

## Isolation and Characterization of Mesophilic Bacteria from Rhizosphere of Plant Rice (*Oryza sativa*) from Lodhran, Pakistan

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The present study demonstrated isolation and characterization of 48 bacterial strains (ABOs01-ABOs48) from rhizosphere of rice plant (*Oryza sativa*) of Rind Jada (Kahrora Pacca), Punjab, Pakistan. Morphological studies including colony color, bacterial shape and gram staining were performed and colonies were observed to be either orange yellow, light yellow, pink, greenish yellow, white, or off-white in appearance. Gram staining showed that out of 48 isolates, 38 were gram positive and 10 were gram negative. Various Biochemical tests were performed to identify these strains; the results were used to identify these strains at the species levels. These strains belong to the following species *Erwinia stewartii* (13), *Klebsiella terrigena* (9), *Klebsiella pneumoniae* susp. *Ozaene* (8), *Serratia plymuthica* (6), *Yersinia* (5), *Escherichia blattae* (5), 1 *Edwardsiella ictaluri* (1), and *Obesumbacterium proteus* (1). Additionally, amylase test showed that 39 strains were positive while 9 were negative. Conversely, all strains were negative for cellulase production. Finally, Antibiotics resistance showed 23 isolates were sensitive vs 25 resistant to ampicillin and 4 isolates were resistant vs 44 sensitive to penicillin. These findings suggested a great microbial diversity in rice plant rhizosphere which demands more investigations for agricultural and industrial purposes.

**Keywords:** Biochemical; Characterization; Morphological; *Oryza sativa*; Rhizospheric Bacteria.

Rice (*Oryza sativa*) is a major cash crop that has been cultivated for more than 7000 years and represents the staple food in different parts of the world (Lakshmanan *et al.* 2015). Rice is known to be grown in watery environments. The upper surface of paddy soil density is considered very important for good crop production (Kazemi *et al.* 2010).

Rhizosphere is the zone of soil in close proximity to the surface of plant root (Morgan *et*

*al.* 2005). The rhizosphere is colonized by huge and diverse microbial community where the microbial cell density may exceed cell density of the plants itself. The plant-associated microorganisms are known as plant microbiome. The rhizospheric microbiome may harbor viruses, bacteria, fungi, protozoa, and nematodes, thus making the rhizosphere a very dynamic environment (Barea *et al.* 2005). The plant secretes a number of compounds collectively known as exudates

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through its roots to the rhizosphere which may include sugars, organic acids, vitamins, hormones, enzymes, and secondary metabolites (Berendsen *et al.* 2012; Huang *et al.* 2014). The microbiome, on the other hand, influence seed germination, seedling vigor, nutrients uptake, plant growth and development, and plant health and resistance to diseases (de Zelicourt *et al.* 2013; Osorio Vega 2007).

Mesophilic bacteria grow at moderate temperature i.e., 25-40°C with optimal growth occurs at 37°C (normal body temperature) and most of the pathogenic bacteria belong to this group. Representative mesophilic bacteria include *Streptococcus pneumoniae*, *Streptococcus pyroenes*, *Staphylococcus aureus*, *Escherichia coli*, etc. Mesophilic bacteria are used in various biotechnological applications such as production of vinegar, yogurt, bread, cheese, amino acids, lactic acid and antibiotics production (streptomycin, erythromycin, chloromycetin, terramycin, tetracycline) (Shi and Zhu 2009), biodegradation of the environmental pollutants (Salt *et al.* 1998), and production of industrially and medically important enzymes such as amylase, cellulase, lipase, xylanase, arginase, L-asparaginase (Turner *et al.* 2007). Rhizospheric bacteria also have a wide range of applications in agriculture, and ecology. For instance, Plant Growth Promoting Rhizobacteria (PGPR) is used in agriculture to improve plant health and productivity or as pesticides and fertilizers (Josic and Kovac 2008). In this context, rhizospheric soil of rice plant may host diverse microbial community with potential industrial and agricultural applications (Muangham *et al.* 2019).

The present work aimed at isolation and characterization of rhizospheric bacteria from rice plant of Rind Jada village, Lodhran, Pakistan. These bacterial strains may be explored further for various applications such as fermentation, baking process and agriculture production.

## MATERIAL AND METHODS

### Isolation of Mesophilic Bacteria

Samples of *Oryza sativa* with attached soil was pulled out from the paddy rice field in the month of November, temperature 37°C of Rind Jada village, Kahrora Pacca, Lodhran, Pakistan,

and transferred to the Biochemistry laboratory of the Islamia University of Bahawalpur, in sterilized plastic jars. Plant roots were cut into 1-2 cm small pieces and submerged in sterile saline solution (0.9% NaCl). This suspension was serially diluted from 10<sup>-1</sup> to 10<sup>-5</sup> to obtain reasonably well separated colonies on petri plates. A volume of 50 µl of this dilution was spread on sterile nutrient agar medium under aseptic conditions and incubated overnight at 37°C for 24 h (Kaur *et al.*, 2012). Next, well separated colonies were picked into test tubes containing sterilized nutrient broth medium and cultivated on shaking incubator at 37°C for 20-24 h. These cultures were centrifuged and suspended in 50% LB-glycerol and stored culture stock at -70°C (Cappuccino and Sherman 1999).

### Characterization of Isolated Strains

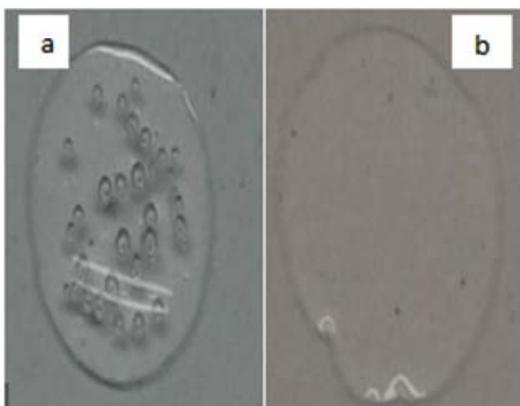
The isolated bacterial strains designated as ABOs were characterized morphologically for colony color, shape, and Gram-staining. Additionally the same strains were subjected to biochemical tests including indole production (IND), lactose fermentation (LAC), methyl red (MR), Vogues Proskauer (VP), citrate production (CIT), hydrogen sulphide (H<sub>2</sub>S), phenylalanine deaminase (PDA), ornithine decarboxylase (ODC), motility (MT), catalase (CAT), and lysine decarboxylase (LDC). The resistance to some common antibiotics (ampicillin and penicillin) was also studied. Furthermore, productions of some industrial enzymes such as amylase and cellulase were also performed on CMC and starch nutrient agar media, respectively, following standard microbiological protocol (Kaur *et al.* 2012; Kim *et al.* 2005; Xuan *et al.* 2016)

## RESULTS

A total of 48 bacterial strains designated as ABOs01-ABOs48 were isolated and characterized for both morphological and biochemical properties. For morphological characterization, different colonies showed different color (Table 1). Also, isolated bacterial strains have rod shape with 38 strains exhibited a gram-positive staining whereas 10 strains were gram negative (Table. 1). For biochemical characterization, the results obtained were documented as either positive (+), negative (-), vary positively (v) or uncertain (u) and

submitted to <https://instr.bact.wisc.edu/inst/index.php> to predict the species (Table 2).

Additionally, the catalase test showed 35 strains as catalase-producers and 13 strains showed no catalase activity as indicated by the presence or absence of bubble, respectively (Fig. 1). In starch containing nutrient agar media, 39 strains were amylase producer and 9 strains showed no amylase



**Fig. 1.** Catalase test: (a) bubbles were formed due to production of oxygen by catalase, (b) no bubbles were formed because of absence of catalase activity

activity (Fig. 2). Conversely, none of the isolated strains were positive for cellulase production on CMC-agar nutrient media (data not shown). Finally, a total of 23 strains were sensitive and 25 resistant to ampicillin (Fig. 4a-c) and 44 strains resistant vs 4 strains sensitive to penicillin (Fig. 5a-c).

## DISCUSSION

*Oryza sativa* is a cereal food crop which belongs to family Poaceae of the plant kingdom. This crop can be more easily grown in tropics associated with humid climate (Yu *et al.* 2002). The rhizosphere or the zone of influence around roots contains many microorganisms which affect both plant health and disease status (Loon *et al.* 1998). The present study was conducted to isolate and identify bacteria from rice paddy field to have an idea about the microbial diversity of rice plant rhizosphere. These strains exhibited diverse morphological and biochemical properties (Table 2) indicating that they belongs to the following species *Yersinia rohdei*, *Klebsiella terrigena*, *Serratia plymuthica*, *Escherichia blattae*, *Edwardsiella ictaluri*, *Erwinia stewarti*,

**Table 1.** Summary of Morphological characterization of ABOs bacterial strains

Gram staining		Shape			Colony color				
Positive	Negative	Rod like	Orange yellow	pink	Light yellow	Greenish-yellow	white	Off-white	
38	10	48	10	5	10	5	10	8	

**Table 2.** Identification of isolated bacterial strains based on Biochemical characterization

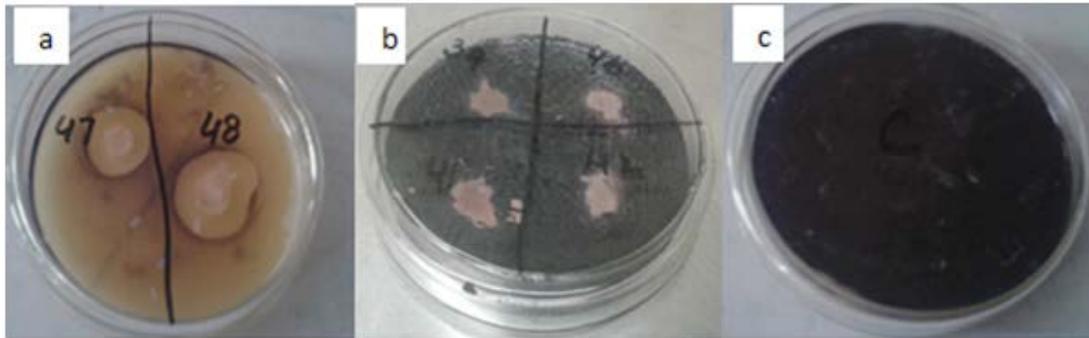
Predicted species	Biochemical test										No. of strains
	MT	IND	CIT	MR	VP	LAC	H <sub>2</sub> S	LDC	PDA	ODC	
<i>Yersinia rohdei</i>	-	-	-	v	-	-	-	-	-	-	2
<i>Klebsiella terrigena</i>	-	-	v	v	+	+	-	+	-	-	9
<i>Serratia plymuthica</i>	v	-	v	+	+	+	-	-	-	-	6
<i>Klebsiella pneumoniae susp.</i>	-	-	v	+	-	v	-	v	-	-	8
<i>Ozaena</i>											
<i>Escherichia blattae</i>	-	-	v	+	u	-	-	+	-	+	5
<i>Edwardsiella ictaluri</i>	-	-	-	-	-	-	-	+	-	v	1
<i>Erwinia stewarti</i>	-	-	u	u	u	+	-	-	-	-	13
<i>Yersinia intermedia</i>	-	+	-	+	-	v	-	-	-	+	1
<i>Obesumbacterium proteus</i>	-	-	-	-	-	-	-	+	-	+	1
<i>Yersinia enterocolitica</i>	-	v	-	+	-	-	-	-	-	+	1
<i>Yersinia ruckeri</i>	-	-	-	+	-	-	-	v	-	+	1

(+) positive, (-) negative, (v) vary positively and (u) uncertain

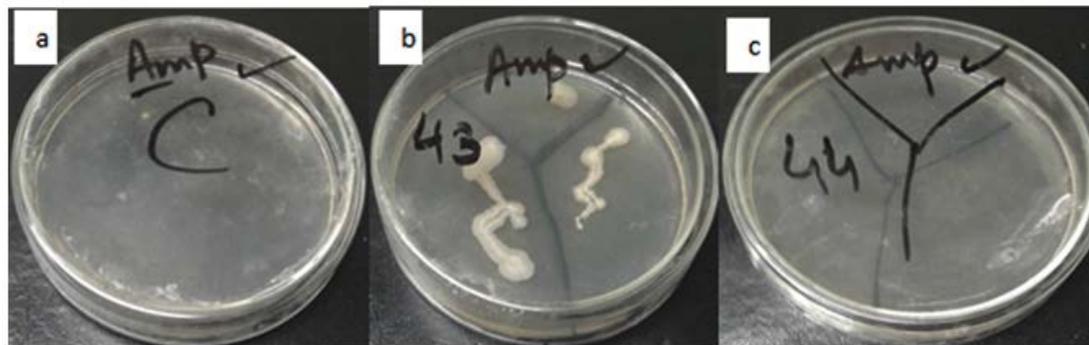
*Yersinia intermedia*, *Obesumbacterium proteus*, *Yersinia ruckeri*, and *Klebsiella pneumoniae* *ssp. Ozaene*. Different studies have isolated different bacterial species from rice plants (Adnan *et al.* 2016); e.g., *Herbaspirillum*, *Geobacter* and *Anaeromyxobacter* (Breidenbach *et al.* 2016), or *Pseudomonas*, *Sphingomonas*, *Ancylobacter*, *Enterobacter*, *Advenella*, and *Proteobacterium* (Shahi *et al.* 2011). The rice plant of paddy field of

Rind Jada village used in our study seems to harbor a unique microbial community and this deserves further investigations to elucidate their interaction with plant rhizosphere and to explore their potential for plant growth promotion.

Additionally, it is interesting that majority of these strains grown on starch containing agar media and showed a zone of starch hydrolysis activity upon staining with iodine tests indicating



**Fig. 2.** Amylase test. (a) amylase producer, (b) amylase non-producer and (c) negative control



**Fig. 3.** Ampicillin resistance: (a) bacteria and antibiotic free media free, (b) Ampicillin resistance, and (c) Ampicillin sensitive



**Fig. 4.** Penicillin resistance: (a) bacteria and antibiotic free media free, (b) Penicillin resistance, and (c) Penicillin sensitive

their ability to produce amylase enzyme. Amylases have wide applications in various industries such as starch hydrolysis, textile industry, laundry and detergent industry, syrup production (Saini *et al.* 2016; Souza and Magalhães 2010). However, none of isolated strains showed a cellulase activity by hydrolyzing CMC nutrient agar media. The absence of cellulase enzyme may suggest that these strains rely on the sugar release by the plant through roots to rhizosphere; however, this speculation needs further investigations. Cellulase enzymes are crucially important enzymes used in tremendous applications such as biofuel production, paper and pulp industry, detergent industry, and beverage industry (Ahmed *et al.* 2017; Ahmed *et al.* 2018).

### CONCLUSION

The present study demonstrated that *Oryza sativa* is naturally associated with a variety of rhizospheric microbes having different physiological, morphological, and biochemical characteristics. Further studies are demanded to identify these bacteria at molecular level. Additionally, these bacteria may be of importance for the rice plant physiology and resistance to diseases and require further investigations to be carried out to understand the association between microbes and plants; the findings of which might be employed for agricultural development. On the other hand, these microbes may be studied for production of some industrially importance enzymes such as amylase, lipase, L-asparaginase, antibiotics and secondary metabolites production which can be brought into pharmaceutical markets.

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