ISOLATION AND IDENTIFICATION OF *AGROBACTERIUM* FROM THE TERRITORY OF TAJIKISTAN

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Vitis vinifera (wine grape) represents an economically and culturally important agricultural crop for which microbial activity plays critical roles in grape [1] and wine production and quality [7,11]. However, scant evidence exists for microbial distribution patterns in grapes and wines [1,10] and the factors driving microbial assemblages on grape surfaces are unknown. Given that many of the same environmental conditions that govern regional variations in grapevine growth and development [6] also alter microbial communities across space and time [8], it follows that biogeographical assemblages of grape-surface microbiota may exist, potentially influencing grapevine health and wine quality.

The extraordinary *Agrobacterium* research story started from the search for the causative agent of crown gall disease more than 100 years ago. *Agrobacterium tumefaciens* was first isolated from grapevine galls in 1897 and later isolated from Paris daisy in 1907 [3]. The *Agrobacterium* infection mechanism involves processing and transfer of a specific DNA fragment (the transferred-DNA, T-DNA) from a bacterial tumor-inducing (Ti) plasmid. Transfer to the plant occurs via a type IV secretion system (T4SS), after which T-DNA is integrated into the plant host genome [5].

Bacterial cancer disease caused by these bacteria is among the most important bacterial diseases that affect the yield and production of different plants, including grapevine. Despite it's high importance in agriculture, *Agrobacterium* spp. strains have not been isolated and studied in Tajikistan yet.

The aim of the study was to isolate *Agrobacterium* spp. strains from different plant materials and soil samples collected from the Farm "Vatan 2008", District Tursunzode, Yangibog.

To achieve this goal the following objectives were set: 1) isolation of pure bacterial cultures from soil and diseased plant samples; 2) Gram staining of bacterial isolates; 3) Biochemical identification of isolates [9]; 4) Identification of selected strains using API 20E and 20NE identification systems; 5) Antibiotic susceptibility testing of selected strains using Kirby-Bauer Disc diffusion test.

The set of experiments have been conducted to isolate *Agrobacterium* spp., from soil and different plant materials (fruits, leaves, tumors). In total, 22 samples (3

from soil, 5 from fruits, 5 from leaves and 9 from tumors) have been used for experiments. From 48 primary isolates on Roy-Sasser medium [4], 25 strains with colony properties similar to standard *Agrobacterium larrymoorei* strains, have been selected for further characterization. The selected strains have been subjected to biochemical identification according to the *Agrobacterium* spp. identification scheme [2] (Figure 1). Based on obtained results, 5 strains showed biochemical properties typical for *Agrobacterium*. These 5 strains and additional 3 randomly selected strains have been identified using API 20E and API NE systems [14]. According to API test results, the majority of selected strains were attributed to *Pseudomonas* spp. [12], (two *Pseudomonas luteola* and three *P. fluorescens* strains) one isolate was identified as our target species *Agrobacterium (Rhizobium) radiobacter*. Other environmental bacteria, such as *Enterobacter cloacae* and *Aeromonas hydrophila* have also been identified.

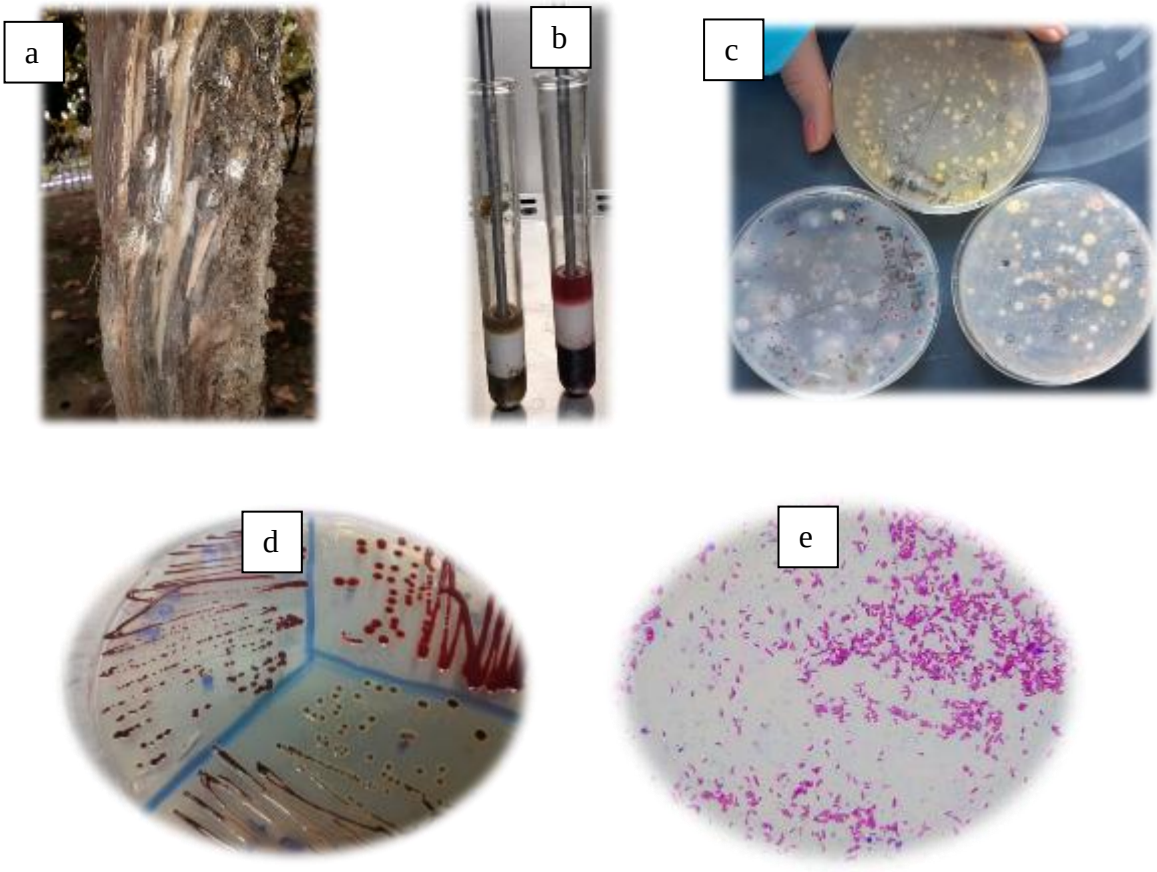

Figure 1 – Stages of isolation of pure culture: a) samples from tumor and soil; b) samples from leaves and fruits; c) plate Roy-Sasser medium; d) pure culture plate on Roy-Sasser medium; e) Gram negative bacteria.

The antibiotic susceptibility testing of 8 identified strains using Kirby-Bauer disc diffusion test showed that the *R. radiobacter* strain was sensitive to 21 tested

antibiotics out of 24, while *A. hydrophila* and *P. fluorescens* were sensitive to 19 and 15 antibiotics, respectively. *E. cloacae* and *P. luteola* showed higher resistance to the tested antibiotics.

To confirm the identity of the *Agrobacterium* isolate, DNA was extracted from the isolate and 16S rRNA genes were amplified by PCR using universal primers. The amplicons were successfully sequenced and obtained sequence was compared with sequences available in Gen Bank using BLASTn tool. As a result, the sequence shared 99% similarity with representatives of genus *Agrobacterium larrymoorei* [13].

Based on obtained results, we can conclude that *Agrobacterium larrymoore* inhabit the plant farm "Vatan 2008" agroecosystem that might lead to the development of crown gall disease in the grapevine.

This is the first work on plant pathogenic bacteria isolated from plant and soil samples collected in Tajikistan.

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