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# Isolation, purification and characterization of *Gluconacetobacter* and Potash Mobilizing Bacteria (KMB) from rhizosphere of Sugarcane (*Saccharum officinarum*)

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**A B S T R A C T**

The laboratory studies were conducted at Department of Plant Pathology and Microbiology, Post Graduate Institute, MPKV, Rahuri for the isolation, purification and characterization of isolates obtained from rhizosphere of Sugarcane. Completely randomized design (CRD) was used for laboratory studies. The present investigation was carried out to isolate and characterize *Gluconacetobacter* and potassium mobilizing bacteria (KMB) from the rhizosphere and endosphere of sugarcane. A total of 11 *Gluconacetobacter* and 5 KMB isolates were obtained from different locations of Ahilyanagar district, Maharashtra. The isolates were characterized based on morphological, cultural, and biochemical characteristics. All isolates were Gram-negative, rod-shaped, motile, and showed positive KOH reaction. Cultural characterization revealed variation in colony morphology on different media, with isolates $GSD_{15}$ and $KSA_{10}$ showing better growth and mucoid nature. Functional evaluation indicated significant variation in nutrient mobilization, wherein Gluconacetobacter isolate $GSD_{15}$ recorded the highest nitrogen fixation (25.40 µg $ml^{-1}$), while KMB isolate $KSA_{10}$ showed maximum potassium solubilization (34 µg $ml^{-1}$) and solubilization index (3.77). The results indicate that these isolates have potential for use as bioinoculants in sustainable sugarcane cultivation.

## Introduction

*Gluconacetobacter* is a nitrogen-fixing, Gram-negative, rod-shaped endophytic bacterium that lives inside sugarcane tissues without harming the plant. It plays a key role in sustainable sugarcane cultivation by naturally supplying nitrogen and promoting plant health. *Gluconacetobacter* enhances plant growth through multiple pathways, such as direct mechanisms including atmospheric nitrogen fixation, production of growth-promoting phytohormones, phosphate solubilization, and suppression of stress-induced ethylene; and indirect mechanisms by strengthening the plant's natural defences against pathogens through induced systemic resistance (Bhattacharya and Jha, 2012).

Potassium mobilizing bacteria (KMB) represent an important advancement in sustainable agriculture, acting

as natural biofertilizers that enhance plant nutrition and soil fertility. These beneficial microbes convert insoluble potassium reserves in the soil into bioavailable forms, thereby improving nutrient uptake and crop performance. KMB employ a variety of biochemical mechanisms to mobilize potassium, including the secretion of organic acids such as citric, oxalic, and gluconic acids, which dissolve potassium-bearing minerals. Additionally, they facilitate the release of potassium through enzymatic degradation of silicate matrices, as well as through chelation an acidolysis processes involving proton exchange reactions (Meena *et al.,* 2014).

Therefore, isolation and characterization of these beneficial microorganisms from the sugarcane rhizosphere are essential for their effective utilization in sustainable crop production.

## Material and Methods

### Isolation and maintenance of isolates of *Gluconacetobacter* and KMB

### Collection of soil samples

Fresh rhizosphere soil samples of sugarcane and along with sugarcane plant samples (sets), were collected from different locations and transported to the laboratory for the isolation of rhizospheric and endophytic microorganisms. A total of 30 rhizosphere soil samples and 15 endosphere plant samples (sets) were collected from Ahilyanagar district of Maharashtra for the isolation of *Gluconacetobacter*, KMB. Air-dried soil samples with low moisture content were stored at 4 °C. For the isolation of Gluconacetobacter spp., a total of 15 sugarcane plant samples were collected from the Ahilyanagar district of Maharashtra. Sugarcane sets aged 6–8 months were randomly selected from each location. The sets were washed under tap water, and the outer portions were removed. Inner active tissues from each set were then excised and placed on cotton in screw-cap plastic vials, with calcium carbonate used as a desiccant beneath the cotton at the bottom. The vials were stored in a refrigerator until further isolation procedures were carried out.

### Preparation of culture media

LGIP medium was prepared for isolation and maintenance of *Gluconacetobacter* and Alexandrov's isolates. Culture medium with following composition were used and sterilized at 15lbs pressure for 15 min at 121ºC temperature. After that the molten media used for culturing the bacterium

### Isolation of *Gluconacetobacter*

Isolation of *Gluconacetobacter* was carried out using LGIP selective agar medium. As *Gluconacetobacter* is an endophytic bacterium, isolation was performed using sugarcane stem samples (sets). The sugarcane sets were washed with sterile distilled water and surface-sterilized with 5% sodium hypochlorite (NaOCl) for 5 minutes, followed by five successive washes with sterile distilled water. The surface-sterilized samples were weighed and homogenized in 1% sterile sucrose solution using a sterile pestle and mortar. One millilitre of the homogenate was aseptically transferred into 9 mL of sterile water blank using a micropipette fitted with a sterile tip and thoroughly mixed using a vortex mixer. From this suspension, 1 mL was further transferred into another 9 mL sterile water blank to obtain a $10^{-2}$ dilution. Subsequent serial dilutions up to $10^{-7}$ were prepared in a similar manner. From the $10^{-4}$, $10^{-5}$, and $10^{-6}$ dilutions, 1 mL aliquots were transferred into sterile Petri plates. LGIP selective agar medium adjusted to pH 5.5 was sterilized by autoclaving at 121 °C and poured into the Petri plates. The contents were mixed by gently rotating the plates, ensuring that the medium did not come into contact with the lids, and allowed to solidify. After solidification, the plates were incubated at 28 ± 2 °C in a BOD incubator for 3 4 days. Following incubation, colonies exhibiting yellow coloration were selected and streaked onto the same medium for further characterization studies.

### Isolation of Potassium Mobilizing Bacteria

Isolation of potassium-mobilizing bacteria (KMB) was carried out using Alexandrov's agar medium. KMB were isolated from collected sugarcane rhizospheric soil samples, which were stored in polythene bags at 4 °C, by employing the serial dilution and pour plate technique. From the homogenized soil sample, 1 g was aseptically transferred into 9 mL of sterile water blank and thoroughly mixed using a vortex mixer. Subsequently, 1 mL of this suspension was transferred into another 9 mL sterile water blank to obtain a $10^{-2}$ dilution. Further serial dilutions up to $10^{-7}$ were prepared in a similar manner. From the $10^{-4}$, $10^{-5}$, and $10^{-6}$ dilutions, 1 mL aliquots

were transferred into sterile Petri plates. Alexandrov's agar medium adjusted to pH 7 was sterilized by autoclaving at 121 °C and poured into the plates. The contents were mixed by gently rotating the plates, ensuring that the medium did not touch the lids, and allowed to solidify. After solidification, the plates were incubated at 28 ± 2 °C in a BOD incubator for 2 days. Colonies exhibiting clear solubilization zones were selected and subcultured on Alexandrov's agar medium for further characterization studies.

## Purification, Multiplication and Maintenance of *Gluconacetobacter* and KMB isolates

All isolates of *Gluconacetobacter* and KMB were purified on nitrogen-free LGIP selective agar medium and Alexandrov's agar medium, respectively. Pure colonies were selected and further purified using the streak plate method. The purified bacterial isolates were maintained on their respective media as slants for future use.

## Characterization of *Gluconacetobacter* and KMB Isolates

## Cultural and morphological characterization of *Gluconacetobacter* and KMB isolates

The Gluconacetobacter and KMB isolates were examined for their morphological characteristics, including Gram staining, cell shape, staining reaction, cell morphology, motility, and the 3% KOH test, following standard procedures. Overnight-grown cultures of the selected isolates were streaked on different media, namely Alexandrov's medium, GYCA medium, LGIP selective agar medium, nutrient agar medium, and PDA medium.

After incubation at 28 ± 2 °C for 48 hours, the cultures were observed for colony morphology, including colony colour, shape, texture, margin, size (mm), and elevation.

## KOH (Potassium Hydroxide) test

A loopful of bacterial culture from a week-old colony was mixed with a drop of 3% aqueous KOH solution on a clean glass slide and stirred in a rapid circular motion for 5–10 seconds using a sterile needle. To confirm the Gram reaction, the needle was lifted a few centimetres above the slide and observed for the formation of viscid strands. The appearance of thread-like slime indicated the presence of Gram-negative bacteria, as described by Suslow *et al.,* (1982) and Schaad (1980).

## Motility test

Test tubes were filled with 5 mL of SIM agar medium and sterilized by autoclaving at 121 °C for 20 minutes. Fresh cultures from isolated colonies were inoculated using a sterile needle by puncturing the medium to within 1 cm of the bottom of the tube to assess motility. Care was taken to ensure that the needle entered and exited the medium along the same line. The inoculated tubes were incubated at 27 °C for 18–48 hours or until visible growth appeared. The presence of diffuse growth radiating away from the line of inoculation indicated a positive motility reaction (Tittsler and Sandholzer, 1936).

## Biochemical characterization of *Gluconacetobacter* and KMB isolates

Biochemical characterization of the isolates was carried out using tests such as the methyl red test, catalase test, starch hydrolysis test, gelatin hydrolysis test, gas production test, $H_2S$ production test, and glucose fermentation test.

## Gram staining

The isolates were characterized for Gram staining following the procedure described by Cerny (1976). A loopful of culture was placed on a clean, dry glass slide to prepare a smear, which was air-dried and heat-fixed. Two drops of crystal violet were applied and allowed to stand for 30 seconds, followed by rinsing with distilled water. Subsequently, 1–2 drops of Gram's iodine were added and kept for 60 seconds, and the slide was then washed with 95% ethyl alcohol. Safranin was applied as a counterstain for 30 seconds, after which the slide was rinsed with distilled water. The stained smear was dried using blotting paper and examined under a compound microscope using an oil immersion lens. Based on the staining reaction, bacterial cells were categorized as Gram-negative ($G^-$), Gram-positive ($G^+$), or Gram-variable ($G\pm$) strains.

## Methyl Red (MR) test

One-third loopful of the culture was inoculated into a labelled test tube containing MR-VP broth and incubated at 37 °C for 24–48 hours. After incubation, five drops of

methyl red indicator were added to the culture. A red colour in the medium indicated a positive methyl red test, corresponding to a pH of around 4. The development of a yellow colour at a pH of approximately 6 indicated a negative reaction, reflecting the presence of acid at a lower hydrogen ion concentration.

## Catalase test

To determine catalase activity, 24-hour-old cultures were placed on clean, labelled glass slides, and a drop of 3% hydrogen peroxide ($H_2O_2$) was added. The immediate formation of gas bubbles indicated a positive catalase reaction.

## Starch hydrolysis test

The isolates were inoculated onto plates containing sterilized starch agar medium using the quadrant point method and incubated at 28 ± 2 °C for two days. After incubation, the plates were flooded with Lugol's iodine solution. The appearance of a clear zone around the bacterial colonies indicated a positive starch hydrolysis test. The isolates were inoculated onto plates containing sterilized starch agar medium using the quadrant point method and incubated at 28 ± 2 °C for two days. After incubation, the plates were flooded with Lugol's iodine solution. The appearance of a clear zone around the bacterial colonies indicated a positive starch hydrolysis test.

## Gelatin hydrolysis test

Gelatin stab media were prepared, and freshly grown cultures of the isolates were inoculated aseptically into the stabs. The inoculated tubes were incubated at 28 °C for 48 hours. After incubation, the gelatin tubes were placed in a refrigerator at 4 °C for approximately 20 minutes and then examined to determine whether the medium remained solid or became liquid. Liquefaction of the medium indicated a positive gelatin hydrolysis test.

## Gas production test

Gas production by the isolates was assessed by growing them in nutrient broth containing 2% glucose. An inverted Durham's tube was placed inside each test tube containing the broth, and the medium was sterilized by autoclaving at 121 °C for 20 minutes. The tubes were inoculated with 0.5 mL of bacterial suspension and incubated at room temperature (28 ± 2 °C) for seven days. The presence of air bubbles in the inverted Durham's tube indicated gas production.

## $H_2S$ production test

The culture was inoculated into labelled test tubes containing SIM agar medium by stab inoculation and incubated at 37 °C for 24–48 hours. Ferrous ammonium sulfate present in the SIM agar acted as an indicator by reacting with hydrogen sulfide gas to form an insoluble black ferrous sulfide precipitate along the line of inoculation, indicating a positive $H_2S$ production test. The absence of black precipitate indicated a negative reaction.

## Glucose fermentation test

Phenol red glucose broth was dispensed into sterile test tubes containing Durham tubes and inoculated with pure bacterial cultures. The inoculated tubes were incubated at 30 °C for 24–48 hours under aerobic conditions. After incubation, the tubes were examined for changes in the colour of the medium and for gas production in the Durham tubes.

## Assessment of the Efficacy of *Gluconacetobacter* and KMB

## Screening of Gluconacetobacter isolates for Nitrogen-fixing efficiency

The 48-hour-old cultures of freshly isolated *Gluconacetobacter* isolates were inoculated into 5 mL of LGIP selective liquid medium and incubated for 48 hours. Subsequently, 1 mL of this culture was transferred to 50 mL of LGIP selective liquid medium and incubated for 15 days. From these broths, 10 mL aliquots were used for nitrogen estimation following the standard Microkjeldahl technique as described by Reis *et al.,* (1994). The formula used for $N_2$ estimation is as follows:

$$N_2\ (\mu g/mL) = \frac{\text{ml of } H_2SO_4 \times \text{ Normality of } H_2SO_4 \times 14.01}{\text{volume of the sample (10ml broth)}}$$

## Screening of KMB isolates for Potassium solubilizing activity using solubilizing index (SI)

For this purpose, GYCaA medium supplemented with 1% feldspar was prepared and sterilized in an autoclave

at 15 lbs pressure for 15 minutes. The sterilized medium was poured into sterile Petri plates and allowed to solidify. Under aseptic conditions, the isolates were inoculated onto the surface of the glucose yeast calcium agar medium and incubated at 28 ± 2 °C. After an incubation period of five days, observations were recorded for the development of clearance zones, and screening was performed using Khandeparkar's selection ratio as described by (Prajapati and Modi 2012).

$$\text{Zone activity} = \frac{\text{Diameter of zone of clearance (D)}}{\text{Diameter of growth (d)}}$$

## Quantitative estimation of Potassium solubilization by KMB isolates

The isolates exhibiting zones of solubilization were further evaluated for their potassium solubilizing ability. For this purpose, the isolates were inoculated into 25 mL of Alexandrov's medium and incubated at 28 ± 2 °C for 10 days. Following incubation, the broth was centrifuged at 7,000 rpm for 10 minutes to separate the supernatant from cell biomass and insoluble potassium.

One millilitre of the supernatant was transferred to a 50 mL volumetric flask, and the volume was made up to 50 mL. The soluble potassium content was then estimated using a flame photometer, following the method described by Parmar *et al.,* (2016).

## Results and Discussion

## Morphological characters of *Gluconacetobacter* isolates

The morphological characterization of eleven *Gluconacetobacter* isolates showed a high degree of uniformity in their basic cellular features. All isolates exhibited a negative Gram reaction and appeared pink upon Gram staining, confirming their Gram-negative nature. Microscopic examination revealed that all isolates possessed a rod-shaped, bacillus-type morphology, which is a characteristic feature of the genus Gluconacetobacter. Variation was observed in cell arrangement, with isolates $GAW_3$, $GPT_4$, $GRW_8$, $GSJ_{10}$ and $GNB_{14}$ occurring predominantly in chains, while $GMO_2$, $GRB_5$, $GSB_7$, $GKK_9$, $GAK_{11}$ and $GSD_{15}$ were mainly present as scattered single cells. All isolates showed positive motility and a positive KOH reaction, further confirming their Gram-negative cell wall characteristics. Overall, the morphological traits clearly established that all isolates shared typical features of *Gluconacetobacter*.

## Morphological characters of potassium mobilizing bacterial isolates

The morphological characterization of five KMB isolates revealed a high degree of uniformity in their basic cellular characteristics (Table 4). All isolates exhibited a negative Gram reaction and appeared pink upon Gram staining, confirming their Gram-negative nature. Microscopic examination showed that all isolates were rod-shaped with a bacillus-type cell morphology, which is typical of Gram-negative plant-associated bacteria. Variation was observed in cell arrangement among the isolates.

Isolates $KAH_3$, $KKA_6$ and $KSA_{10}$ occurred predominantly as scattered single cells, whereas $KRJ_5$ and $KRS_8$ were mainly arranged in chains, indicating strain level diversity in cellular organization. All isolates exhibited positive motility, suggesting the presence of flagella that may contribute to effective colonization and adaptation in the rhizosphere environment. All KMB isolates showed a positive KOH reaction, further confirming their Gram-negative cell wall characteristics.

Overall, the uniformity in Gram reaction, cell shape, morphology, motility, and KOH reaction, along with minor variations in cell arrangement, clearly establishes that the KMB isolates share similar morphological traits and validates their suitability for further biochemical and functional characterization

## Cultural characterization of *Gluconacetobacter* isolates

Cultural characterization of *Gluconacetobacter* isolates $GPT_4$ and $GSD_{15}$ revealed clear variation in colony morphology across different media (Table 5). Isolate $GPT_4$ produced yellow to transparent white colonies with irregular shape, slimy texture, and predominantly wavy margins, with colony size ranging from 7.0 to 9.0 mm. In contrast, $GSD_{15}$ exhibited larger colonies (8.0–14.0 mm), with circular to irregular shape, smooth margins, and mucoid texture. Colony elevation in both isolates varied from flat to convex depending on the medium. Maximum colony growth was observed on LGIP medium for both isolates. Overall, $GSD_{15}$ showed better growth and more pronounced mucoid characteristics compared to GPT.

## Cultural characterization of KMB isolate

Cultural characterization of KMB isolate $KSA_{10}$ on different media revealed distinct variations in colony morphology (Table 6). Colonies were predominantly whitish creamy in colour with round shape, while irregular shape was observed on PDA medium. Texture varied from slimy to mucoid, with wavy margins recorded on nutrient agar and PDA, and smooth margins on other media. Colony elevation remained flat across all media. Colony size ranged from 7.0 mm on LGIP medium to 12.5 mm on Aleksandrov's medium, indicating maximum growth on Aleksandrov's medium.

## Biochemical tests of *Gluconacetobacte*r isolates

The biochemical characterization of eleven *Gluconacetobacter* isolates revealed a largely uniform biochemical profile with certain strain-level variations (Table 7). All isolates showed a positive methyl red reaction, indicating their ability to produce stable acidic end products during glucose metabolism, a characteristic feature of acetic acid bacteria. Glucose fermentation was also positive in all isolates, confirming their efficient carbohydrate utilization capability. Catalase activity was observed in most of the isolates; however, isolates $GAW_3$ and $GAK_{11}$ showed a negative catalase reaction, indicating variability in oxidative stress tolerance among the strains.

All isolates were negative for starch hydrolysis, suggesting the absence of extracellular amylase activity, which is consistent with the known biochemical behaviour of *Gluconacetobacter* spp. Gelatin hydrolysis showed variable responses among the isolates. Positive gelatin liquefaction was observed in $GPT_4$, $GRB_5$, $GSB_7$ and $GSD_{15}$, while the remaining isolates were negative, reflecting differences in proteolytic enzyme production at the strain level. Hydrogen sulfide production was positive in most isolates, except $GAW_3$ and $GAK_{11}$, indicating variability in sulphur metabolism. All isolates exhibited positive gas production, further supporting their fermentative metabolic activity.

Overall, the biochemical test results closely align with the established biochemical characteristics of the genus Gluconacetobacter. The observed variations among certain isolates highlight strain-level diversity while collectively supporting their identification as *Gluconacetobacter* spp. prior to molecular confirmation.

## Biochemical tests of potassium mobilizing bacterial isolates

The biochemical characteristics of five KMB isolates were evaluated using standard biochemical tests, and the results are presented in Table 8. The isolates exhibited a largely uniform biochemical profile, indicating close physiological similarity among them. All KMB isolates showed a positive methyl red reaction, demonstrating their ability to produce stable acidic end products during carbohydrate metabolism. Catalase activity was also positive in all isolates, suggesting their capacity to neutralize oxidative stress, which is a common feature of aerobic and facultative aerobic bacteria. All isolates were negative for starch hydrolysis, indicating the absence of extracellular amylase activity. Gelatin hydrolysis showed minor variation among the isolates; $KAH_3$, $KRJ_5$, $KKA_6$ and $KSA_{10}$ exhibited positive gelatin liquefaction, whereas isolate $KRS_8$ was negative, reflecting strain-level differences in proteolytic enzyme production.

Hydrogen sulfide production was positive in all isolates, indicating active sulphur metabolism. Similarly, all isolates showed positive gas production and glucose fermentation, confirming their efficient utilization of glucose as a carbon source and their fermentative metabolic capability.

Overall, the biochemical test results are consistent with the characteristic biochemical behavior of potassium-mobilizing bacteria. The high degree of uniformity observed among the KMB isolates, along with minor variations in gelatin hydrolysis, supports their close relatedness and validates their selection for further functional evaluation.

## Nitrogen fixation ability of *Gluconacetobacter* isolates in N free broth (µg/ ml)

The nitrogen fixation potential of selected *Gluconacetobacter* isolates was evaluated by estimating the amount of nitrogen fixed, expressed as µg $ml^{-1}$ (Table 9). The results revealed considerable variation in nitrogen fixation capacity among the isolates, indicating strain-level differences in diazotrophic efficiency. Among the tested isolates, $GPT_4$ exhibited a relatively high nitrogen fixation ability, recording 24.80 µg $ml^{-1}$, while $GRB_5$ and $GAW_3$ fixed 18.50 µg $ml^{-1}$ and 17.20 µg $ml^{-1}$ nitrogen, respectively. Isolate $GNB_{14}$ showed moderate nitrogen fixation activity with a value of 20.30 µg $ml^{-1}$.

**Table.1** *Gluconacetobacter* isolates obtained from sugarcane sets in different tehsils of Ahilyanagar district.

| Sr. No | Tahasil Name | Village name | *Gluconacetobacter* Isolates Designation |
|---|---|---|---|
| **1** | MPKV | OFRTC field | $GMO_2$ |
| **2** | Ahmednagar | Walki | $GAW_3$ |
| **3** | Pathardi | Tisgaon | $GPT_4$ |
| **4** | Rahuri | Bramhani | $GRB_5$ |
| **5** | Shrirampur | Belapur | $GSB_7$ |
| **6** | Rahata | Wakadi | $GRW_8$ |
| **7** | Kopergaon | Kopergaon | $GKK_9$ |
| **8** | Sangamner | Jorve | $GSJ_{10}$ |
| **9** | Akole | Kalas Bk | $GAK_{11}$ |
| **10** | Nevasa | Bahirwadi | $GNB_{14}$ |
| **11** | Shevgoan | Devtakali | $GSD_{15}$ |

**Table.2** Potassium mobilizing bacterial isolates obtained from sugarcane rhizosphere soil in different tehsils of Ahilyanagar district.

| Sr. No | Tahasil Name | Village name | KMB Isolates Designation |
|---|---|---|---|
| **1** | Ahmednagar | Hingangaon | $KAH_3$ |
| **2** | Rahuri | Jambhali | $KRJ_5$ |
| **3** | Karjat | Ambijalgaon | $KKA_6$ |
| **4** | Rahata | Sakori | $KRS_8$ |
| **5** | Sangamner | Ashvi Kd | $KSA_{10}$ |

**Table.3** Morphological characters of *Gluconacetobacter* isolates

| Sr. no | Isolates | Morphological characters of *Gluconacetobacter* isolates | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | **Gram reaction** | **Stain colour** | **Cell shape** | **Cell morphology** | **Cell arrangement** | **Motility test** | **KOH Test** |
| **1** | $GMO_2$ | -ve | Pink | Rod | Bacillus | Scattered single | + ve | + ve |
| **2** | $GAW_3$ | -ve | Pink | Rod | Bacillus | Arranged in chain | + ve | + ve |
| **3** | $GPT_4$ | -ve | Pink | Rod | Bacillus | Arranged in chain | + ve | + ve |
| **4** | $GRB_5$ | -ve | Pink | Rod | Bacillus | Scattered single | + ve | + ve |
| **5** | $GSB_7$ | -ve | Pink | Rod | Bacillus | Scattered single | + ve | + ve |
| **6** | $GRW_8$ | -ve | Pink | Rod | Bacillus | Arranged in chain | + ve | + ve |
| **7** | $GKK_9$ | -ve | Pink | Rod | Bacillus | Scattered single | + ve | + ve |
| **8** | $GSJ_{10}$ | -ve | Pink | Rod | Bacillus | Arranged in chain | + ve | + ve |
| **9** | $GAK_{11}$ | -ve | Pink | Rod | Bacillus | Scattered single | + ve | + ve |
| **10** | $GNB_{14}$ | -ve | Pink | Rod | Bacillus | Arranged in chain | + ve | + ve |
| **11** | $GSD_{15}$ | -ve | Pink | Rod | Bacillus | Scattered single | + ve | + ve |

**Table.4** Morphological characters of potassium mobilizing bacterial isolates

| Sr. no | Isolates | Morphological characters of KMB isolates | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | Gram reaction | Stain colour | Cell shape | Cell morphology | Cell arrangement | Motility test | KOH Test |
| 1 | $KAH_3$ | -ve | Pink | Rod | Bacillus | Scattered single | + ve | + ve |
| 2 | $KRJ_5$ | -ve | Pink | Rod | Bacillus | Arranged in chain | + ve | + ve |
| 3 | $KKA_6$ | -ve | Pink | Rod | Bacillus | Scattered single | + ve | + ve |
| 4 | $KRS_8$ | -ve | Pink | Rod | Bacillus | Arranged in chain | + ve | + ve |
| 5 | $KSA_{10}$ | -ve | Pink | Rod | Bacillus | Scattered single | + ve | + ve |

**Table.5** Cultural characterization of efficient *Gluconacetobacter* isolates on different media.

| Sr. no | Isolates | Media | Colony characters of *Gluconacetobacter* isolates | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | | Colour | Shape | Texture | Margin | Elevation | Colony size (mm) |
| 1. | $GPT_4$ | 1. LGIP Media | Yellow | Irregular | Slime | Wavy | Convex | 9.0 |
| | | 2. Nutrient Agar media | Transparent White | Irregular | Slime | Wavy | Convex | 8.5 |
| | | 3. PDA media | White | Irregular | Slime | Smooth | Flat | 7.5 |
| | | 4. GYCA media | Transparent White | Irregular | Slime | Wavy | Convex | 8.0 |
| | | 5. Alexandrov's media | Transparent White | Irregular | Slime | Smooth | Flat | 7.0 |
| 2. | $GSD_{15}$ | 1. LGIP Media | Yellow | Circular | Slime | Smooth | Convex | 14.0 |
| | | 2. Nutrient Agar media | Whitish creamy | Round | Mucoid | Smooth | Flat | 9.5 |
| | | 3. PDA media | White creamy | Irregular | Mucoid | Smooth | Convex | 8.0 |
| | | 4. GYCA media | Transparent White | Round | Mucoid | Smooth | Flat | 10 |
| | | 5. Alexandrov's media | Transparent White | Irregular | Mucoid | Smooth | Convex | 8.5 |

**Table.6** Cultural characterization of efficient KMB isolates on different media.

| Sr. no | Isolates | Media | Colony characters of KMB isolates | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | | Colour | Shape | Texture | Margin | Elevation | Colony size (mm) |
| 1. | $KSA_{10}$ | 1.Alexandrov's media | Whitish creamy | Round | Slime | Smooth | Flat | 12.5 |
| | | 2.Nutrient Agar media | Whitish creamy | Round | Mucoid | Wavy | Flat | 8.5 |
| | | 3. PDA media | Whitish creamy | Irregular | Mucoid | Wavy | Flat | 7.5 |
| | | 4.GYCA media | White | Round | Slime | Smooth | Flat | 12.0 |
| | | 5. LGIP Media | Whitish creamy | Round | Slime | Smooth | Flat | 7.0 |

**Table.7** Biochemical tests of *Gluconacetobacter* isolates.

| Sr. no | Isolates | Biochemical tests of *Gluconacetobacter* isolates | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | Methyl red test | Catalase test | Starch hydrolysis test | Gelatin hydrolysis test | $H_2S$ Production test | Gas production test | Glucose fermentation test |
| 1 | $GMO_2$ | +ve | +ve | -ve | -ve | +ve | +ve | +ve |
| 2 | $GAW_3$ | +ve | -ve | -ve | -ve | -ve | +ve | +ve |
| 3 | $GPT_4$ | +ve | +ve | -ve | +ve | +ve | +ve | +ve |
| 4 | $GRB_5$ | +ve | +ve | -ve | +ve | +ve | +ve | +ve |
| 5 | $GSB_7$ | +ve | +ve | -ve | +ve | +ve | +ve | +ve |
| 6 | $GRW_8$ | +ve | +ve | -ve | -ve | +ve | +ve | +ve |
| 7 | $GKK_9$ | +ve | +ve | -ve | -ve | +ve | +ve | +ve |
| 8 | $GSJ_{10}$ | +ve | +ve | -ve | -ve | +ve | +ve | +ve |
| 9 | $GAK_{11}$ | +ve | -ve | -ve | -ve | -ve | +ve | +ve |
| 10 | $GNB_{14}$ | +ve | +ve | -ve | -ve | +ve | +ve | +ve |
| 11 | $GSD_{15}$ | +ve | +ve | -ve | +ve | +ve | +ve | +ve |

**Table.8** Biochemical tests of potassium mobilizing bacterial isolates.

| Sr. no | Isolates | Biochemical tests of KMB isolates | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | Methyl red test | Catalase test | Starch hydrolysis test | Gelatin hydrolysis test | $H_2S$ Production test | Gas production test | Glucose fermentation test |
| 1 | $KAH_3$ | +ve | +ve | -ve | +ve | +ve | +ve | +ve |
| 2 | $KRJ_5$ | +ve | +ve | -ve | +ve | +ve | +ve | +ve |
| 3 | $KKA_6$ | +ve | +ve | -ve | +ve | +ve | +ve | +ve |
| 4 | $KRS_8$ | +ve | +ve | -ve | -ve | +ve | +ve | +ve |
| 5 | $KSA_{10}$ | +ve | +ve | -ve | +ve | +ve | +ve | +ve |

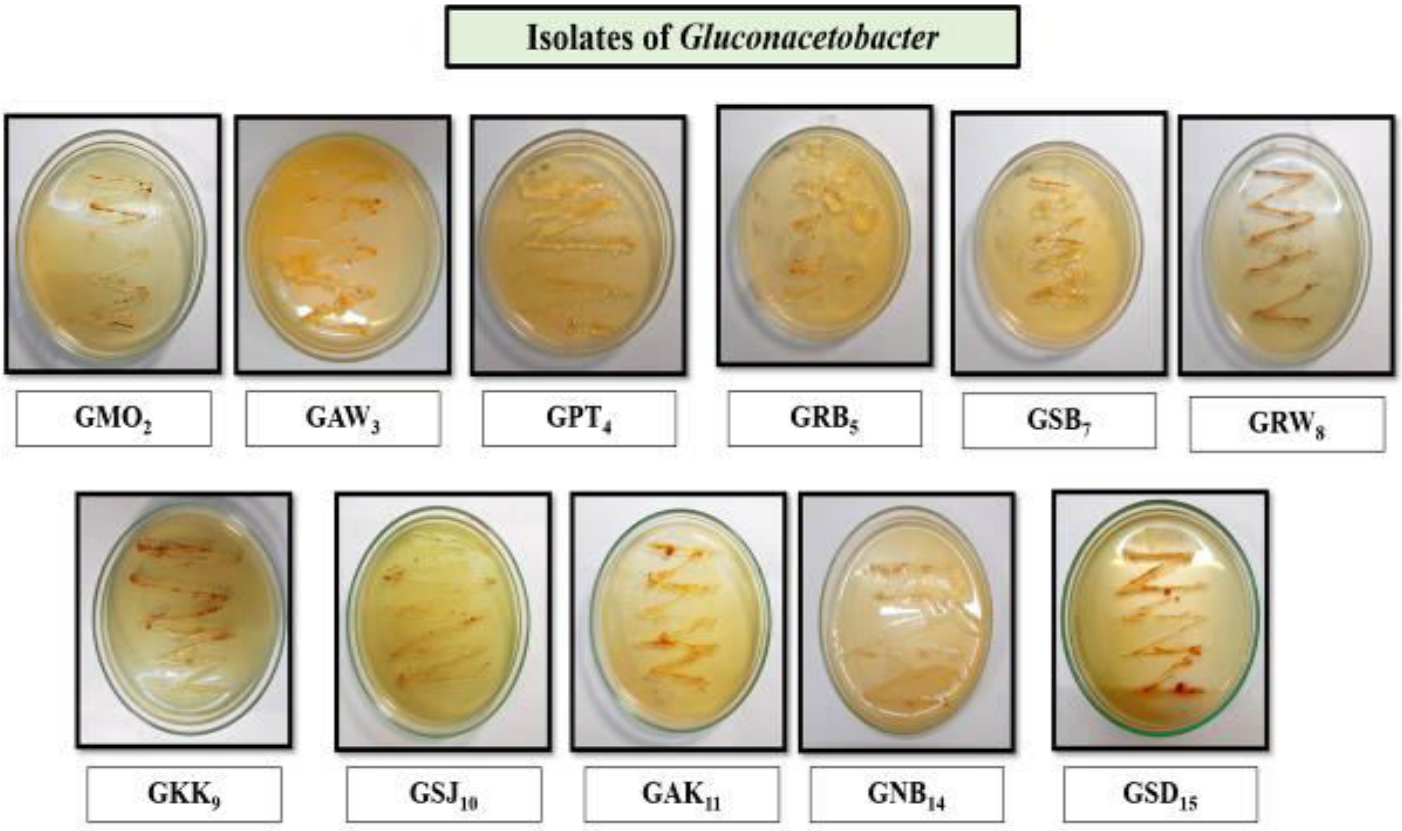
**Table.9** Nitrogen fixation ability of *Gluconacetobacter* isolates in N free broth (µg/ ml)

| Sr. No. | Isolates | Nitrogen fixation (µg/ ml) |
|---|---|---|
| 1 | $GMO_2$ | 19.10 |
| 2 | $GAW_3$ | 17.20 |
| 3 | $GPT_4$ | 24.80 |
| 4 | $GRB_5$ | 18.50 |
| 5 | $GSB_7$ | 18.60 |
| 6 | $GRW_8$ | 22.20 |
| 7 | $GKK_9$ | 21.70 |
| 8 | $GSJ_{10}$ | 18.00 |
| 9 | $GAK_{11}$ | 21.50 |
| 10 | $GNB_{14}$ | 20.30 |
| 11 | $GSD_{15}$ | 25.40 |
| SEm | | 0.28 |
| CD at 1% | | 0.82 |
| CV | | 2.35% |

**Table.10** Potassium solubilizing ability of Potassium mobilizing bacterial (KMB) isolates.

| Sr. No. | Isolates | Potassium solubilization (µg/ ml) | Diameter of zone of clearance (D) mm | Diameter of growth (d) mm | D/d (ratio) |
|---|---|---|---|---|---|
| **1** | $KAH_3$ | 22.5 | 14 | 6.5 | 2.15 |
| **2** | $KRJ_5$ | 28.1 | 19 | 7.2 | 2.64 |
| **3** | $KKA_6$ | 31.4 | 27.8 | 8.2 | 3.39 |
| **4** | $KRS_8$ | 26.5 | 20.5 | 8.5 | 2.41 |
| **5** | $KSA_{10}$ | 34 | 34 | 9 | 3.77 |
| **SEm±** | | 0.37 | | | |
| **CD at 1%** | | 1.17 | | | |
| **CV%** | | 2.26 | | | |

**Plate.1** Isolates of *Gluconacetobacter* and Potash Mobilizing Bacteria (KMB)



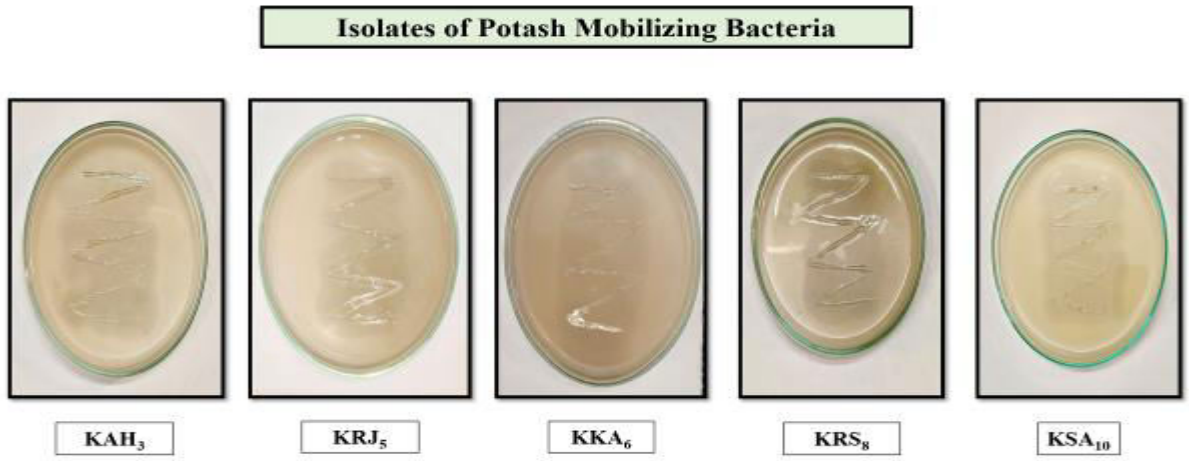
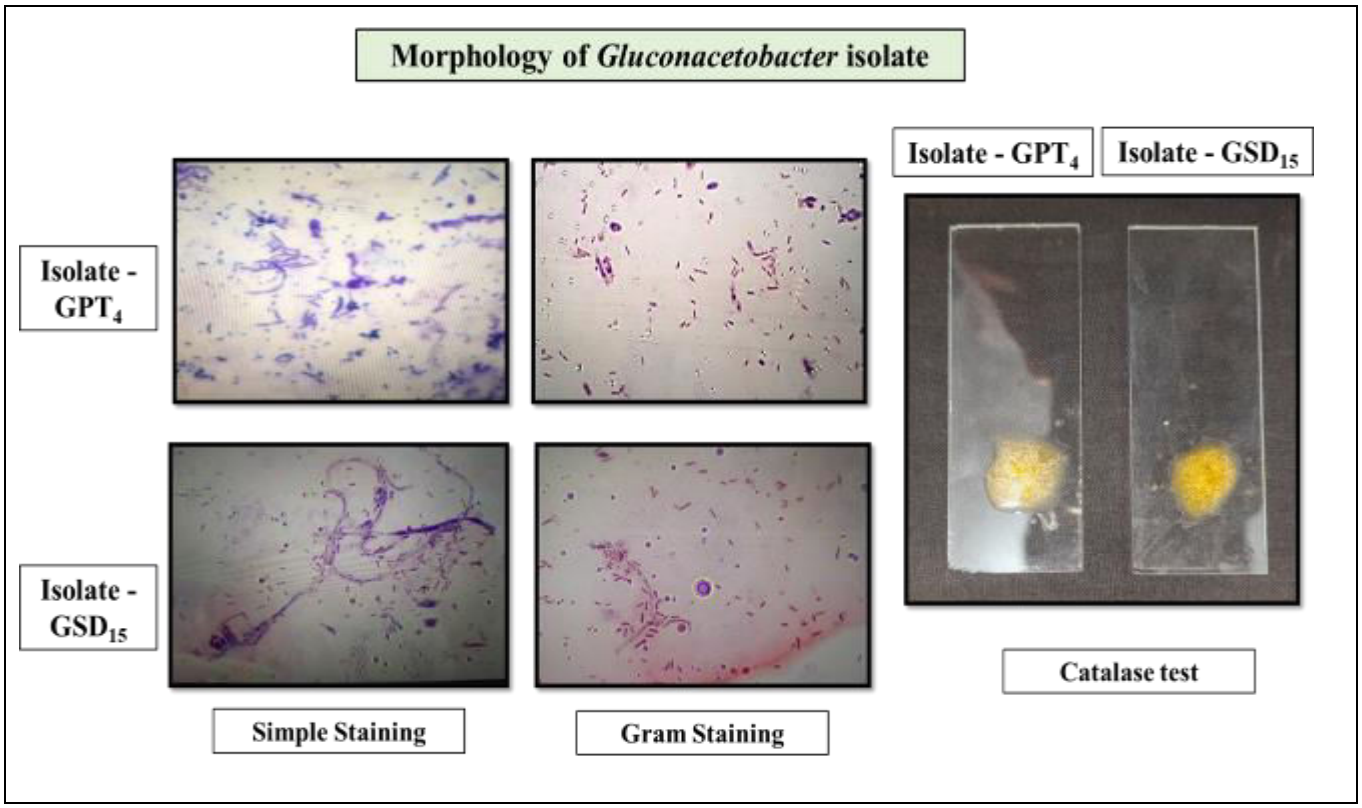



**Plate.2** Morphological characters of efficient isolates of *Gluconacetobacter* and Potash Mobilizing Bacteria (KMB)



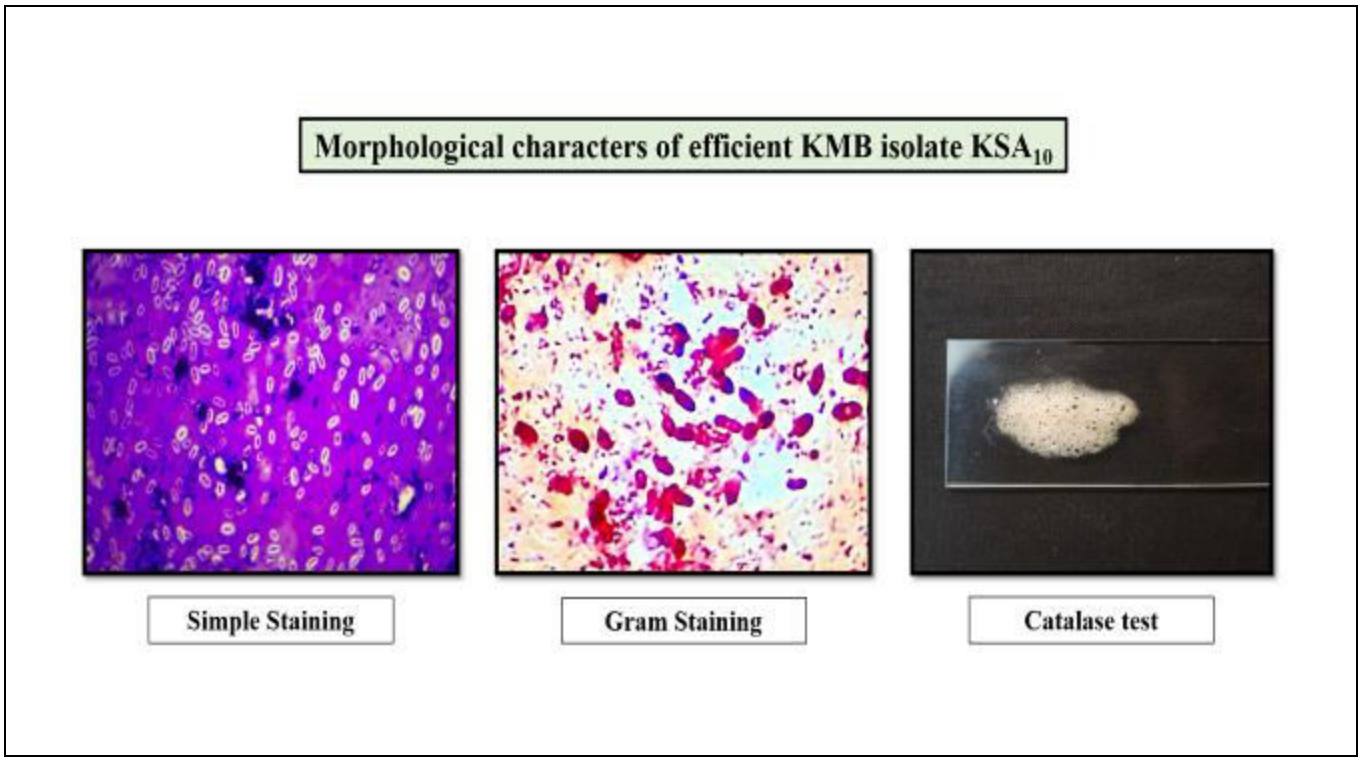
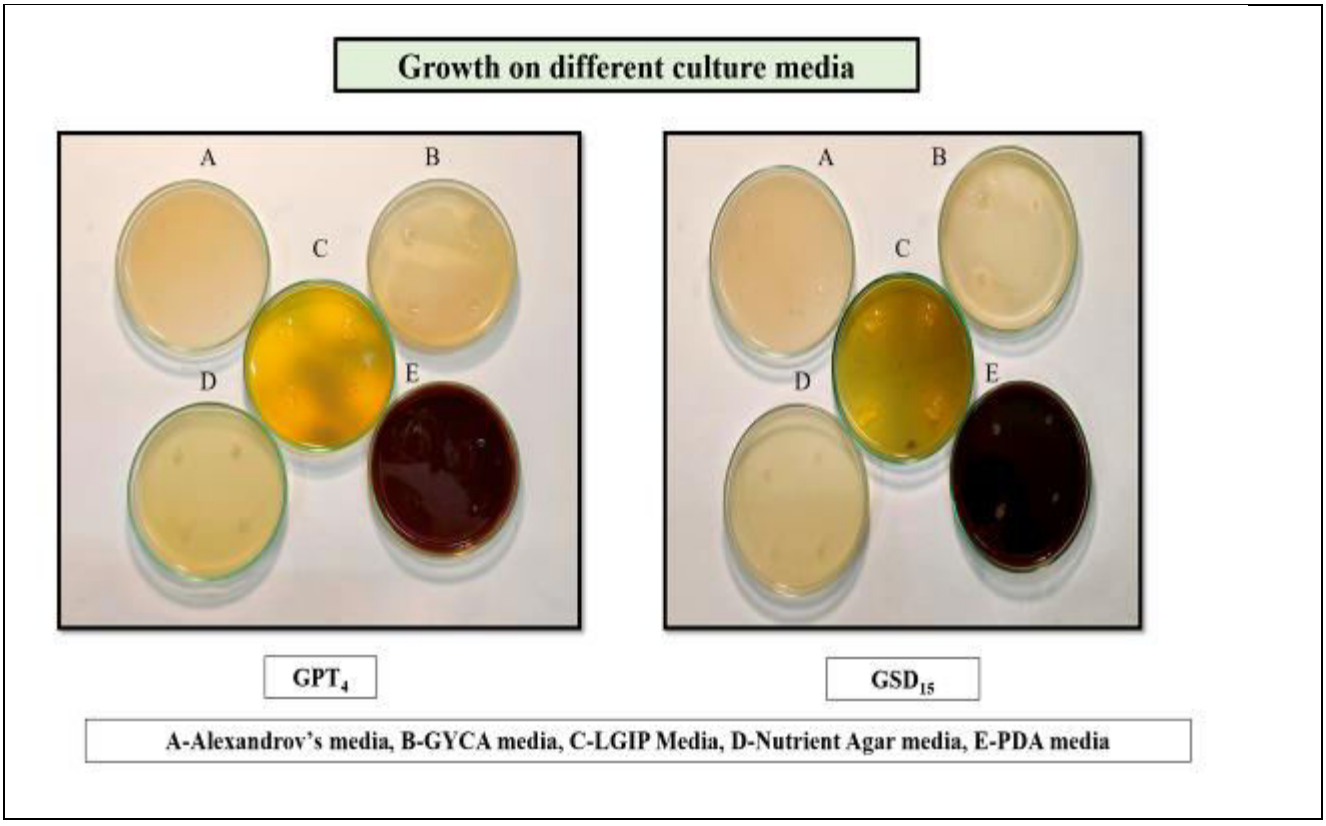



**Plate.3** Cultural characterization of efficient isolates of *Gluconacetobacter* and Potash Mobilizing Bacteria (KMB) on different media



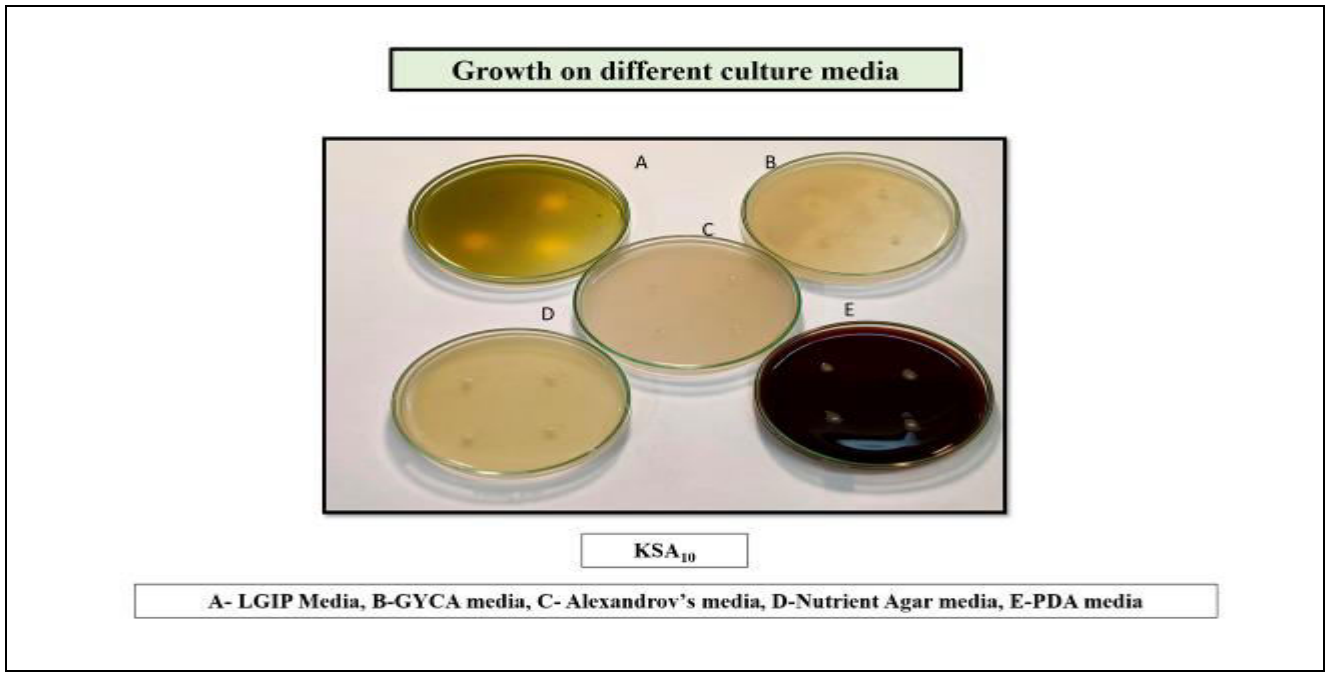



**Plate.4** Biochemical characterization of efficient isolates of *Gluconacetobacter* and Potash Mobilizing Bacteria (KMB) on different media.

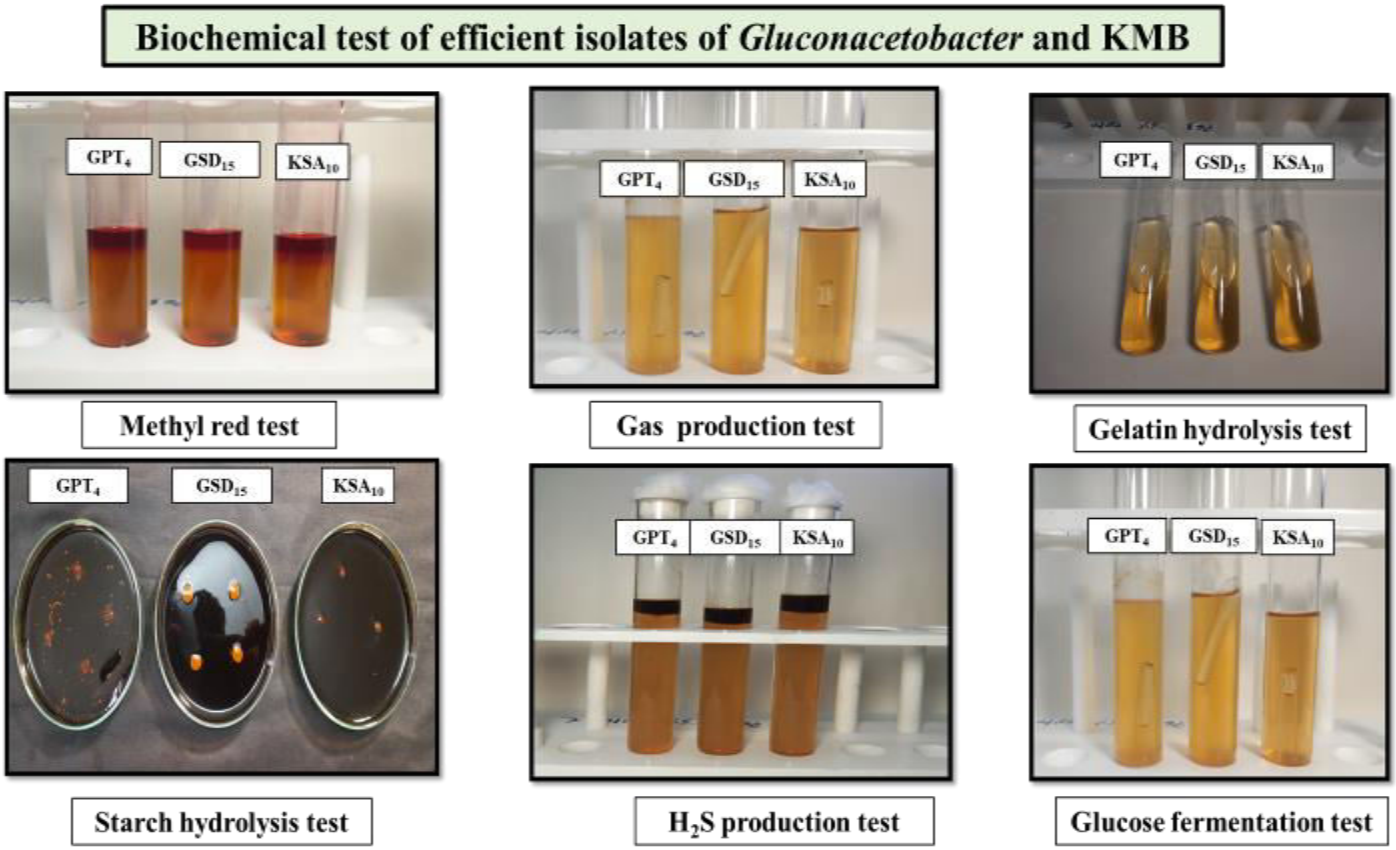
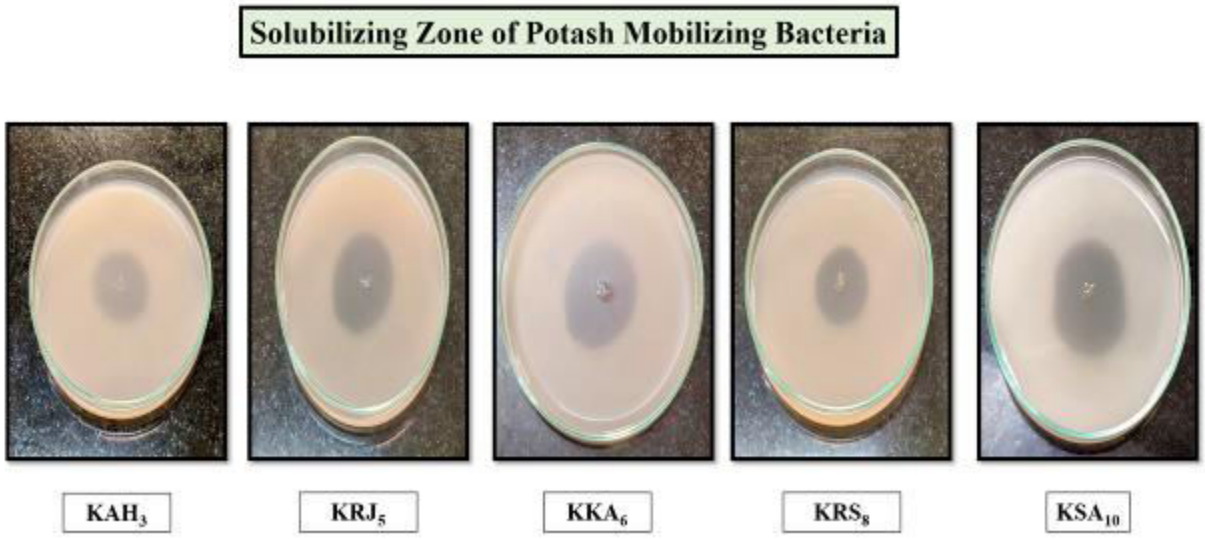


**Plate.5** Solublizing zone of Potash Mobilizing Bacteria (KMB)




The highest nitrogen fixation potential was observed in isolate $GSD_{15}$, which recorded a maximum value of 25.40 µg $ml^{-1}$, indicating its superior diazotrophic performance among the evaluated isolates. The observed variability in nitrogen fixation among the isolates may be attributed to differences in nitrogenase enzyme activity and genetic diversity within the *Gluconacetobacter* population. These findings agree with earlier reports that highlight strain-dependent variation in nitrogen-fixing efficiency among *Gluconacetobacter* spp., particularly those associated with plant rhizospheres and endophytic niches. Overall, the results confirm the ability of the tested *Gluconacetobacter* isolates to fix atmospheric nitrogen, with isolate $GSD_{15}$ emerging as the most efficient nitrogen fixer. This characteristic, combined with their favourable morphological and biochemical traits, suggests their potential application as plant growth-promoting bacteria, warranting further molecular and functional evaluation.

## Potassium solubilizing index of potassium mobilizing bacterial isolates.

The potassium solubilization efficiency of selected KMB isolates was further assessed by measuring the diameter of the zone of clearance (D), diameter of bacterial growth (d), and calculating the D/d ratio on potassium solubilization medium (Table 10). The D/d ratio is an important indicator of solubilization efficiency, as it reflects the ability of an isolate to solubilize insoluble potassium relative to its growth. Among the tested isolates, $KSA_{10}$ exhibited the highest solubilization efficiency, producing the maximum zone of clearance (34 mm) with a growth diameter of 9 mm, resulting in the highest D/d ratio of 3.77.

This was followed by isolate $KKA_6$, which showed a zone of clearance of 27.8 mm, growth diameter of 8.2 mm, and a D/d ratio of 3.39, indicating strong potassium solubilization potential. Moderate solubilization efficiency was observed in isolate $KRJ_5$, which recorded a zone of clearance of 19 mm and a growth diameter of 7.2 mm, with a corresponding D/d ratio of 2.64. Isolate $KRS_8$ showed the lowest solubilization efficiency among the tested isolates, with a zone of clearance of 20.5 mm, growth diameter of 8.5 mm, and a D/d ratio of 2.41. The variation observed in zone of clearance and D/d ratio among the isolates indicates strain-specific differences in potassium solubilization mechanisms, possibly related to differential production of organic acids and mineral dissolution capabilities. Overall, isolate $KSA_{10}$ demonstrated superior potassium solubilization efficiency, corroborating the quantitative potassium solubilization results and highlighting its potential as an efficient potassium-mobilizing bioinoculant.

## Quantitative Potassium solubilizing ability of KMB isolates

The potassium solubilization potential of selected KMB isolates was evaluated by estimating the amount of potassium released into the medium, expressed as µg $ml^{-1}$, and the results are presented in Table 10. The

isolates exhibited notable variation in potassium solubilization efficiency, indicating differences in their functional capabilities. Among the tested isolates, $KSA_{10}$ exhibited the highest potassium solubilization activity, recording a maximum value of 34 µg $ml^{-1}$. This was followed by isolate $KKA_6$, which solubilized 31.4 µg $ml^{-1}$ of potassium. Moderate potassium solubilization was observed in isolate $KRJ_5$ (28.1 µg $ml^{-1}$), whereas isolate $KRS_8$ recorded the lowest solubilization value of 26.5 µg $ml^{-1}$ among the tested isolates. The observed variation in potassium solubilization ability may be attributed to differences in organic acid production and mineral dissolution mechanisms among the isolates. The superior performance of isolate $KSA_{10}$ suggests its enhanced ability to mobilize insoluble potassium sources, making it a promising candidate for use as a potassium-mobilizing bioinoculant. Overall, the results confirm the potassium solubilization potential of the KMB isolates, with isolate $KSA_{10}$ emerging as the most efficient potassium solubilizer. When considered along with their favourable morphological and biochemical traits, these isolates show potential for application in sustainable nutrient management and biofertilizer development.

The present study confirmed the presence of *Gluconacetobacter* and potassium mobilizing bacteria (KMB) in the sugarcane rhizosphere and endosphere, indicating their role as plant growth–promoting microorganisms. The isolates showed uniform morphological and biochemical characteristics with minor variations, reflecting strain-level diversity. Cultural characterization revealed differences in colony texture and size, with $GSD_{15}$ and $KSA_{10}$ exhibiting better growth and mucoid nature, suggesting higher metabolic activity. Functional evaluation further indicated that $GSD_{15}$ recorded the highest nitrogen fixation, while $KSA_{10}$ showed superior potassium solubilization efficiency. These findings highlight the potential of these isolates as bioinoculants for sustainable nutrient management in sugarcane cultivation (Meena *et al.,* 2014).

In conclusion, the present study successfully isolated and characterized *Gluconacetobacter* and potassium mobilizing bacteria from the sugarcane rhizosphere and endosphere. The isolates exhibited typical morphological, cultural, and biochemical characteristics with minor variations, indicating strain-level diversity. Among the isolates, *Gluconacetobacter* isolate $GSD_{15}$ showed the highest nitrogen fixation potential, while KMB isolate $KSA_{10}$ demonstrated superior potassium solubilization efficiency. These findings highlight the potential of these efficient isolates as bioinoculants for sustainable sugarcane cultivation and improved nutrient management, thereby reducing dependence on chemical fertilizers.

## Author Contributions

M. D. Raut: Investigation, formal analysis, writing—original draft. A. M. Nawale: Validation, methodology, writing—reviewing. R. T. Gaikwad:—Formal analysis, writing—review and editing. P. H. Gaikwad: Investigation, writing—reviewing.

## Data Availability

The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

## Declarations

**Ethical Approval** Not applicable.

**Consent to Participate** Not applicable.

**Consent to Publish** Not applicable.

**Conflict of Interest** The authors declare no competing interests.

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