%)	Clay (%)
Mostaganem	7.7	1,087	0.1257	0.97	25.8	1.32	0.64	1.76	12.1	6.76	7	11.6
Bouira	7.68	5,091	0.1352	0.01	20.2	0.09	1.52	0.76	19	12.54	3.78	4.5
Bejaia	4.58	2,087	4,968	0.58	21.6	0.56	0 ,83	1.54	21.5	8.53	6.1	5.5

Table: 1. Results of physico-chemical analyzes of soil composites of each site

Strains	Dry Weight of Aerial parts (G/plant)		Dry Weight of Parties Resprouts (G/plant)		Dry Weight of Aerial parts (G/plant)		Dry Weight of Parties Resprouts (G/plant)	
	-Iron	+Iron	-Iron	+Iron	Ph 6.8	Ph4.5	Ph6.8	Ph4.5
F243	9 ,32	24.43	11.43	39.79	30.75	25.76	27.76	25.14
F203	11,43	28	10.54	39	32.41	26 ,23	37.65	35.84
F228	14.7	23 ,76	15.8	34	32.54	29.99	26.76	25.32
F154	10.6	23 ,65	15.87	32	31.54	35	36.54	28.65
F229	14	24.54	18.8	38	33.15	27.65	31.83	28.14
F204	9.54	16.32	16.65	41	32.48	27.65	29.01	25.01
F229	9.32	25.65	12.65	40	35.45	31	36.76	35.41
F235	7.54	28.65	15.65	36	€ 30.78	28	42.98	35.74
F142	11.64	29.09	12.6	34	30.74	24.65	40.76	31.57
F207	21.3	34.21	14.43	36.54	29.75	21	46.65	35.17
F197	20	26.43	12.6	49, 43	29.45	25.76	42.87	38.47
F239	19.6	27.56	11.43	31	31.27	29	38.65	35.17
F134	18.8	28.76	10.54	40	34.75	28.87	29.87	25.47
F141	15.08	26.76	14 ,54	29	35.49	32.65	31.26	25.64
F155	16.76	24.65	13.54	41	25.74	21.84	27.55	21.98
F263	9 ,32	29.34	12.43	49	26.17	22	29.76	25.64
F238	11, 43	27.08	17.6	45	29.47	30 ,76	26.43	19.45
F213	14.7	28.87	7.54	52	31.62	26 ,09	24.98	21.41
F190	10.6	27 ,98	11.64	42	29.74	24.43	21.98	19.75
F237	14	31.76	9.76	34	35.41	28	28.87	27.65
F197	9.54	24.43	11.43	39.79	28.74	25.76	27.76	25.45

Table: 2. Influence of pH and iron deficiency on the symbiosis of *Lablab purpureus*-rhizobia after two months of culture in the Greenhouse

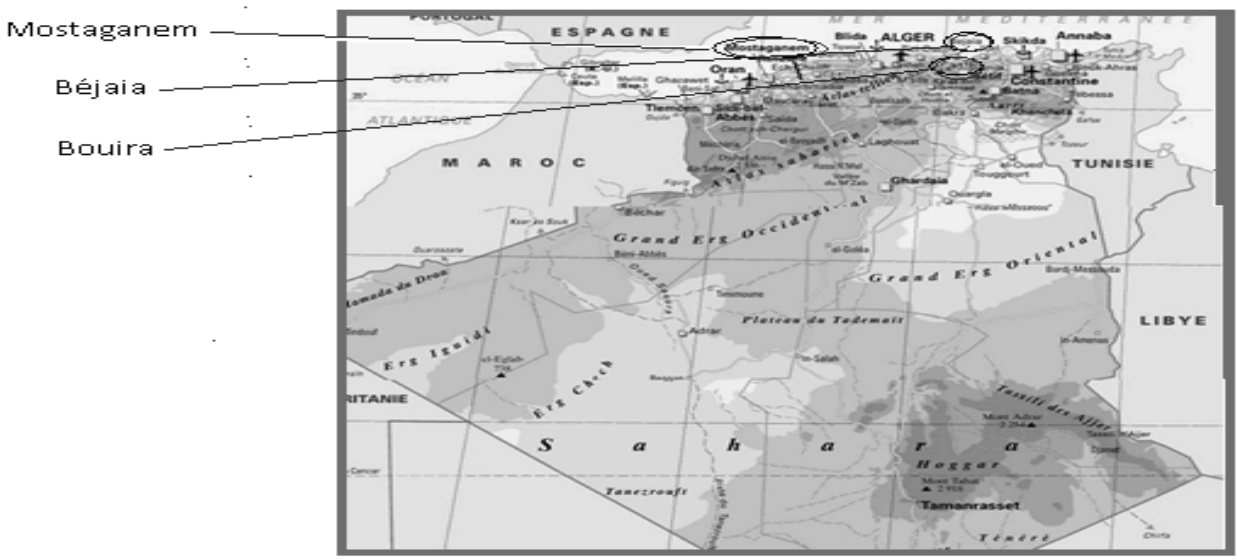

Fig: 1. geographical map of Algeria represents the geographical position of sites of samplings (Mostaganem, Bejaia, Bouira).

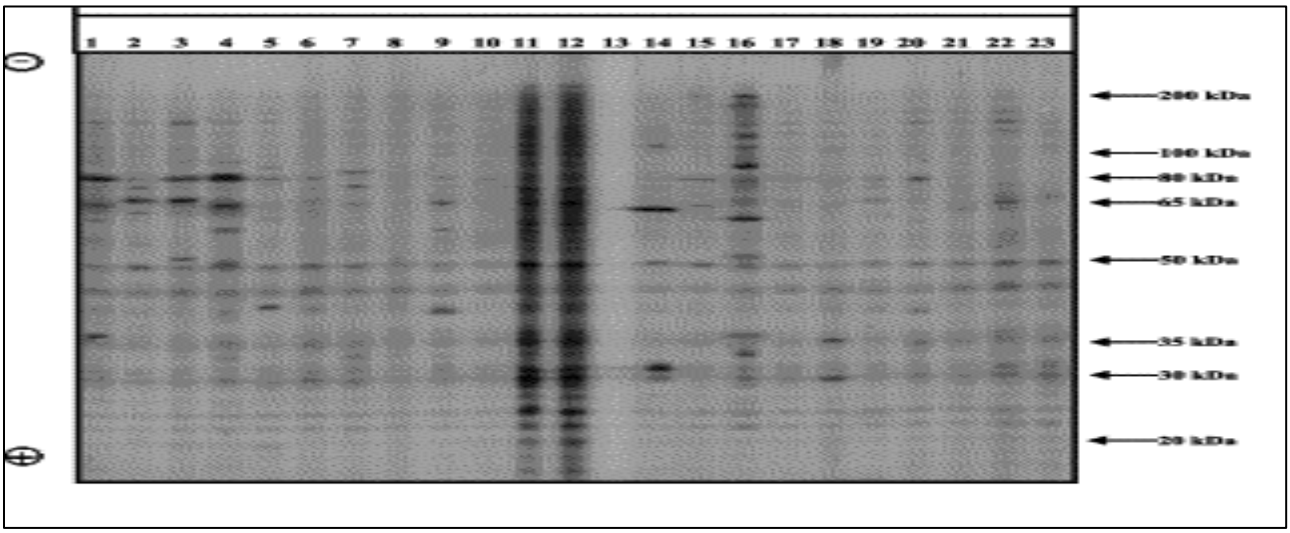

Fig: 2. Profile SDS-PAGE of total protein (Laemmli, 1970) of the strains isolated from *Lablab purpureus* from different regions of Algeria (**(1)F203, (2)F2282 , (3)F179 , (4)F229 , (5)F142 , (5)F235, (6)F237 , (7)F261 , (8)F155 , (9)F213, (10) F154 (13)F262 , (14)F204, (15)F238, Mesorhizobium sp, (17)F141 , (18)F190 , (19) F236, (20) F207,(22) Bradyrhizobium sp,)**

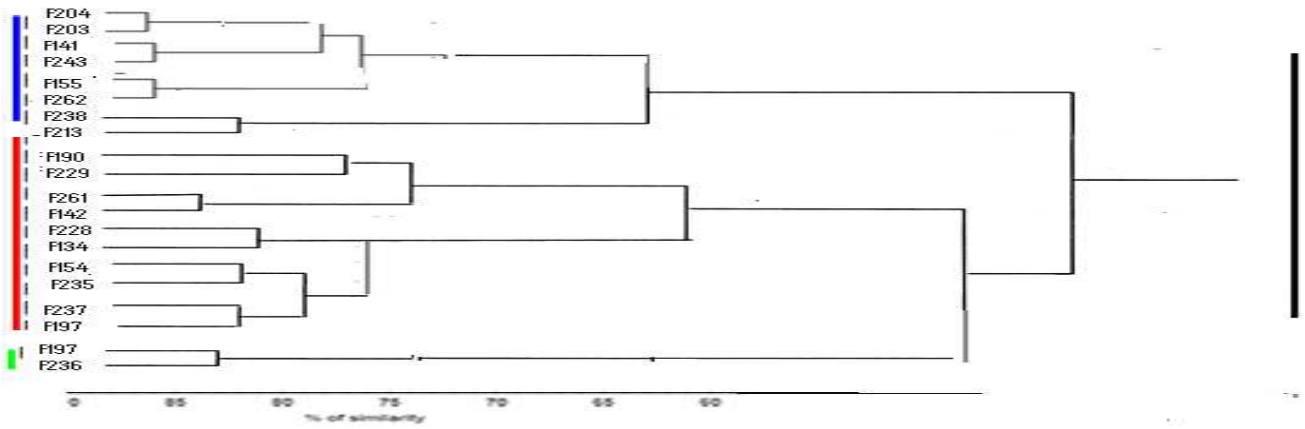

Fig: 6. Phenodendrogramme indicating the phenotypic similarities between the strains nodulant *Lablab purpureus* from different parts of Algeria on the basis of the numerical analysis of 45 phenotypic characters by statistical software using Minitab.

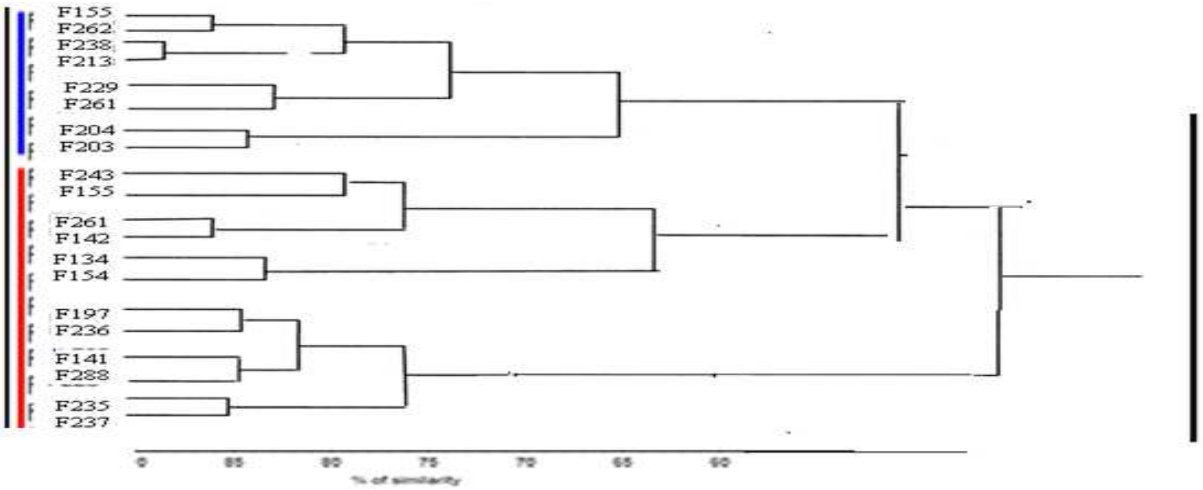

Fig: 5. Dendrogramme indicating the similarities between the strains nodulant *L. purpureus* from different parts of Algeria and the reference strains analyzed by the profile SDS-PAGE of total protein according to Laemmli (1970).

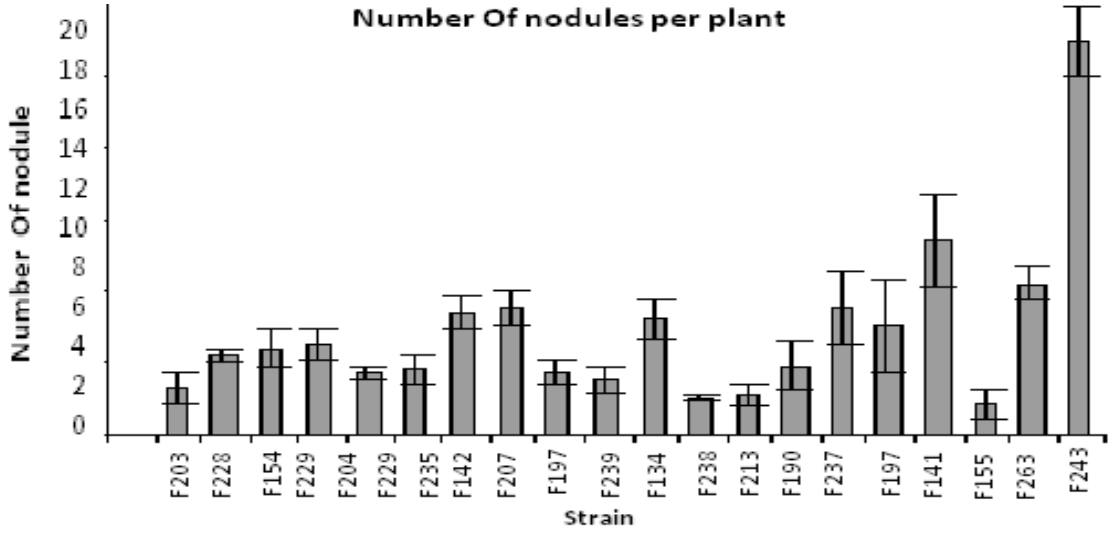

Fig: 3. Infectivity of strains nodulant lablab purpureus

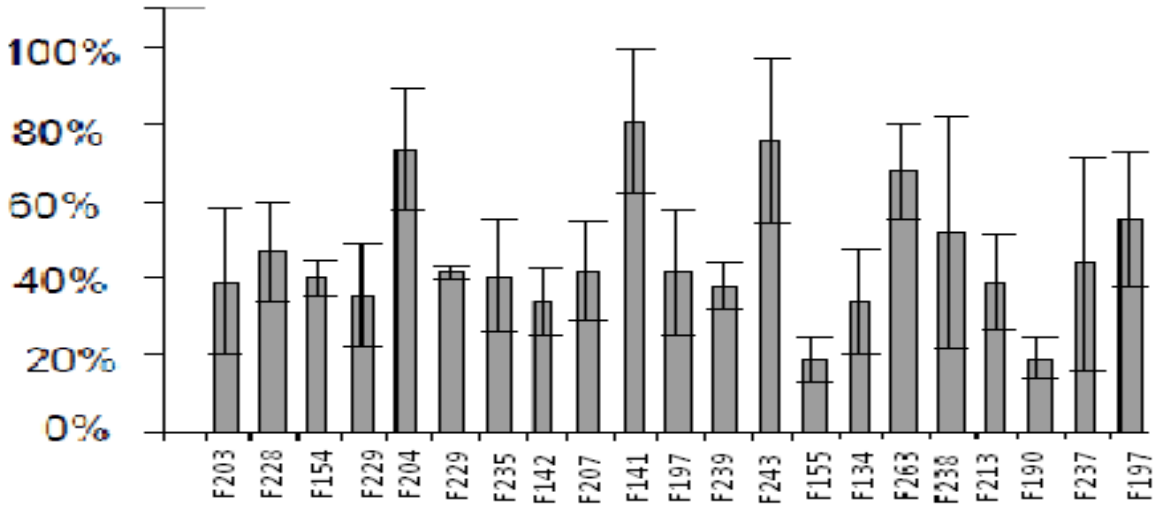

Fig: 4. Relative Efficiency of strains nodulant lablab purpureus after describing two months of inoculation

	Température (°C)							
Isolats	2	4	10	20	25	30	35	42
F243	-	+	+	++	+++	+++	+++	++
F203	-	+	+	++	+++	+++	+++	++
F228	-	+	+	++	+++	+++	+++	++
F154	-	+	+	++	+++	+++	+++	++
F229	-	+	+	++	+++	+++	+++	+
F204	-	+	+	++	+++	+++	+++	_
F229	-	+	+	++	+++	+++	+++	+
F235	-	+	+	++	+++	+++	+++	+
F142	-	+	+	++	+++	+++	+++	_
F207	-	+	+	++	+++	+++	+++	_
F197	-	+	+	++	+++	+++	+++	+++
F239	-	+	+	++	+++	+++	+++	+
F134	-	+	+	++	+++	+++	+++	+
F141	-	+	+	++	+++	+++	+++	+
F155	-	+	+	++	+++	+++	+++	-
F263	-	+	+	+	+++	+++	+++	+
F238	-	+	+	++	+++	+++	+++	++
F213	-	+	+	++	+++	+++	+++	-

Table: 3. Effect of temperature (°C) on the growth of strains.

-Not tolerant, + averagely tolerant, ++tolerate, +++very strong tolerance

	pH							**NaCl (%)**			
Isolats	2	4	6	6,8	8	10	12	4%	6%	8%	10%
F243	0.245	0.3487	0.587	0.6897	0.7987	0.514	0.451	0.254	0.458	0.685	0.715
F203	0,278	0.258	0.524	0.654	0.7518	0.4284	0.3652	0.2168	0.4975	0.7581	0.795
F228	0.258	0.3475	0.5236	0.6248	0.7851	0.4252	0.2658	0.23658	0.4147	0.6254	0.752
F154	0.216	0.2358	0.5174	0.6245	0.7962	0.4120	0.2471	0.3147	0.5178	0.7412	0.841
F229	0.387	0.3486	0.5246	0.5999	0.6851	0.3524	0.2654	0.1568	0.3075	0.7584	0.834
F204	0.249	0.3174	0.6487	0.7014	0.7951	0.5174	0.3584	0.234	0.5245	0.7604	0.872
F229	0.742	0.369	0.4287	0.6214	0.7625	0.5245	0.3014	0.2014	0.5647	0.7827	0.7951
F235	0.269	0.3429	0.3875	0.5978	0.6872	0.4201	0.2645	0.2987	0.5647	0.7142	0.8640
F142	0.1999	0.3487	0.4025	0.6487	0.7255	0.4208	0.2058	0.2345	0.5214	0.7629	0.862
F207	0.2087	0.3147	0.4521	0.7584	0.7985	0.4201	0.2014	0.364	0.6248	0.8056	0.8985
F197	0.2358	0.3269	0.4271	0.5148	0.6851	0.4258	0.231	0.3754	0.7548	0.9710	0.997
F239	0.2468	0.3475	0.3876	0.5987	0.6782	0.4127	0.2301	0.3178	0.6478	0.8750	0.9751
F134	0.3548	0.4587	0.5429	0.6875	0.7851	0.4012	0.2034	0.2548	0.5648	0.7813	0.872
F141	0.6987	0.2198	0.4257	0.5785	0.6248	0.3014	0.2015	0.3159	0.6248	0.8246	0.964
F155	0.2489	0.3654	0.4758	0.5987	0.6875	0.3014	0.2015	0.1987	0.5478	0.7854	0.857
F263	0.2478	0.3742	0.4576	0.5468	0.6987	0.5148	0 ,254	0.2546	0.5328	0.8650	0.927
F238	0.1678	0.3957	0.4258	0.5648	0.6218	0.3751	0.1785	0.2597	0.5642	0.8634	0.914
F213	0.2589	0.3527	0.4257	0.5628	0.6728	0.3540	0.1025	0.2754	0.5872	0.8619	0.974
F190	0.2457	0.3487	0.5876	0.6897	0.7987	0.5142	0.4518	0.2958	0.5642	0.8964	0.901
F237	0.278	0.2589	0.5248	0.6548	0.7518	0.4284	0.3652	0.2521	0.5724	0.8653	0.975

Table: 4. Effect of pH and NaCl on the growth of strains (DO)