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MICROBIAL DIVERSITY OF THE RHIZOSPHERE OF KOCHIA (*KOCHIA INDICA*) GROWING UNDER SALINE CONDITIONS

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Abstract

Study was conducted to find out the microbial diversity of the rhizosphere and rhizoplane of *Kochia indica* (halophyte plant). Strains were isolated on four different media viz., LB, AP, NFM and HaP. Maximum diversity was observed in rhizosphere as compare to rhizoplane. Total 14 types of genera were found to reside in rhizosphere and rhizoplane while Predominent genera found were *Klebisella* and *Vibrio*. All the isolates were morphologically characterized and screened in vitro for their plant growth promoting traits like production of indoleacetic acid (IAA) and phosphate solubilization. From the rhizosphere 65% strains and from the rhizoplane 62% strains were able to solubilize inorganic phosphate. From the rhizosphere, 35% isolates and from the rhizoplane 74% isolates showed IAA production. One of the isolates from rhizoplane (NFP 9) showed maximum production (35 µM/ml) of IAA. Bacterial isolates were also screened out for the presence of fluorescent Pseudomonads and only two strains exhibited fluorescence under the UV light.

Introduction

Most of the areas of Pakistan are arid to semiarid with low annual precipitation, and 6.3 million hectares land is affected to varying degree of salinity (Ashraf *et al.*, 2006). Because of this salinity problem, we are facing scarcity in crop yields and plant productivity. The world population is increasing rapidly, and there is a great demand of food supply but due to the shortage of water (Abbas, 2006) and increasing salinization of agricultural lands, the food supply is at alarming level. Moreover, farmers are not utilizing these lands for cultivation and ultimately turning these lands into wastelands. According to Yensen (2008), fresh water resources are diminishing with the passage of time. It results in the expansion of saline areas which are suitable for halophyte crops. Therefore, it is expected that 21st century will likely be the century of halophyte agriculture expansion. It is necessary to utilize these lands by growing halophytes (salt tolerant plants) on salt affected wastelands, which may bring socio-economic gains.

Halophytes are salt tolerant plants that can grow easily in saline soil (Ungar, 1991). There is a great diversity of halophytes in Pakistan. The Chenopodiaceae family includes the maximum number of halophytes. In Pakistan 19% of the flora consist of halophytes. Balochistan plains have the highest diversity of halophytic species (182) while deserts have less diversity of halophytic species (65). Many other halophytes are used in the production of vegetable oil. Many desert animals depend upon halophytes as their food (Ajmal & Qaiser, 2006; Qasim *et al*., 2010).

There is a great diversity of microorganisms in the rhizosphere because of root exudates provide nutrients and carbon sources to microorganisms, and make the rhizosphere an attractive ecological niche. In the rhizosphere, there is an interaction between the root and microbes, and interaction among the microorganisms. These microorganisms can have a neutral, pathogenic or beneficial interaction with their host plant (Hartmann *et al.,* 2009; Jha *et al*., 2011; Miransari, 2011; Khan *et al*., 2012).

The microorganisms residing in the rhizosphere are very beneficial for the growth of the plants and are called plant growth promoting rhizobacteria (PGPR). They are also responsible for managing soil as well as plant health (Han *et al.,* 2009). These PGPR transform organic substrates, release mineral elements, and hence strongly influence plant growth. These PGPR can also be used as bioinoculants for other crops in order to enhance their yield in saline soil. This leads to expansion in saline agriculture.

The aim of current study was to find the microbial diversity of the rhizosphere and rhizoplane of *Kochia indica* (halophyte plant) and screening of halophilic and alkaliphilic strains for PGP abilities.

Material and Methods

Sampling of soil: Soil samples were collected from plant of Kochia *(Kochia indica),* growing under controlled conditions at the Biosaline Research Station (BSRS) of Nuclear Institute for Agriculture and Biology (NIAB), Faisalabad. The rhizosphere soil profile was EC1:1 (dSm-1) 2.14 +0.29-4.07+0.84, pH 8.76+ 0.21- 8.59+0.05, depth (cm) 0-30-30-60 and SAR value 35.9+9.0- 45.6+8.0.

Isolation and characterization of bacteria from *Kochia indica*: For the isolation of rhizospheric bacteria*,* 1g of rhizospheric soil was mixed with 9ml of saline and incubated in shaker for about half an hour. Bacterial fraction from rhizoplane was isolated by shifting 0.6 gram of washed root to a falcon tube containing 9ml saline along with some pebbles and incubated in a shaker for 30 minutes (Bilal & Malik, 1987). Serial dilutions (10^{-1}-10^{-10}) were the method described by Somasegaran & Hoben (1994). About 100µl sample from 10^{-1} to 10^{-8} dilutions were plated onto LB, Alkaliphilic medium (AP) and Halophilic medium (HaP) agar plates. Same volume of sample from 10^{-1} to 10^{-10} dilutions was inoculated into NFM semi-solid vials. Plates were incubated at 28°C until the appearance of visible bacterial colonies. The bacterial colonies were counted and number of cells per gram of soil was calculated (Somasegaran & Hoben, 1994). The formation of pellicles was observed in the vials. The pellicles were streaked on NFM solid media plates. Purification of the bacteria was done by repeated sub culturing of single colonies. CFU and MPN were calculated. Bacterial colonies were characterized on the basis of morphological characters (form, color, surface, texture, elevation, and margins).

Plant growth-promoting traits: To check the PGP traits, the isolated strains from *Kochia indica* were analysed for their ability to solubilize phosphate, and produce indole acetic acid (IAA).

Qualitative and quantitative IAA production: Production of IAA by bacterial isolates from the rhizosphere and rhizoplane of *Kochia indica* was assayed as described by Patten & Glick (1996). Bacterial cultures were grown on their respective media with 100 and 200 µg/ml of L-tryptophan at 28°C for 48h. Bacterial cells were removed by centrifugation at 4,000 rpm for 20 min at 4ºC. About1ml of the supernatant was mixed with 4ml of Salkowski's reagent in the ratio of 1:4 and incubated at room temperature for 20 min. For the quantification of IAA, absorbance of supernatant mixture (supernatant + Salkowski's reagent) was measured for indole production at 535nm. The quantity of IAA was determined comparing with a standard curve using an IAA standard graph.

Phosphate solubilization: Phosphate solubilizing ability of the bacterial strains was checked as described by Pikovskaya (1948) on Pikovskaya's agar containing tricalcium phosphate as insoluble P source. The plates were incubated for one week at 28ºC and observed for the formation of transparent halo zone of solubilized phosphate around the inoculated surface of bacteria.

Isolation of fluorescent pseudomonads: The bacterial isolates from the rhizosphere and rhizoplane of *Kochia indica* were checked for fluorescence. For this purpose they were streaked on King's B medium (King *et al*., 1954). The plates were incubated at 28°C for 48 hours. They were observed under UV light transilluminator to check the fluorescence.

Biochemical characterization of bacterial strains isolated from the rhizosphere and rhizoplane of *Kochia indica*: Physiological and biochemical tests of bacterial isolates were performed using QTS-24 miniaturized identification system (DESTO Laboratories Karachi, Pakistan). For these tests 24h old bacterial cultures were used and then checked the results after 18h of incubation at 30°C.

Results and Discussion

The present research work was conducted to evaluate the microbial diversity and to exploit the plant growth promoting rhizobacteria (PGPR) residing in the rhizosphere of *Kochia indica* growing under saline conditions. For this purpose, bacterial strains were isolated from the rhizosphere as well as from the rhizoplane on four different media (LB. AP, HaP and NFM media) in order to get the maximum bacterial diversity.

In case of rhizosphere the maximum number 19% of bacterial colonies on LB medium, 17% on AP medium while 16% on HaP medium was observed. The CFU value was highest on LB medium and the lowest on HaP medium. In case of rhizoplane**,** the maximum number 29% of bacterial colonies on LB medium, 12% on AP medium while 21 % on HaP medium was observed. The CFU value was highest on LB medium and the lowest on AP medium (Table 1). These findings are similar to the reports of Han *et al*., (2009) who found the maximum number of bacteria on LB medium. MPN (most probable number) was calculated for both rhizosphere as well as rhizoplane (Table 2). The nitrogen fixers in the rhizosphere of *Kochia indica* were more abundant (48%) as compare to rhizoplane (38%).

Table 1. Log number of CFU (colony forming unit) obtained from the rhizosphere and rhizoplane of *Kochia indica* on different growth media (LB, AP, and HaP).

Sample	Media used	CFU 10^4
Rhizospheric Soil	LB	1230
	AP	10
	HaP	7
	Media used	**CFU 10^3**
Rhizoplane	LB	20205
	AP	70
	HaP	1845

Table 2. MPN from the rhizosphere and rhizoplane of *Kochia indica*

Sample code	MPN 10^6
Rhizosphere	1.6
Sample code	**MPN 10^2**
Rhizoplane	408

Discrete bacterial colonies were morphologically characterized. Bacterial colonies were different in form, surface, texture elevation and colour. Majority were circular, glistening, mucoid, raised and offwhite. One of the isolates (NFP9) was dark pink. Majority of the isolates were rods while some were cocci, few were spirilla and pleomorphic. Majority of the isolates were motile and very few were non-motile (Tables 3-5). Biochemical test were conducted for PGPRs for their tentative identification. They showed variations in the result. Total 14 different types of genera were found having Klebsiella and vibrio as dominant genera. Biochemical characters of the isolates were tested using microbial identification kits QTS-24. Many isolates showed positive results for oxidase test, ONPG test while few were positive for urease activity and nitrogen reduction. Most of the isolates gave negative results for CIT, MALO, ADH, VP and TDA. Most of the bacterial strains had ability to utilize sugars like glucose, sucrose, mannitol, lactose, arabinose and sorbitol present in the medium but a few had ability to produce acid from maltose and inositol. Many isolates had ability to hydrolyze gelatin (Tables 6 & 7). Most of the isolates were facultative anaerobes having cytochrome oxidase test positive while few isolates gave negative results. This work of characterization is in agreement with the work reported by Kleeberger *et al.,* (1983) and Mirza *et al.,* (2001). Bacterial strains forming clear halo zones on Pikoviskaya's medium and producing phytohormones (IAA) were termed as PGPR. Phosphate solubilizing bacterial (PSB) strains may be used in the production of biofertilizer in areas where P availability is limited or P is fixed and is unavailable (Yasmin & Bano, 2011). In PGP properties, 65% strains from the rhizosphere and 62% strains from the rhizoplane were able to solubilize inorganic phosphate (Tables 3-5). Our findings were similar to the reports of Vazquez *et al.,* (2000) who isolated the phosphate solubilizing strains from the rhizosphere of 2 species of mangroves. Yasmeen *et al*., (2012) reported significant increase in biochemical and physiological characteristic of mung bean (Vigna radiata) inoculated with non-nodulating PSBs. From the

rhizosphere 35% isolates and 74% isolates from the rhizoplane produced IAA. Quantification of IAA was estimated for selected strains. Strain NFP9 showed maximum production (35µM/ml) of IAA followed by NFP3, NFP2, NFP1, NFP5, NFP4, NFP6 and NFP7 produced 27, 20.5, 19.2, 18, 17.8 and 17.5µM/ml IAA respectively (Tables 3-5). Some alkaliphilic strains also showed good production of IAA i.e., APP1 (25µM/ml), APP7 (22µM/ml) and APP8 (22.01µM/ml). The results are also justified through the work of Fischer *et al*., (2007) in terms of isolation of bacteria from the rhizosphere and characterized them on the basis of IAA production and other PGP traits. One of the nitrogen fixer (NFP9) showed different morphological characters than others. Its cells were pleomorphic i.e., vibrio to S-shaped and motile. Dark pink and wrinkled colony was observed on LB medium when it was streaked on coloured and colourless NFM retarded growth was observed. On basis of physiological, morphological and biochemical characters this strain was identified as *Azospirillum halopraeferans*. It was also characterized as a phosphate solubilizing bacteria as it produced the clear halo zones and gave maximum IAA production. The above mentioned results are in agreement with the work reported by Reinhold *et al*., (1987) according to which *A. halopraeferans* were associated with the roots of Kallar grass that also showed somewhat similar characteristics. Azospirilum spp., are reported to promote plant growth under normal and stress conditions (Ilyas *et al*., 2012). This work of evaluating PGPR is consistent with the work of Meuchang *et al.*, (2006) who isolated PGPR from paddy fields and checked their phosphate solubilizing activity and IAA production. Isolation of fluorescent *Pseudomonads* was also done by using King's B medium. Among all the isolates from the rhizosphere and rhizoplane of *Kochia indica*, only two strains RP7 and HaPP1 produce fluorescence under the UV light. While none of the rest of strains could produce fluorescence. This work is consistent with the work of Rangarajan *et al.,* (2002) who isolated the *Pseudomonas* from the rhizosphere of rice (*Oryza sativa*) growing under saline conditions.

Table 3. Cell morphology and PGP traits of nitrogen fixers isolated from the rhizosphere of *Kochia indica* on NFM medium

Strains	Origin	Cell Morphology		PGP traits	
		Shape	Motility	IAA production	Phosphate solubilization
NF1	Rhizo	Rods	+ve	-ve	+ve
NF2	Rhizo	Rods	+ve	-ve	+ve
NF3	Rhizo	Rods	+ve	-ve	+ve
NF4	Rhizo	Rods	+ve	+ve	+ve
NF5	Rhizo	Rods	+ve	+ve	+ve
NF6	Rhizo	Vibrio	-ve	-ve	-ve
NF7	Rhizo	Rods	+ve	-ve	+ve
NF8	Rhizo	Rods	+ve	+ve	+ve
NF9	Rhizo	Rods	+ve	-ve	-ve
NF10	Rhizo	Rods	+ve	-ve	-ve
NF11	Rhizo	Cocci	+ve	-ve	-ve
NF12	Rhizo	Rods	+ve	+ve	+ve
NF13	Rhizo	Rods	+ve	+ve	+ve
NF14	Rhizo	Rods	-ve	-ve	+ve
NF15	Rhizo	Cocci	+ve	-ve	+ve
NF16	Rhizo	Rods	-ve	-ve	+ve
NF17	Rhizo	Rods	-ve	-ve	+ve
NF18	Rhizo	Rods	+ve	-ve	+ve
NF19	Rhizo	Rods	+ve	-ve	+ve
NF20	Rhizo	Rods	-ve	-ve	+ve
NF21	Rhizo	Rods	+ve	-ve	+ve
NF22	Rhizo	Rods	-ve	-ve	+ve
NF23	Rhizo	Rods	+ve	-ve	+ve
NF24	Rhizo	Rods	+ve	-ve	+ve
NF25	Rhizo	Rods	+ve	-ve	+ve
NF26	Rhizo	Vibrio	+ve	-ve	+ve
NF27	Rhizo	Rods	+ve	-ve	+ve
NF28	Rhizo	vibrio	+ve	+ve	+ve
NF29	Rhizo	Rods	+ve	+ve	+ve
NF30	Rhizo	Rods	+ve	+ve	+ve
NF31	Rhizo	Rods	+ve	-ve	+ve
NF32	Rhizo	Rods	+ve	+ve	+ve
NF33	Rhizo	Rods	+ve	-ve	+ve
NF34	Rhizo	Rods	+ve	-ve	+ve
NF36	Rhizo	Cocci	+ve	-ve	+ve
NF37	Rhizo	Vibrio	+ve	+ve	+ve
NF38	Rhizo	Vibrio	+ve	-ve	+ve
NF39	Rhizo	Spiril	+ve	-ve	+ve

Table 4. Cell morphological characteristics and PGP traits of bacterial strains isolated from the rhizosphere and rhizoplane of *Kochia indica* on LB, AP and HaP media.

Strains	Origin	Cell Morphology		PGP traits	
		Shape	Motility	IAA production	Phosphate solubilization
RS1	Rhizo	Rods	+ve	-ve	-ve
RS2	Rhizo	Rods	+ve	-ve	-ve
RS3	Rhizo	Rods	+ve	-ve	+ve
RS4	Rhizo	Rods	+ve	-ve	-ve
RS5	Rhizo	Vibrio	-ve	-ve	-ve
RS6	Rhizo	Rods	+ve	-ve	-ve
RS7	Rhizo	Rods	+ve	+ve	+ve
RS8	Rhizo	Rods	+ve	-ve	-ve
RS9	Rhizo	Rods	+ve	-ve	-ve
RS10	Rhizo	Rods	+ve	-ve	-ve
RS11	Rhizo	Rods	+ve	-ve	-ve
RS12	Rhizo	Rods	+ve	-ve	+ve
RS13	Rhizo	Rods	+ve	-ve	-ve
RS14	Rhizo	Rods	+ve	+ve	-ve
RS15	Rhizo	Rods	+ve	-ve	+ve
RP1	Rhizop	Rods	+ve	+ve	-ve
RP2	Rhizop	ND	-ve	+ve	+ve
RP3	Rhizop	Rods	+ve	-ve	+ve
RP4	Rhizop	Rods	+ve	-ve	+ve
RP5	Rhizop	Rods	+ve	+ve	+ve
RP6	Rhizop	Ple	+ve	-ve	-ve
RP7	Rhizop	Rods	+ve	+ve	+ve
RP8	Rhizop	Rods	+ve	+ve	-ve
RP9	Rhizop	Rods	+ve	+ve	+ve
RP10	Rhizop	Rods	+ve	+ve	-ve
AP1	Rhizo	Rods	+ve	-ve	-ve
AP3	Rhizo	Rods	+ve	-ve	-ve
AP4	Rhizo	Rods	+ve	-ve	-ve
AP5	Rhizo	Rods	+ve	+ve	-ve
AP6	Rhizo	Rods	+ve	-ve	-ve
AP8	Rhizo	Cocci	+ve	+ve	+ve
AP9	Rhizo	Rods	+ve	-ve	-ve
AP10	Rhizo	Cocci	+ve	+ve	+ve
AP11	Rhizo	Rods	+ve	-ve	-ve
AP12	Rhizo	ND	ND	-ve	+ve
AP13	Rhizo	Rods	+ve	-ve	-ve
AP14	Rhizo	ND	ND	+ve	+ve
AP16	Rhizo	ND	ND	+ve	+ve
AP18	Rhizo	ND	ND	+ve	+ve
APP1	Rhizop	Rods	+ve	+ve	+ve
APP7	Rhizop	Rods	+ve	+ve	-ve
APP8	Rhizop	Rods	-ve	+ve	+ve
APP9	Rhizop	Cocci	+ve	-ve	+ve
HaP1	Rhizo	Cocci	+ve	-ve	-ve
HaP2	Rhizo	Rods	+ve	+ve	-ve
HaP3	Rhizo	Rods	+ve	-ve	-ve
HaP4	Rhizo	Rods	+ve	+ve	-ve
HaP5	Rhizo	Rods	+ve	+ve	-ve
HaP6	Rhizo	Rods	+ve	+ve	-ve
HaP7	Rhizo	Rods	-ve	+ve	+ve
HaP8	Rhizo	ND	ND	-ve	+ve
HaP9	Rhizo	Cocci	+ve	-ve	+ve
HaP10	Rhizo	Rods	+ve	-ve	+ve
HaP11	Rhizo	Rods	+ve	+ve	+ve
HaP12	Rhizo	Cocci	+ve	+ve	-ve
HaP14	Rhizo	Rods	+ve	+ve	-ve
HaPP1	Rhizop	Rods	+ve	+ve	-ve
HaPP2	Rhizop	Rods	+ve	+ve	+ve
HaPP3	Rhizop	Rods	+ve	-ve	-ve
HaPP4	Rhizop	Rods	+ve	-ve	+ve
HaPP5	Rhizop	Rods	+ve	-ve	+ve
HaPP6	Rhizop	Rods	+ve	+ve	+ve
HaPP7	Rhizop	Rods	+ve	+ve	+ve

Table 5. Cell morphological characteristics and PGP traits of bacterial strains isolated from the rhizoplane of *Kochia indica* on NFM medium.

Strains	Origin	Cell Morphology		PGP traits		
		Shape	Motility	IAA production	IAA production (µM/ml)	Phosphate solubilization
NFP1	Rhizop	Rods	+ve	+ve	20	-ve
NFP2	Rhizop	Rods	+ve	+ve	20.5	-ve
NFP3	Rhizop	Rods	+ve	+ve	27	-ve
NFP4	Rhizop	Rods	+ve	+ve	18	-ve
NFP5	Rhizop	Rods	+ve	+ve	19.2	-ve
NFP6	Rhizop	Rods	+ve	+ve	17.8	+ve
NFP7	Rhizop	Rods	+ve	+ve	17.5	+ve
NFP9	Rhizop	Ple	+ve	+ve	35	+ve
NFP11	Rhizop	Rods	+ve	-ve	-	-ve
NFP12	Rhizop	Rods	+ve	+ve	-	+ve
NFP14	Rhizop	Rods	+ve	-ve	-	+ve
NFP15	Rhizop	Rods	+ve	-ve	-	+ve
NFP16	Rhizop	Rods	+ve	+ve	-	+ve

Table 6. Biochemical characterization and tentative identification of selected bacterial strains isolated from the rhizosphere and rhizoplane of *Kochia indica.*

Sample	ONPG	CIT	MALO	LDC	ODC	ADH	UREA	H2S	TDA	CO	VP	GEL	NO_2/NO_3	Tentative identification
AP8	+	-	-	-	-	-	-	-	+	-	-	+	-/+	*Streptococcus* sp.
AP10	+	-	-	-	-	-	-	-	+	+	-	+	-/+	*Staphylococcus* sp.
AP12	+	-	-	-	-	-	-	-	-	-	-	+	-/+	*Moraxella* sp.
AP16	+	-	-	-	-	-	-	-	+	-	-	+	-/+	*Brucella* sp.
AP18	+	-	-	-	-	-	-	-	+	+	-	-	-/+	*Acinetobacter* sp.
HaP5	-	-	-	-	-	-	-	-	+	-	-	+	-/+	*Citrobacter diversus*
HaP7	+	-	-	-	-	-	+	-	+	+	+	+	-/+	*Vibrio orientalis*
HaP11	+	-	-	-	-	-	-	-	+	+	-	+	-/+	*Pseudomonas* sp.
NF32	-	+	+	-	+	-	-	-	+	+	+	+	-/+	*Klebsiella oxytoca*
NF36	+	-	+	-	-	-	-	-	+	-	-	-	-/+	*Staphylococcus aureus*
RP2	+	-	-	-	-	ND	-	ND	+	+	-	+	+/+	*Pseudomonas* sp.
RP7	+	+	-	-	-	ND	+	ND	+	-	-	-	+/+	*Klebsiella* sp.
RP9	+	-	+	-	-	ND	-	ND	-	+	-	-	+/-	*Vibrio* sp.
APP8	+	-	-	-	-	-	-	-	+	-	-	+	-/+	*Klebsiella oxytoca*
HaPP2	+	+	+	-	-	+	-	-	+	-	+	-	+/-	*Erwinia chrysanthemi*
HaPP6	+	-	-	-	-	+	-	-	+	-	-	+	-/-	*Vibrio fischeri*
HaPP7	+	-	-	-	-	-	-	-	+	-	+	-	+/+	*Citrobacter diversus*
NFP6	+	+	+	-	-	+	-	-	+	+	+	-	-/-	*Alcaligenes* sp.
NFP7	+	+	+	-	-	+	-	-	+	-	+	-	-/-	*Klebsiella* sp.
NFP9	+	+	-	+	+	-	-	-	+	+	+	+	-/-	*Azospirillum halopraeferans*
NFP12	+	+	+	+	-	+	-	-	+	+	+	+	-/-	*Chromobacter* sp.
NFP16	+	+	+	-	+	+	-	-	+	-	+	+	-/-	*Citrobacter* sp.

Symbols: ONPG, ortho-Nitrophenyl-β-galactoside ;CIT, Sodium Citrate; MALO, Sodium Malonate; LDC, Lysine Decarboxylase; ADH, Arginine Dihydrolase; ODC, Ornithine Decarboxylase; H_2S, H_2S production; URE, Urea; TDA,Tryptophane Deaminase; IND, Indole; VP, Voges-Proskauer test (Acetation); GEL, Gelation Hydrolysis; GLU, Acid from Glucose; NO_3, Nitrate reduction; CO, Cytochrome Oxidase.

Table 7. Carbon source utilization of selected bacterial strains isolated from the rhizosphere and rhizoplane of *Kochia indica.*

Sample	GLU	MALT	SUC	MANN	ARAB	RHAM	SORB	INOS	ADO	MEL	RAF
AP8	+	+	-	-	+	+	+	+	-	+	+
AP10	+	+	+	-	+	+	+	-	-	+	+
AP12	+	+	-	+	+	+	-	-	-	+	-
AP16	+	+	-	+	+	+	+	-	-	+	-
AP18	-	+	+	-	+	-	-	-	-	+	+
HaP5	+	+	-	+	+	+	+	-	-	+	+
HaP7	+	+	+	-	+	+	-	-	-	-	+
HaP11	-	+	+	+	+	+	-	-	-	+	+
NF32	+	+	+	+	+	+	-	+	-	+	-
NF36	+	+	-	-	+	+	-	+	-	+	-
RP2	-	-	-	-	+	+	-	-	-	+	-
RP7	-	+	-	+	+	-	+	-	-	+	+
RP9	+	+	+	+	+	-	+	-	-	+	+
APP8	+	+	+	-	+	+	-	-	-	+	+
HaPP2	+	+	+	+	+	+	+	-	-	+	+
HaPP6	+	-	-	+	+	-	-	-	-	+	+
HaPP7	-	+	-	-	+	-	+	-	-	+	+
NFP6	+	+	+	+	+	+	+	-	-	+	+
NFP7	+	+	+	+	+	+	+	-	-	+	+
NFP9	+	+	+	-	+	-	+	-	-	+	+
NFP12	+	+	+	+	+	+	+	-	-	+	+
NFP16	+	+	+	+	+	+	+	-	-	+	+

Symbols: MAL, Acid from Maltose; SUC, Acid from Sucrose; MAN, Acid from Mannitol; ARA, Acid from Arabinose; RHA, Acid from Rhamnose; SOR, Acid from Sorbitol; INO, Acid from Inositol; ADON, Acid from Adonitol; MEL, Acid from Melibiose; RAF, Acid from Raffinose.

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