RESULT AND DISCUSSION:

1) ISOLATION OF EFFICIENT RHIZOBIUM ISOLATES:

Four Rhizobium strains were isolated from

2) MORPHOLOGICAL CHARECTERISTICS OF RHIZOBIUM ISOLATES:

Gram- negative and rod shaped isolates were present. Rhizobium colonies in Congo red medium were white, transluscent, glistening, raised and, in comparison to stained colonies of non-Rhizobium isolates, smaller.

3) BIOCHEMICAL TEST:

Oxidase test:

An organism’s ability to produce cytochrome oxidase is assessed using the oxidase test. Neisseria, Moraxella, Campylobacter, and Pasteurella species (oxidase positive) are distinguished using the test.

Result Interpretation of Oxidase Test

Positive Result

Oxidase production is indicated by the emergence of a rich purple-blue/blue colour within 5–10 seconds.

Negative Result

There is no purple-blue colour or colour shift.

Catalase test:

Catalase-producing organisms are discovered using this assay. By dissolving hydrogen peroxide into water and oxygen gas, this enzyme detoxifies it.



The presence of oxygen gas bubbles definitely indicates a catalase-positive outcome.

Result Interpretation of catalase Test:

• Positive outcome: Adding hydrogen peroxide causes bubbles to appear on the bacterial suspension. This demonstrates that the Rhizobium culture has catalase activity.

• Negative outcome: After adding hydrogen peroxide, no bubbles appear on the bacterial solution. This shows that the Rhizobium culture lacks catalase activity.

Citrate test:

The ability of an organism to use citrate as a source of energy is examined using citrate agar. Citrate is the only source of carbon in the medium, while inorganic ammonium salts (NH4H2PO4) are the only source of nitrogen

Result Interpretation of citrate Test:

Positive Reaction: Growth with color change from green to intense blue along the slant.

Negative Reaction: Slant remains green; there is no expansion or colour change.

Gelitanase test:

This test is used to assess an organism’s capacity to create the gelatinases, which are extracellular proteolytic enzymes that break down gelatin, a substance found in vertebrate connective tissue.

Result Interpretation of gelitanase Test:

• Positive outcome: The existence of a clean area around the bacterial growth shows that the Rhizobium culture is producing gelatinase.

• Negative outcome: The absence of a distinct zone around the bacterial growth suggests that the Rhizobium culture is not producing gelatinase.

Indole test:

The indole test evaluates an organism’s capacity to break down the amino acid tryptophan and generate indole.Bacteria that express the tryptophanase enzyme can deaminate and hydrolyze the amino acid tryptophan. The main prerequisite for cultivating an organism before running the indole test is that the medium has enough tryptophan. When a microorganism is cultivated in a medium containing tryptophan, the presence of Indole indicates that the organism has the ability to breakdown tryptophan.

Result Interpretation of Indole Test:

Positive: Within seconds of introducing the reagent, the medium’s top layer of the reagent layer develops a pink to red hue (a “cherry-red ring”).

Negative: No colour change even after the proper reagent has been added.

Congo red test:

Rhizobia cannot be distinguished from other bacteria by congo red absorption, although it may be useful as a strain marker. It is an all-purpose biochemical test that can be used to distinguish and identify different bacteria. However, it is frequently used to evaluate the capacity of specific bacterial species, such as Rhizobium, to make exopolysaccharides (EPS) or capsular material.

EPS are crucial for the development of root nodules in Rhizobium, nitrogen-fixing bacteria that associate symbiotically with leguminous plants. The bacteria are housed in these nodules, which also offer a setting favourable for nitrogen fixation. For the bacteria to create and keep up this symbiotic interaction, EPS generation is essential.

Result Interpretation of Congo red Test:

Positive outcome (EPS production): A positive outcome is indicated if the bacterial colonies are pink or red in colour. This indicates that EPS was created by the bacteria and the Congo The polysaccharides included in the EPS matrix have bound red dye. The dense, mucoid, or gummy consistency linked to EPS formation is generally indicated by the pink or red colour.

A negative result (lack of EPS generation) is indicated if the bacterial colonies are white or colourless. This shows that the bacteria either create very little EPS or none at all. Without EPS, the Congo red dye is unable to attach to any polysaccharides and does not produce any colour.

Methyl Red test:

The fundamental goal of an MR test is to determine if an organism can produce and maintain an adequate quantity of stable acid as a byproduct of the fermentation of glucose while also outpacing the system’s buffering ability.This test is designed to identify the fermentation route that uses glucose as an energy source.

Result Interpretation of Methyl red Test:

Positive Response: A pronounced crimson hue

Negative Response: A yellow reaction is unfavourable.

Starch hydrolysis test:

We use starch agar, a different nutritive media, in the starch hydrolysis test (also known as the amylase test). The test organisms are plated onto a starch surface and allowed to develop for up to 48 hours at 30°C. An iodine solution is then poured into the Petri dish.

Depending on the iodine content, Gram’s iodine combines with starch to produce a dark blue, purple, or black complex.

Result Interpretation of Starch Hydrolysis Test:

Positive test: When iodine solution is added, a clear zone forms around the line of development, proving that the organism has hydrolyzed starch.

Negative test: A medium that is either blue, purple, or black in colour (depending on the iodine content).

Bromothymol blue test:

To distinguish between Rhizobium and Agrobacterium, this test is necessary.Rhizobium bacteria have the ability to produce acidic metabolic byproducts like organic acids when they are cultivated in a medium with a carbon source that can be fermented. The pH of the growing medium can be lowered by these byproducts. The colour change can be used as an indicator to determine whether Rhizobium is producing acid by utilising bromothymol blue.This experiment aims to assess the acid generation rate of Rhizobium’s metabolic activity. It can be used to contrast several Rhizobium strains or evaluate the effects of diverse growth circumstances on their metabolic activity.

Result Interpretation of Bromothymol blue Test:

Colour change to yellow or green: A noticeable pH reduction and a colour change to yellow or green both imply that rhizobium bacteria are actively fixing nitrogen. This shift in colour denotes a successful nitrogen-fixing process.

Little to no colour change: If there is little to no colour change, it can be a sign of poor metabolic activity or unfavourable nitrogen fixation circumstances. The rhizobium bacteria in this situation might not be fixing nitrogen

Voges proskauer test:

The capacity of some microbes to produce the neutral end product 2,3 butanediol from the fermentation of glucose is tested. It is used to identify organisms that convert glucose to acetylmethyl carbinol. Acetylmethyl carbinol, if present, undergoes diacetylation in the presence of -naphthol, strong alkali (40% KOH), and oxygen from the atmosphere.

Result Interpretation of Voges proskauer test:

Positive Reaction: A surface that is pink-red in colour

Negative Reaction: Lack of a pink-red hue causes a negative reaction.

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| --- | --- | --- | --- | --- |
| BIOCHEMICAL TEST | ISOLATE 1 | ISOLATE 2 | ISOLATE 3 | ISOLATE 4 |
| OXIDASE TEST | + | + | + | + |
| CATALASE TEST | + | + | + | + |
| CITRATE TEST | + | + | + | + |
| GELATINASE TEST | - | - | - | - |
| INDOLE TEST | - | - | - | - |
| CONGO RED TEST | - | - | - | - |
| METHYL TEST | - | - | - | - |
| STARCH HYDROLYSIS TEST | - | - | - | - |
| VOGES PROSKAUER TEST | + | + | + | + |

(+) positive result, (-) negative result

4) TESTING EFFICIENCY OF SELECTED RHIZOBIUM ISOLATES:

Rhizobium isolates had a significant positive effect on growth and nodulation of groundnut plants compared to the non-inoculated control 45 days after plant emergence.

Experimental results of selected Rhizobium isolates indicate how well these isolates promote symbiotic nitrogen fixation in legumes. Rhizobium is a group of soil bacteria that form a mutualistic relationship with legumes, allowing them to convert atmospheric nitrogen into a usable form that benefits both the bacteria and the plant.

Several factors can be considered when evaluating the performance of testing selected Rhizobium isolates:

Nitrogen-fixing ability: The main function of Rhizobium isolates is to fix atmospheric nitrogen in ammonia by the enzyme nitrogenase. Efficacy testing involves evaluating the ability of selected isolates to perform this crucial task. This can be measured by the amount of nitrogen fixed by bacteria and its subsequent transfer to host plants.

Nodulation efficiency: Rhizobium forms nodules on legume roots, providing a special environment for nitrogen fixation. The efficacy of selected isolates can be evaluated by evaluating their nodulation ability. This includes studying the number, size and activity of nodes formed on plant roots and their ability to maintain a symbiotic relationship with the host.

Plant growth and yield: The ultimate goal of efficient nitrogen fixation is to improve plant growth and yield. Parameters such as plant height, shoot and root biomass, chlorophyll content and yield of legumes inoculated with selected Rhizobium isolates are measured to evaluate the effectiveness of the experiment. Comparison with non-inoculated or ineffective isolates can provide valuable insights.

Compatibility with host plants: Different Rhizobium isolates have different compatibility with certain legume species. Performance testing involves determining which isolates work best for a particular legume. Compatibility can be assessed by examining nodulation and performance, as well as the general health and vigor of the host plant.

Genetic characterization: Genetic characterization can be performed to better understand selected Rhizobium isolates. This involves analyzing genetic structure, such as the presence of specific genes related to nitrogen fixation and nodulation. Genetic analysis can provide insight into the potential and compatibility of isolates with specific legume cultivars.

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| --- | --- | --- | --- | --- |
| ISOLATES ID | GERMINATION RATE (%) | SHOOT LENGTH (cm) | ROOT LENGTH (cm) | BIOMASS (g) |
| ISOLATE 1 | 90 | 15 | 12 | 0.8 |
| ISOLATE 2 | 85 | 12 | 10 | 0.6 |
| ISOLATE 3 | 70 | 8 | 6 | 0.4 |
| ISOLATE 4 | 95 | 18 | 14 | 1.0 |

Germination rate: This column indicates presence of seeds that successfully germinated and developed into healthy seedling when inoculated with respective isolate.

Shoot length: The length of above-ground shoot of seedlings is recorded in this column. This indicates the vigor and growth potential of the plant.

Root length: This column measures length of seedling’s root system. It reflects the development of root structure and its ability to absorb nutrients and water from soil.

Biomass: This column indicates the total biomass of seedlings, including both shoot and root components. It provides a general indicator of plant growth and productivity.

Compared with other isolates, maximum amount of biomass found in Isolate 4, followed by Isolate 1, Isolate 2 and Isolate 3. Isolate 4 has higher shoot and root length with higher rate of germination.

5) CONCLUSION:

Rhizobium isolates were isolated from in a groundnut plant based experiment. Once the Isolate has been purified and its characterized by morphological and biochemical parameters. Isolate 4 was superior to other strain in terms of germination rate and biomass and was selected for future field trial. Increasing the yield of groundnut requires isolation of suitable and effective microbial strains. The method used in this study helps in screening isolate local rhizobium and test their effectiveness local peanut varieties. After all, there is a goal to improve groundmut productivity.