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Efficiency of Antagonistic PGPR isolates of Bt and non Bt cotton rhizosphere from Telangana on seed germination and seedling vigor index

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Abstract: *Plant growth promoting rhizobacteria (PGPR) are beneficial bacteria that colonize plant roots and enhance plant growth by a wide variety of mechanisms. In the present study, a total of 76 bacterial strains isolated from the rhizosphere of Bt and Non Bt cotton (Gossypium hirsutum* L.*) plants were screened preliminarily for antifungal activity against Fusarium oxysporum f. sp. vasinifectum and 23 were selected. Study of seed germination and seedling vigour index under the influence of 23 antifungal selected isolates was performed. Further they were characterized on the basis of colony morphology, gram staining. These PGPR strains were observed to be effective in improving seed germination and vigour index. Thus, use of biological application of these free living plant growth promoting rhizobacteria (PGPR) may help to minimize the amounts of chemical fertilizers to be added, to improve plant growth, thereby decrease the production cost and environmental risks.*

Keywords: *PGPR, cotton, antifungal activity, seed germination, vigor Index.*

I. Introduction

Plant growth promoting rhizobacteria (PGPR) are a group of bacteria that actively colonize plant roots and increase plant growth and yield [1]. PGPR are free-living, soil-borne bacteria, isolated from the rhizosphere, which, when applied to seeds or crops, enhance the growth of the plant or reduce the damage from soil-borne plant pathogens [2]. Plant growth stimulation by PGPR involves diverse mechanisms not entirely elucidated. They may release key hormones for plant development such as indole acetic acid (IAA) [3], iron sequestration [4], solubilization of mineral phosphate [5] and enzymatic lowering of plant ethylene level [6]. Several studies have been carried out to determine the effect of PGPR on plant but very few have improved para-meters of seed germination well as plant growth. Nowadays, the plants inoculation with rhizobacteria PGPR is a major asset for biological agriculture. This environmental biotechnology is also receiving attention as a way to reduce chemical fertilizer doses without affecting crop yield. The first observations of PGPR effects on the seeds have been realized with Pseudomonas spp. isolated from roots. In California and Idaho, [7] obtained a statistically significant increase of potato (*Solanum tuberosum* L.) yield from 14% to 33% in 59 fields with seeds treated with *Pseudomonas fluorescens* and *Pseudomonas putida* suspension. It has been also shown that *Pseudomonas putida* and *Pseudomonas fluorescens* strains can increase root and shoot elongation in canola [8], wheat and potato [9] [10].

Cotton is the most important fiber crop and also oil crop cultivated worldwide. Cotton is a soft, staple fiber that grows in a form known as a boll around the seeds of the cotton plant. Cotton is used in textile industry as well as cotton paper and in book binding [11]. After ginning, cotton seed is used to produce cotton seed oil. India is the second largest producer of the cotton in the world after China. It is a cash crop

Thus the aim of this study was to find out the antagonistic PGPR strains and determine their effect for the improvement of seed germination and vigor index of cotton (Gossypium hirsutum)

II. Materials and Methods

2.1. Isolation of PGPR from cotton rhizosphere

The rhizosphere soil samples were collected from cotton growing fields of Telangana, India. These samples were used for isolating most predominant plant growth promoting rhizobacteria (PGPR) bacteria using serial dilution plate method [12] on nutrient agar (NA) and King's B (KB) medium. Bacterial cultures were maintained on the respective media slants media and stored at 4°C for further use.

2.2. Preliminary screening of the bacterial isolates

The bacterial isolates were assessed for their antifungal activity of against the cotton wilt pathogen *Fusarium oxysporum* f. sp. *vasinifectum* by dual culture plate technique [13] on potato dextrose agar (PDA) medium. The diameter of the zone of inhibition was measured after 3-5 days incubation at 28± 2^0 C. The percent inhibition was calculated using the formula:

% inhibition = (R - r) / R ×100

Where' r' is radial growth of the fungal colony opposite the bacterial colony and, R is the radial growth of the pathogen in control plate.

2.3. In vitro seed germination assay

The antagonistic bacterial strains were bioassayed for their ability to promote seed germination and growth of cotton seedlings using standard methods. The seeds were surface sterilized with 0.1% $HgCl_2$ for 3 min. followed by successive washing with sterile distilled water and 2 then the water was decanted. The seeds were then added to cultures grown in their respective medium for 48 hr., kept for 10 minutes in the culture and the medium was decanted. The seeds were kept on soft agar (1%) plates and incubated at 30^o C for 2-7 days. The seeds treated with sterilized medium alone were used as control. After 3 days, the shoot and root lengths were recorded [14]. Seed vigour index was calculated by using the formula given by Abdul-Baki and Anderson [15].

2.4. Morphological characterization of isolates

Morphological features of the chosen bacterial isolates were examined on their respective media.. After 3 days of incubation, different characteristics of the colonies such as colour, shape, elevation, surface, motility and gram staining etc. were recorded [16].

III. Results and Discussion

Rhizosphere soils of Gossypium hirsutum L. from Telangana, India were explored for PGPR and a total of 76 bacterial strains were isolated. Among these bacterial isolates 40 were isolated from Bt cotton and 36 being from non Bt cotton plants. Out of these 76 isolates 23 exhibited antifungal activity against *Fusarium oxysporum* f.sp. *vasinfectum*. Among antagonistic isolates OUR1, OUR3, OUR11, OUR12, OUR13, OUR17, OUR27, OUR36, OUR39, OUR40 were from Bt cotton, OUN3, OUN8, OUN14, OUN23, OUN25, OUN26, OUN29, OUN30, OUN31, OUN32 and OUN35 from non Bt cotton exhibited antifungal activity. Highest percentage of inhibition was observed by OUN26 (73%), next in OUR13 (67%) (Table 1).

On the basis of antagonistic property, 23 antifungal bacterial isolates were selected for seed germination assay. Compared to untreated control, PGPR treated were performed better and enhanced the plumule length of cotton. About 25% of the isolates showed 100% germination, 50% showed more than 50% and about 25% of the isolates showed less than 50% germination. Maximum % germination (100%) and vigour index (Bt-1927, Non Bt-2307) was recorded in isolate OUN26, followed by the isolates OUR13 (Bt-1895, Non Bt-2285) (Table 1).

The morphological characteristics of PGPR isolates widely varied (Table 2). Most of the isolates produced irregular and circular shaped and slightly raised colonies having smooth shiny and dry powdery surface with undulated and lobate margin. No pigmentation was observed on NA media. They differed in white colour but all were odourless. Microscopic observations were performed to investigate the characteristics of PGPR isolates such as shape, gram reaction and motility. All the isolates were positive for gram's reaction, rod shaped and showing motility.

The term biological control was first adopted into the field of plant pathology by Smith [17]. Baker and Cook [18] defined biological control as the reduction of inoculum or disease producing activity of a pathogen accomplished by or through one or more organisms other than man. The term biological control clearly implies control of diseases through some biological agent means antagonist, a living organisms which may be micro or macro organisms other than the diseased or damaged plant acting as host and the pathogen causing the disease. These biological agents that provide benefit to plant can be either symbiotic or free living in soil, but are found in abundance near the roots are termed as PGPR.

Plant growth promoting effects of PGPR strains in different crops were clearly demonstrated [1]. PGPR promote plant growth through more than one mechanisms, including production of growth stimulating hormones and suppression of plant pathogens. Bacterial inoculants are able to increase plant growth and germination rate, improve seedling emergence, responses to external stress factors and protect plants from disease [19]. This present investigation confirms the earlier works. It revealed that under in vitro conditions, seed treatment with PGPR strains improved seed germination, seedling vigor, seedling emergence and seedling stand over the control. Similar improvement of seed germination parameters by rhizobacteria has been reported in cotton [20].

In my present experiment 76 PGPR strains were isolated from cotton rhizosphere, 23 were selected based on their pathogen growth suppression ability, and further study was conducted to know their effect on seed germination, vigour index. OUR13 and OUN26 showed highest antagonism as well as significant increase in % germination as compared to untreated control. Similar enhancement of seed germination in cotton crop was also reported [21], however vigour index observed in other crops was much lower country to our findings.

Similar improvement of seed germination parameters by rhizobacteria has been reported in other cereals such as sorghum [22] and pearl millet [23] [24]. The improvement in seed germination by PGPR was also found in wheat and sunflower [25] [26]. In some cases achieved increases up to 100% greater than controls.

IV. Conclusion

The present experiment proved that the PGPR strains were effective in inhibiting the growth of the pathogenic disease, improving the growth promoting activities such as seed germination and vigour index. Thus this study confirms the promotory effect of PGPR on germination and the plants growth. The single inoculation of cotton seeds (*Gossypium hirsutum* L.) by the rhizobacteria improved in vitro germination. Among all the tested PGPR strains, the OUR13, OUN26 were most efficient in antagonistic and growth promoting activities. These results suggest the possibility to use these PGPR further as biologic fertilizer to increase the output of cotton.

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References

[1]. S.C. Wu., Z.H. Cao., Z.G. Li., K.C. Cheung and M.H.Wong, Effects of biofertilizer containing N-fixer, P and K solubilizers and AM fungi on maize growth: a greenhouse trial, Geoderma., 125, 2005, 155– 166.
[2]. J.W. Kloepper, and M.N. Schroth, Relationship of in vitro antibiosis of plant growth - promoting rhizobacteria to plant growth and the displacement of root microflora, Phytopathol. 71, 1981, 1020-1024.
[3]. A. Khalid, M. Arshad, and Z.A. Zahir, Screening plant grgrowth_promoting rhizobacteria for Improving growth and yield of wheat, Journal Applied Microbiology, 96, 2004, 473-480.
[4]. J.W. Kloepper, Plant growth promoting Rhizobacteria as biological control agents. In Meeting, F.B. Jr. Soil Microbial Ecology. Applications in Agricultural and Environmental Management. Marcel Dekker, New York, 1993.
[5]. J.R. De Freitas, M.R. Banerjee, and J.J. Germida, Phosphate solubilizing rhizobacteria enhance the growth and yield but not phosphorus uptake of canola (Brassica napus L.), Biology of Fertility Soils, 24, 1997, 358-364.
[6]. B.R. Glick, The enhancement of plant growth by free living bacteria. Canadian Journal of Microbiology, 41, 1995,109-117.
[7]. T.J. Burr, M.N. Schroth and T. Suslow, Increased Potato Yields by Treatment of Seed Pieces with Specific Strains of Pseudomonas fluorescens and P. putida, Phytopathology, 68, 1978, 1377 – 1383.
[8]. B.R. Glick, L. Changping, G. Sibdas and E. B. Dumbroff, Early Development of Canola Seedlings in the Presence of the Plant GrowthPromoting Rhizobacterium Pseudo- monas putida GR12-2, Soil Biology and Biochemistry, 29(8), 1997, 1233-1239.
[9]. M.I. Frommel, J. Nowak and G. Lazarovits, Treatment of Potato Tubers with a Growth Promoting Pseudomonas sp.: Plant Growth Responses and Bacterium Distribution in the Rhizosphere, Plant and Soil, 150(1), 1993,51-60.
[10]. J.R. De Freitas and J.J. Germida, Growth Promotion of Winter Wheat by Pseudomonads fluorescentunder Growth Chamber Conditions, Soil Biology and Biochemistry, 24(11), 1992,1127-1135.
[11]. B.K.Lavine , Chemonmetrics, Anal. Chem. 70, 1998, 209R-228R.
[12]. L.F. Johnson and E.A. Curl, Method for research on ecology of soil borne pathogens, Burgress Soc., 4, 1972, 97-102.
[13]. R. Rabindran, and P. Vidyasekaran, Development of a formulation of Pseudomonas fluorescens PfALR2 for management of rice sheath blight, Crop Protection, 15, 1996, 715-721.
[14]. S.T. Shende, R.G. Aple, and T. Singh, Influence of Azatobacter on germination of rice and cotton seeds, Curr. Sci. 46, 1977, 675 - 676.
[15]. A.A. Abdul-Baki and J.D. Anderson, Vigour determination in soyabeen seed by multiple criteria, Crop Sc., 13(6), 1973, 630-633.
[16]. M. Ashrafuzzaman, F.A. Hossen, M.A. Ismail Hoque, M.Z. Islam, S.M. Shahidullah and S. Meon, Efficiency of Plant GrowthPromoting Rhizobacteria (PGPR) for the enhancement of rice growth, Afr. J. Biotech., 8(7), 2009, 1247-1252.
[17]. H.S.Smith, On some phases of insect control by the biological method, J. Econ. Entamol. 12, 1999, 288 - 292.
[18]. K. F. Baker, and Cook, R. J., Biological control of plant pathogen. San. Fco., Calif., Freeman. 1974, 433.
[19]. B. Lugtenberg, T. Chin-A-Woeng, and G. Bloemberg, Microbe–plant interactions: principles and mechanisms Antonie van Leeuwenhoek, 81, 2002, 373–383.
[20]. Baljeet Singh Saharan and Vibha Nehra, Assessment of plant growth promoting Attributes of cotton (Gossypium hirsutum) Rhizosphere isolates and their Potential as bio-inoculants, Journal of Environmental Research And Development, 5(3), 2011, 575-583.
[21]. M.A. Anjum, M.R. Sajjad, N. Akhtar, M.A. Qureshi, A. Iqbal, A.R. Jami and M. Hasan, Response of cotton to Plant Growth Promoting Rhizobacteria (PGPR) inoculation under different levels of nitrogen, J. Agri. Res., 45(2), , 2007,135-143
[22]. N.S. Raju., S. R. Niranjana., G. R. Janardhana., H. S. Prakash., H. S.Shetty and S.B. Mathur, Improvement of seed quality and field emergence of Fusarium moniliforme infected sorghum seeds using biological agents, J. Sci. Food. Agric., 79, 1999, 206.212.
[23]. S.R. Niranjan., N.P. Shetty and H.S. Shetty, Seed bio-priming with Pseudomonas fluorescens isolates enhances growth of pearl millet plants and induces resistance against downy mildew, J.pest.manage., 50(1), 2004, 41-48. 2004
[24]. S.R. Niranjan., S.A. Deepak., P. Basavaraju., H.S. Shetty., M.S. Reddy and J.W. Kloepper, Comparative performance of formulations of plant growth promoting rhizobacteria in growth promotion and suppression of downy mildew in pearl millet, Crop Protection., 22, 2003, 579– 588.

[25]. K. Shaukat., S. Affrasayab and S. Hasnain, Growth responses of Triticum aestivum to plant growth promoting rhizobacteria used as a biofertilizer. Res. J. Microbiol., 1(4), 2006b, 330-338.
[26]. K. Shaukat., S. Affrasayab and S. Hasnain, Growth responses of Helianthus annus to plant growth promoting rhizobacteria used as a biofertilizer, J.Agri.Res., 1(6), 2006a, 573-581.

Table1: Percent inhibition by PGPR and their effect in seed germination and vigor index.

S.No	Isolate	Antifungal activity zone of inhibition (%)	Seedling length (Shoot +Root)		% of seed germination		Vigour Index	
		Fusarium oxysporum f. sp. vasinfectum	Bt	Non Bt	Bt	Non Bt	Bt	Non Bt
1.	OUR1	52	7.51	8.52	28	25	751	852
2.	OUR3	50	9.79	9.53	35	34	979	953
3.	OUR11	54	10.54	9.56	45	48	1054	956
4.	**OUR12**	**57**	**12.38**	**12.71**	**100**	**100**	**1238**	**1271**
5.	**OUR13**	**67**	**18.95**	**22.85**	**100**	**100**	**1895**	**2285**
6.	OUR17	66	11.52	12.25	95	90	1152	1225
7.	**OUR27**	**57**	**16.56**	**16.27**	**100**	**100**	**1656**	**1627**
8.	OUR32	55	9.85	10.92	55	58	985	1092
9.	OUR36	64	10.25	10.53	85	82	1025	1053
10.	OUR39	59	10.65	11.08	90	92	1065	1108
11.	OUR40	64	9.68	8.83	46	35	968	883
12.	OUN2	52	7.98	8.12	28	30	798	812
13.	**OUN3**	**54**	**16.09**	**18.82**	**100**	**100**	**1609**	**1882**
14.	**OUN8**	**59**	**15.85**	**21.35**	**100**	**100**	**1585**	**2135**
15.	OUN14	47	9.85	8.25	49	45	985	825
16.	OUN23	57	10.48	9.54	65	70	1048	954
17.	OUN25	53	8.56	9.35	78	80	856	935
18.	**OUN26**	**73**	**19.27**	**23.07**	**100**	**100**	**1927**	**2307**
19.	OUN29	57	11.58	10.65	88	85	1158	1065
20.	OUN30	52	8.40	9.54	68	72	840	954
21.	OUN31	59	10.25	10.65	55	52	1025	1165
22.	OUN32	66	10.85	11.65	74	68	1285	1165
23.	OUN35	59	10.52	11.58	75	73	1052	1158

Table 2: Morphological and microscopic characters of Antagonistic PGPR isolates.

Isolate	**Colony colour**	**Form**	**Margins**	**Elevation**	**Surface**	**Pigmentation**	**Gram's reaction**	**Shape**	**Motility**
OUR1	white	Irregular	Lobate	Flat	Rough	None	+ve	Rods	Motile
OUR3	white	Circular	Entire	Flat	Smooth shiny	None	+ve	Rods	Motile
OUR11	white	Circular	Undulate	Slightly Raised	Dry powdery	None	+ve	Rods	Motile
OUR12	white	Circular	Undulate	Flat	Dry powdery	None	+ve	Rods	Motile
OUR13	white	Circular	Entire	Slightly Raised	Smooth, shiny	None	+ve	Rods	Motile
OUR17	white	Irregular	Undulate	Slightly Raised	Rough	None	+ve	Rods	Motile
OUR27	white	Irregular	Lobate	Slightly Raised	Smooth	None	+ve	Rods	Motile
OUR32	white	Circular	Undulate	Flat	Smooth	None	+ve	Rods	Motile
OUR36	white	Irregular	Entire	Flat	Rough	None	+ve	Rods	Motile
OUR39	white	Circular	Undulate	Slightly Raised	Dry	None	+ve	Rods	Motile
OUR40	white	Circular	Entire	Flat	Rough	None	+ve	Rods	Motile
OUN2	white	Irregular	Lobate	Flat	Smooth	None	+ve	Rods	Motile
OUN3	white	Irregular	Undulate	Flat	Smooth	None	+ve	Rods	Motile
OUN8	white	Irregular	Undulate	Flat	Smooth	None	+ve	Rods	Motile
OUN14	white	Circular	Entire	Flat	Rough	None	+ve	Rods	Motile
OUN23	white	Irregular	Undulate	Slightly Raised	Smooth	None	+ve	Rods	Motile
OUN25	white	Circular	Undulate	Flat	Smooth	None	+ve	Rods	Motile
OUN26	white	Irregular	Undulatee	Flat	Dry powdery	None	+ve	Rods	Motile
OUN29	white	Irregular	Lobate	Flat	Rough	None	+ve	Rods	Motile
OUN30	white	Circular	Entire	Slightly	Dry	None	+ve	Rods	Motile

				Raised					
OUN31	white	Circular	Undulate	Flat	Smooth	None	+ve	Rods	Motile
OUN32	white	Irregular	Lobate	Flat	Smooth	None	+ve	Rods	Motile
OUN35	white	Circular	Entire	Flat	Smooth	None	+ve	Rods	Motile

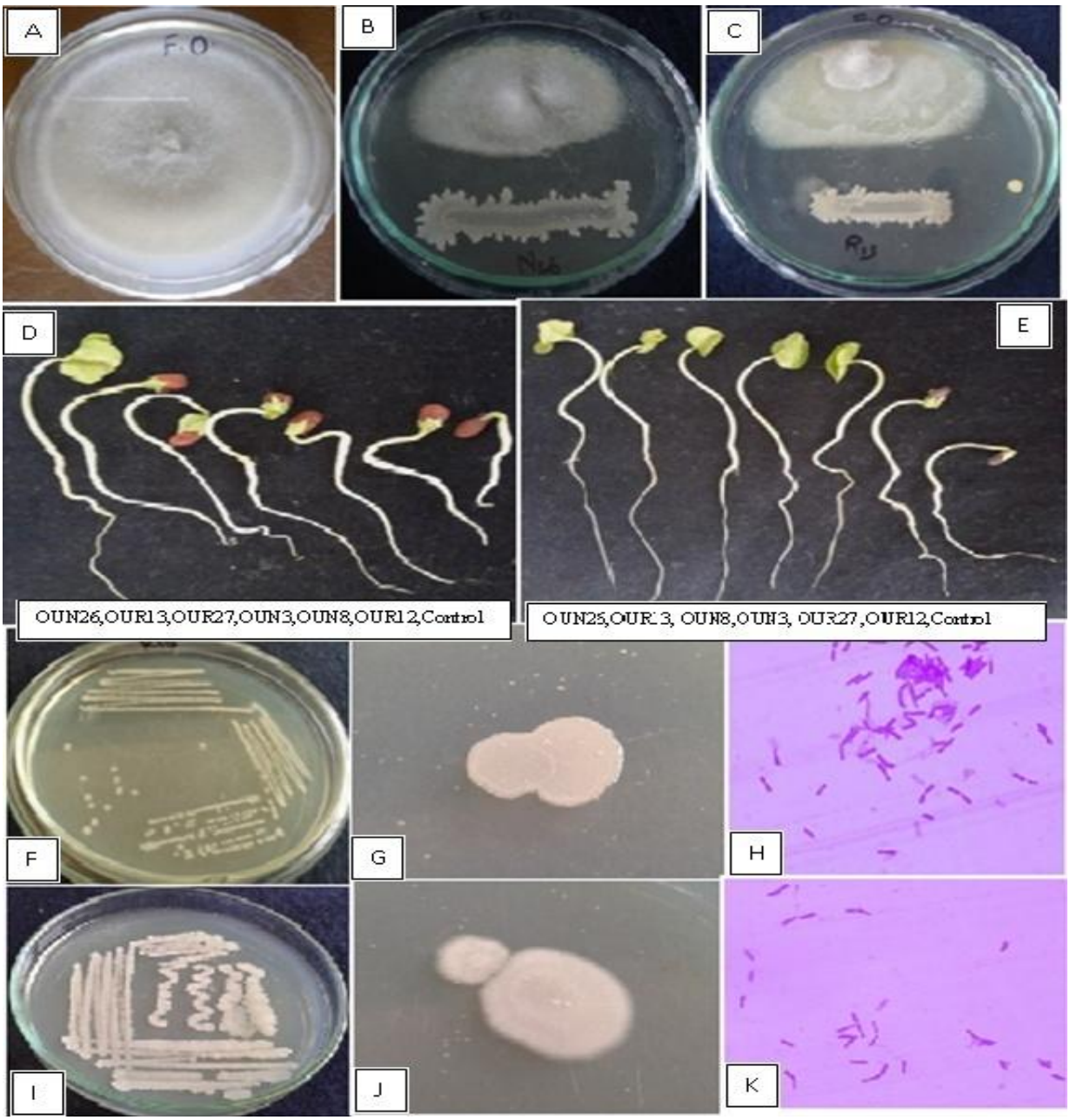

Fig.1 A- *F. oxysporum* f.sp. *vasinfectum* (*F. o*) (control), B- OUN26 and C- OUR13 against *F. o*.; Seed germination ability D- Bt cotton, E. Non Bt cotton; Colony Morphology, Streak plate- F- OUR13, I- OUN26: Individual colony- G- OUR13, J- OUN26; Gram staining, H. OUR13, K. OUN26.