# AL-JOUF REGION OLIVE TREES RHIZOSPHERIC BACTERIA: ENUMERATION, PHENOTYPIC DIVERSITY, AND AMYLASE SCREENING

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**ABSTRACT**

This preliminary bacteriological study of some Al-Jouf region olive trees rhizospheres aimed to investigate their phenotypic bacterial diversity and amylase productivity. The results of olive cultivar age effect on total rhizospheric bacteria were very low in seedlings compared to the adult threes, and generally variable according to the following descending order *Barri > Sorani > Romani > Russaei*. Concerning olive cultivars genotype effect on total rhizospheric bacteria, the results revealed on variable total rhizospheric bacteria associated with investigated cultivars, according to the following descending order *Barri (68.4 %) > Picual (23.5%) > Arbosana (7 %) > Chetoui (1%),* whereas the results of olive rhizospheric bacteria phenotypic diversity were variable according to the following descending order *Arbosana > Chetoui > Picual >Barri*. The phenotypic diversity was characterized by variations in colonies color, size, form, elevation, edges, and texture, with the dominance of pinpoint and red colonies in *Arbosana* and *Picual* cultivars respectively, whereas *Chetoui* cultivar distinguished with brown pigment diffusing colonies, which may be indicated siderophores secretion. In addition, amylase screening results showed the superiority of *Chemchali* and *Chetoui* cultivars as efficient sources for amylase-producing bacteria which can be exploited for further biotechnological applications.

**KEYWORDS:** *Olea. europaea*; Rhizosphere; Bacteria; Enumeration; Phenotypic Diversity; Amylase Screening.

## 1. INTRODUCTION

The rhizosphere may be defined as the narrow zone of soil surrounding plant roots, characterized by intense biological activity and influenced by root exudates, which stimulate or inhibit rhizosphere organisms.[1] Furthermore, contain beneficial microorganisms such as Plant Growth-Promoting Rhizobacteria (PGPR) that improve plant growth, crop yield, health and stresses adaptation, either directly or indirectly.[2]

The plant rhizosphere is a nutrient-rich region that led to a higher microbial density that attracts various predators such as protozoa, microfauna, and nematodes.[3] The complexity and diversity of interactions (plant/faunal, Plant/microbe, and microbe/microbe interactions) in the rhizosphere make this region very dynamics and active.[4]

Olive rhizosphere harbored a diverse and complex microbiota that exerted beneficial or harmful effects, On the other hand, olive released roots exudates that affect microbial population numbers, diversity, and activity. In the last decade, olive rhizosphere microbiota research's focused on four main axes, the first was their importance on plant nutrition, growth, and productivity[5,6] second was the suppression of plant diseases and pathogenesis[7,8] the third microbial diversity[9,10] and the fourth is novel bioactive producing bacteria.[11]

Several studies were conducted by Spanish. Italian and Tunisian scientists on their olive cultivars microbiota, to promote sustainable agriculture, in contrast, Al-Jouf region olive trees rhizospheres are still unexplored, and extensive studies are needed for a clear understanding of their microbial ecology.

In previously published papers, some North African plant and olive rhizospheres were explored and screened for bacterial diversity, exoenzymes, and exopolysaccharides production, respectively,[12,13,5] The focus of this paper is to investigate the phenotypic bacterial diversity of some Al-Jouf region olive trees rhizospheres, moreover, exploring the effect of olive cultivars age and genotype on bacterial abundance, and screening their rhizospheres as inhabiting niches for amylase producing bacteria.

## 2. MATERIAL AND METHODS

### 2.1. Rhizospheric soil Sampling and Olive trees cultivars

Cultivated olive (Olea. europaea var. europaea) rhizospheric soil samples were collected according to[14], whereas olive trees cultivars used in this study were summarized in Tab.1

**Tab. 1: Olive trees cultivars used in this study.**

| N | Olive Cultivar | Origin | Use | Source* |
|---|---|---|---|---|
| 1 | Barri | Saudi Arabia | O | ORU/CPRC |
| 2 | Koroneiki | Greece | O | |
| 3 | Arbosana | Spain | O | |
| 4 | Picual | Spain | O | |
| 5 | Arbequina | Spain | O | |
| 6 | Chemchali | Tunisia | O | |
| 7 | Chemlali | Tunisia | O | |
| 8 | Chetoui | Tunisia | O | |
| 9 | Quaissi | Syria | O | |
| 10 | Djalat | Syria | O | |
| 11 | Sorani | Syria | O/T | |
| 12 | Nabbali | Palestine | O/T | |
| 13 | Romani | Palestine | O | |
| 14 | Russaei | Palestine | O | |
| 15 | Nabbali Muhassan | Jordan | O/T | |

O: for olive oil, T: for table olive, ORU/CPRC: Olive Research Unit - Camel and Pasture Research Center - Sakaka, Al Jouf state - Saudi Arabia

### 2.2. Effect of olive cultivar Age and Genotype on total rhizospheric bacteria

Concerning the effect of olive age, rhizospheric soils from seedlings and threes of four olive cultivars *Sorani, Romani, Russaei, and Barri* were chosen for assessing the total rhizospheric bacteria which were assessed via suspending rhizospheric soil samples in a sterile saline solution; after vortexing, settling, and serial diluting, triplicates of 100µl samples were plated on nutrient agar (NA) and incubated for 48h at 30 °C. For genotype effect, fifteen olive cultivars genotype (Tab.1) were screened for total viable counts (TVC) of rhizospheric bacteria as described above.

### 2.3. Olive Rhizospheric Bacteria Phenotypic characterization

Olive cultivars that show the highest total viable counts (TVC) of rhizospheric bacteria (*Picual, Arbosana, Chetoui, and Barri*) were subjected for phenotypic characterization via studying their colonial characteristics (form, size, color, edges, elevation, and texture).

### 2.4. Screening for amylase producing rhizobacteria

Ten phenotypically distinguished colonies from each olive cultivar (*Chemchali, Chetoui, Koroneiki, Picual, Arbosana*) were selected and screened for amylase production on 0.5% starch agar medium, after 48 h incubation at 30°C, the amylolytic activity expressed by clearing zones appearance surrounding the colonies, when revealed by bi-sublimated iodine.

## 3. RESULTS AND DISCUSSION

### 1.1. Effect of olive Cultivar Age and Genotype on total rhizospheric bacteria

The results of the effect of olive cultivar age on total rhizospheric bacteria represented in Fig.1 showed in general that the total variable count in seedlings was very low compared to the adult threes, which were according to the following descending order *Barri > Sorani > Romani > Russaei*, similar results were obtained by Choubane et al. 2016 (12) on Young Carob tree (*Ceratonia siliqua L*), also Micallef et al., 2009[15] reported that plant age affects rhizosphere bacterial communities of *A. thaliana*, suggested to be due to changes in root exudations.

Concerning the results of olive cultivars genotype effect on total rhizospheric bacteria illustrated in Fig.2 revealed a variable total rhizospheric bacteria associated with investigated cultivars, according to the following descending order Barri (68.4 %) > Picual (23.5%) > Arbosana (7 %) > Chetoui (1%). Comparable results on some olives cultivars rhizosphere bacterial abundance and diversity reported by Cheba and Abdelzaher, 2020.[5] According to Fuchs and Herisse,1999[16] the differences in total bacteria between cultivars rhizospheres may be due to the fact all microorganisms are not attracted in the same way by the roots. Indeed, the roots exudates vary from a plant to another which leads to a variation in type and quantity of rhizospheric microorganisms. Furthermore, Chaparro *et al.* 2013,[17] via combining metatranscriptomics and metabolomics proved that plant root exudates change along the different stages of plant

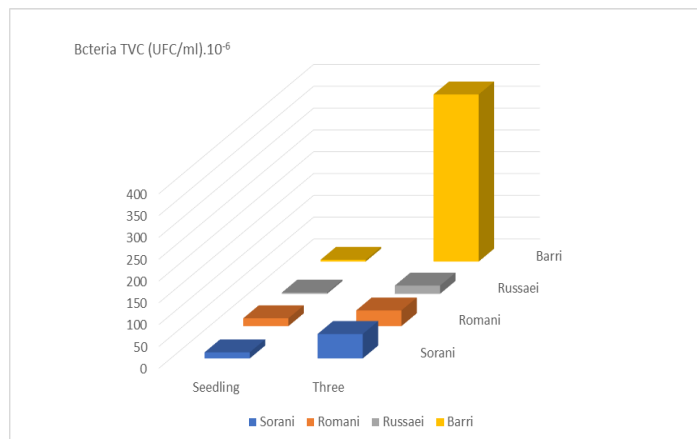development helping them to shape their rhizosphere microbiome to their benefit.



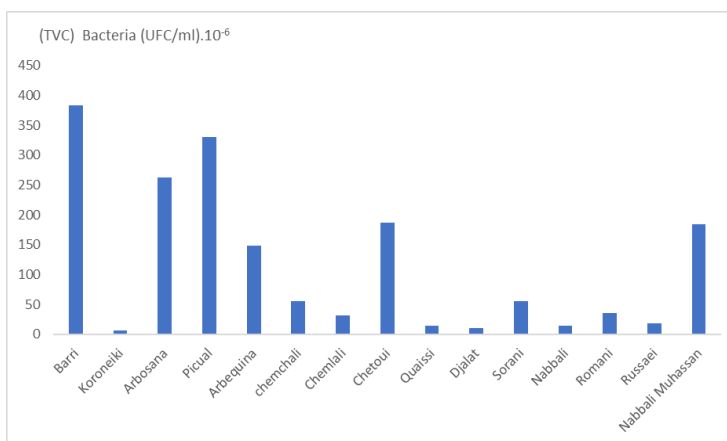**Fig. 1: Effect of olive tree age on total rhizospheric bacteria.**



**Fig. 2: Effect of olive cultivar genotype on total rhizospheric bacteria.**

### 1.2. Olive Rhizospheric Bacteria Phenotypic characterization

The results of olive rhizospheric bacteria phenotypic characterization summarised in Tab.2 and partially illustrated in Fig.3 indicated a variable phenotypic diversity according to the following descending order Arbosana > Chetoui > Picual >Barri.

phenotypic diversity characterized by variations in colonies color, size, form, elevation, edges, and texture, respectively (Tab.2). With the dominance of pinpoint and some pink colonies in *Arbosana* cultivar and red colonies dominance in *Picual* cultivar, whereas *Chetoui* cultivar distinguished with the dominance of colonies with

diffused brown pigment (Fig.3). which may be indicated siderophores secretion.

In general, few kinds of literature were recorded about olive rhizospheric microbial diversity specially at the phenotypic level however, several reports, were found at the molecular level for example Ruano-Rosa, et al. 2017[7] investigated *Picual* olive roots bacterial diversity using 16S rDNA and gyrB gene sequences analysis for pathogens biocontrol purposes. Also, Gómez-Lama Cabanás et al. 2018[8] investigated *Picual* olive rhizobacteria displaying biocontrol activity against *Verticillium* wilt.

**Tab. 2: Phenotypic characterization of bacterial cultures associated with some olive cultivars rhizospheres.**

| Olive Cultivar | Colony Characteristics | | | | | | Observation |
|---|---|---|---|---|---|---|---|
| | *Form* | *Size* | *Color* | *Edges* | *Elevation* | *Texture* | |
| *Picual* | circular | small, minute | bright white, transparent white, reddish-pink, yellow, hazel | entire, regular | flat, biconvex | buttery, latinous | red colonies dominance |
| *Arbosana* | circular, point | large, medium, | bright white, white, light | entire, lobular | flat, convex | buttery, viscous, | the dominance of |

| | | small | transparent, beige, cream, pink | | | sticky | pinpoint and some pink colonies |
|---|---|---|---|---|---|---|---|
| *Chetoui* | circular, point | large, medium, small | bright white, light white, beige, cream, and brown dye diffused | entire, lobular | flat, slightly onvex | buttery | the dominance of colonies with diffused brown pigment, the phenomenon of satellitism |
| *Barri* | circular, point form | large, medium, small | white, beige, cream, light brown | entire, lobular, regular, irregular | flat, convex, slightly height | buttery | - |
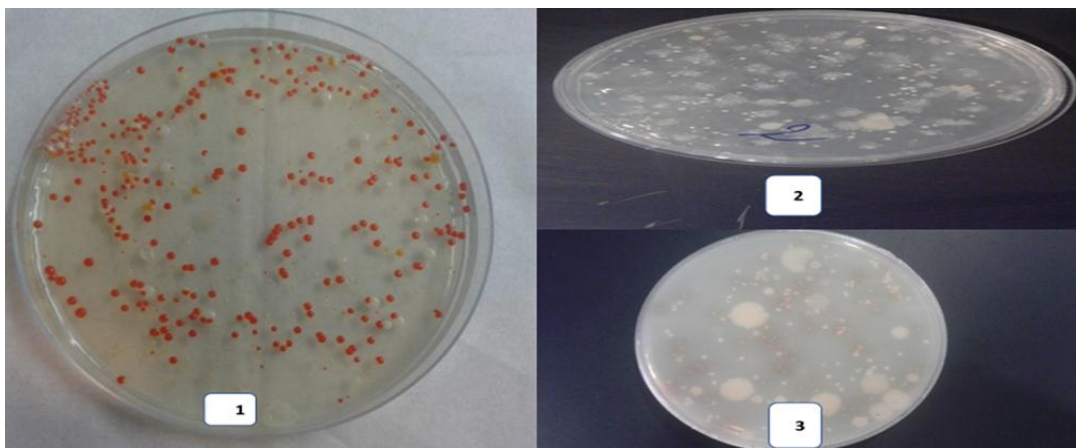


**Fig. 3: Phenotypic characterization of bacterial cultures associated with some olive cultivars rhizospheres 1,2,3 was *Picual, Arbosana, and Chetoui* cultivars respectively.**

### 1.3. Olive rhizospheric bacteria amylase screening

Amylase screening results presented in Fig.4 and partially illustrated in Fig.5 showed the superiority of *Chemchali* and *Chetoui* cultivars as an efficient source for amylase producing bacteria, these results agree with previously reported by Cheba and Abdelzaher,2020[5] on the dominance of *Chetoui* and *Chemlali* Tunisian cultivars rhizospheric bacteria in exoenzymes multiproduction. Also, (Gómez-Lama Cabanás *et al.* 2018[8] reported the multienzymes production of Picual olive rhizobacteria. Furthermore, (Gharsallah *et al.* 2019[18] explored olive orchard's bacterial and metabolic diversity, and screen their enzymatic activities (lipase, protease, and amylase) for biotechnological applications.
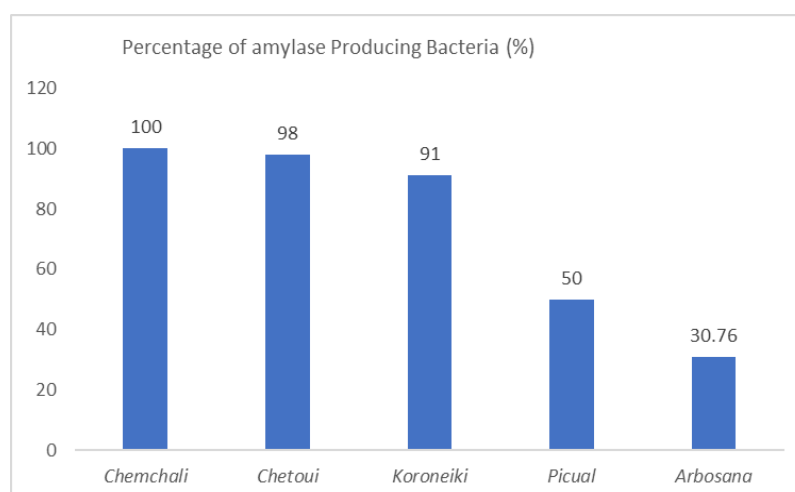


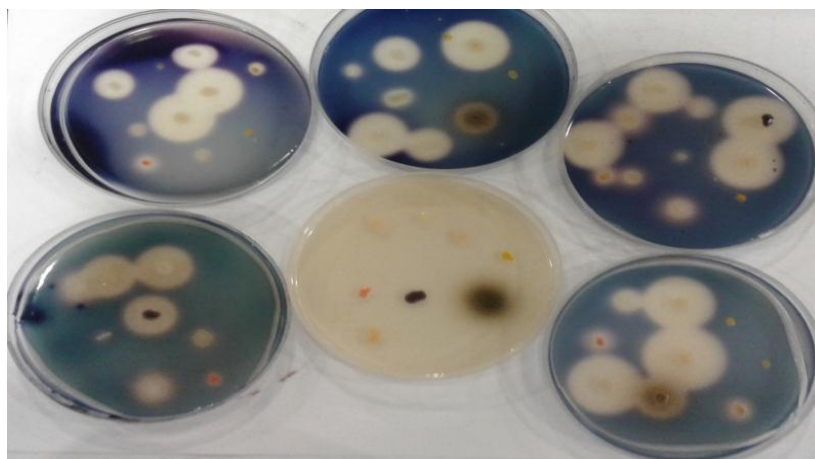**Fig. 4: Percentage of amylase producing bacteria isolated from olive cultivars rhizospheres.**

**Fig. 5: amylase-producing bacteria isolated from olive cultivars rhizospheres.**

## 3. CONCLUSION

The olive rhizosphere is a dynamic and complex niche due to its interactive microbiota; we conclude from this preliminary culture-based investigation that olive cultivar's age and genotype significantly affect the bacterial abundance and phenotypic diversity. furthermore, *Chemchali* and *Chetoui* cultivars were the efficient sources for amylase-producing bacteria which can be exploited biotechnologically. In addition, nonculture-based studies (rhizosphere metagenomic and microbiomic) were very recommended for the deep understanding of olive rhizosphere microbiota complexity and functionality.

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