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ANALYSIS OF THE PATHOGENIC *AGROBACTERIUM RADIOBACTER* GENOME SEQUENCES ISOLATED FROM GRAPES IN TAJIKISTAN

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Research on Agrobacteria began more than 100 years ago with the search for the causative agent pathogenic bacterium that causes crown gall disease, a plant tumor affecting a wide range of plant species. Agrobacterium tumefaciens causes crown gall disease in various plant species by injecting its T-DNA into the genome. Thus, Agrobacterium has been widely studied as a pathogen and an important biotechnological tool. These changes not only lead to abnormally proliferating host cells with heterotrophic and transport-dependent metabolism but also cause differentiation and serve as mechanisms for balancing protection against pathogens and adaptation to abiotic stress conditions, thereby ensuring the coexistence of crown bile and the host plant. Crown gall one of the most serious and common bacterial diseases of vines (Vitis vinifera L.) worldwide, is mainly caused by oncogenic strains of Agrobacterium vitis. Coronation bile is a very destructive plant disease that reduces the viability and yield of infected plants by up to 40%. Typical symptoms of a grapevine gallbladder are tumors and tissue overgrowth in the lower part of the trunk. Agrobacterium radiobacter strain Agro fruit was isolated from an infected fruit from the Dekhkan farm "Vatan", Yangibog site, Tursunzade, Tajikistan. The 5.7-Mbp draft genome sequence, of unique sequence data, was distributed into 35 contigs with an N50 value of 267,803 bp, GC content of 59.44 %, and genome completeness was estimated as 100 %.

Key words: *Agrobacterium radiobacter, Tajikistan, Grape Plant, genome sequences, 16S rRNA gene, Illumina NovaSeq 6000 S2, pathogen.*

Исследования агробактерий начались более 100 лет назад с поиска возбудителя патогенной бактерии, вызывающей болезнь корончатого галла, опухоль растений, поражающую широкий спектр видов растений. Agrobacterium tumefaciens вызывает болезнь корончатого галла у различных видов растений путем инъекции своей Т-ДНК в геном. Таким образом, Agrobacterium широко изучалась как патоген и важный биотехнологический инструмент. Эти изменения не только приводят к аномальной пролиферации клеток-хозяев с гетеротрофным и транспортно-зависимым метаболизмом, но и вызывают дифференцировку и служат механизмами баланса защиты от патогенов и адаптации к абиотическим стрессовым условиям, обеспечивая тем самым сосуществование корональной желчи и растения-хозяина. Корончатый галл, одно из самых серьезных и распространенных бактериальных заболеваний винограда (Vitis vinifera L.) во всем мире, в основном вызывается онкогенными штаммами Agrobacterium vitis. Корончатый галл — очень разрушительное заболевание растений, снижающее жизнеспособность и урожайность зараженных растений до 40 %. Типичными симптомами корончатого галла виноградной лозы являются опухоли и разрастание тканей в нижней части ствола. Штамм Agrobacterium radiobacter Agro Fruit был выделен из инфицированных плодов дехканского хозяйства «Ватан», участок Янгибог, Турсунзаде, Таджикистан. Черновая последовательность генома размером 5,7 Mbp, состоящая из уникальных данных о последовательностях, была распределена на 35 контигов со значением N50 267 803 bp, содержанием GC 59,44 %, а полнота генома оценивалась как 100 %.

Ключевые слова: *Agrobacterium radiobacter, Таджикистан, виноград, геномные последовательности, ген 16S rRNA, Illumina NovaSeq 6000 S2, патоген.*

Introduction

Agrobacterium radiobacter (Beijerinck and van Delden 1902) Conn 1942 (formerly Agrobacterium tumefaciens) [1 p. 574–584] was first isolated from grapevine galls in 1897 as the causative agent of crown galls disease [2 p. 340]. Agrobacterium is known among microbiologists, geneticists, and biotechnologists as a robust and versatile tool used to introduce foreign genes into plants or fungi [3 p. 221–229, 4]. However, most members of this genus are primarily plant pathogens that induce galls on dicotyledonous plants. Formerly, the Agrobacterium genus encompassed various species such as *A. rubi, A. larrymoorei, A. vitis*, and *A. tumefaciens*. The latter species is

now recognized as a complex of several species including A. fabrum to which belongs A. fabrum C58, whose genome was the first sequenced in *Agrobacterium* [5 p. 208–215, 6 p. 373–378, 7]. Collectively, Agrobacteria belong to the family Rhizobiaceae of the class alpha-proteobacteria, members of which are often found in soils of various origins and appear to be among the most common inhabitants of these environments [8 p. 717–721, 9 p. 1283–1289, 10 p. 989–1001, 11 p. 460–470, 12 p. 86–90, 13 p. 91–95]. Interestingly, agrobacteria isolated from soils, including rhizospheric soils, are most often avirulent [8 p. 717–721, 14 p. 617–620], i.e., they do not harbor a Ti plasmid, the key replicon that determines virulence, unless the soil has an history of crown gall disease [15 p. 1310–1317, 16 p. 3358–3365]. The infection mechanism involves processing and transfer of a specific DNA fragment (the transferred-DNA, T-DNA) from a bacterial tumor-inducing (Ti) plasmid via a type IV secretion system (T4SS), after which T-DNA is integrated into the plant host genome [17 p. 265].

The main part

Despite their great importance in agriculture, strains of *Agrobacterium* from Tajikistan have not been studied previously. Within the framework of a project aiming at development of phage therapy for treatment of *Agrobacterium*-infected grapevine trees in Tajikistan, strain *A. radiobacter* was isolated from an infected fruit collected from the Vatan 2008 grape farm in Yangibog, Tajikistan.

The fruit was rinsed three times in sterile dH2O and homogenized. Aliquots were spread onto Roy-Sasser agar plates and incubated at 30oC for three days. Colonies were picked and streaked onto new Roy-Sasser plates [18 p. 399–412] for purification. A series of experiments were conducted to isolate *Rhizobium* spp. (*Agrobacterium* spp.) from soil and various plant materials (fruits, leaves, tumors) (Fig.1).

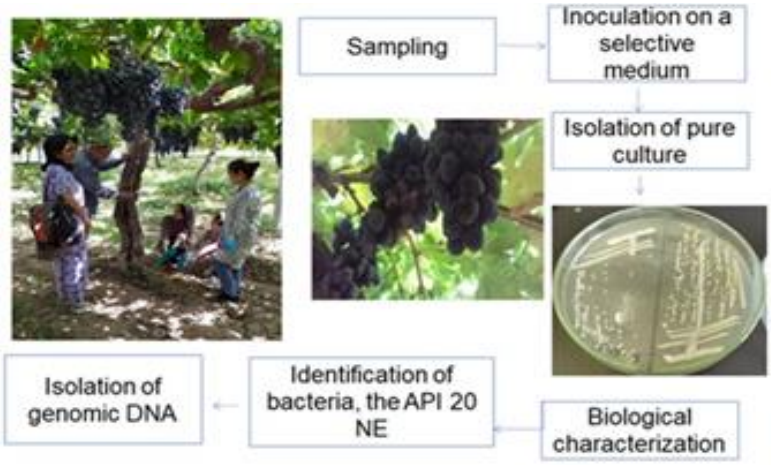

Fig. 1. Scheme of isolation of *Agrobacterium*.

A total of 43 samples (6 soils, 8 fruits, 6 leaves, and 23 tumors) were used for the experiments. From 104 primary isolates on the Roy-Sasser medium, 43 presumptive *Rhizobium* spp. strains were selected for further characterization. The selected strains were biochemically identified according to the *Rhizobium* spp. identification scheme. Based on the obtained results, 19 strains showed biochemical properties consistent with *R. radiobacter* (Fig.2).

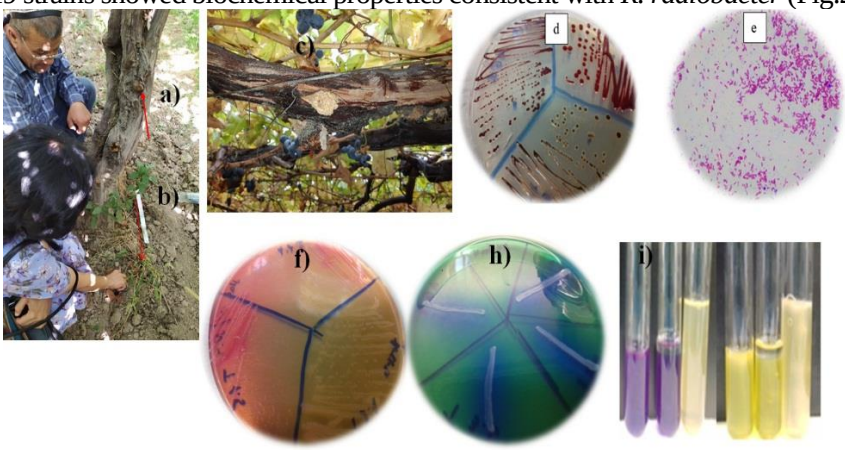

Fig. 2. Stages of isolation of pure culture and biochemical properties consistent with *R. radiobacter*: a) samples from tumor; b) samples from soil; c) samples from leaves and fruits; d) bacterial growth a Roy-Sasser medium; e) pure culture plate on Roy-Sasser medium; f) Gram staining of bacteria; g) Growth of isolates on MacConkey agar; h) citrate utilization test; i) Hugh Leifson Medium and Motility Test Medium

The strain, forming dark red colonies, was identified as *A. radiobacter* by its API 20NE profile (1-4-6-7-7-4-4).

DNA was extracted from cells cultivated in LB for 24 hours at 30°C with shaking using the GenElute bacterial genomic DNA kit (Sigma-Aldrich) [19]. Sequencing of the 16S rRNA gene as described yielded a 1275 nucleotides sequence identical with the *A. radiobacter/tumefaciens* type strain. For genome sequencing by Eurofins Genomics, a NEBNext Ultra II DNA preparation kit was used, and Illumina NovaSeq 6000 S2 paired-end genomic sequencing was performed with a read length of 2 x 150 bp resulting in 5,062,990 reads and a total of 1,518,897,000 sequenced bases. Reads with a maximum of 7 bases with a Phred score below 28 were discarded. Additional quality control was performed using the Trim Reads tool in the CLC Genomics Workbench v. 20.1. Assembly was performed using the CLC de novo assembly tool, resulting in 5,736,602 bp of unique sequence data distributed into 35 contigs with an N50 value of 267,803 bp, and GC content of 59.44 % [20 p. 75]. All software was used with default values. Annotation of the draft genome was done using the NCBI Prokaryotic Genome Annotation Pipeline (PGAP) [21 p. 1020–1028, 22 p. 851–860, 23 p. 6614–6624]. Genome completeness was estimated as 100 % by CheckM v. 1.0.18 [24 p. 1043–1055].

A phylogenomic analysis revealed clustering within the *Agrobacterium*, with pairwise average nucleotide identity (ANI) values of 97.97 % and 83.8 % dDDH values against the type of strain *A. radiobacter* NCPPB 3001. Genome-based phylogenetic affiliation of strain *A. radiobacter* with representative *A. radiobacter* NCPPB 3001 strain and *Agrobacterium* spp., represented as a phylogenetic tree (Fig. 3).

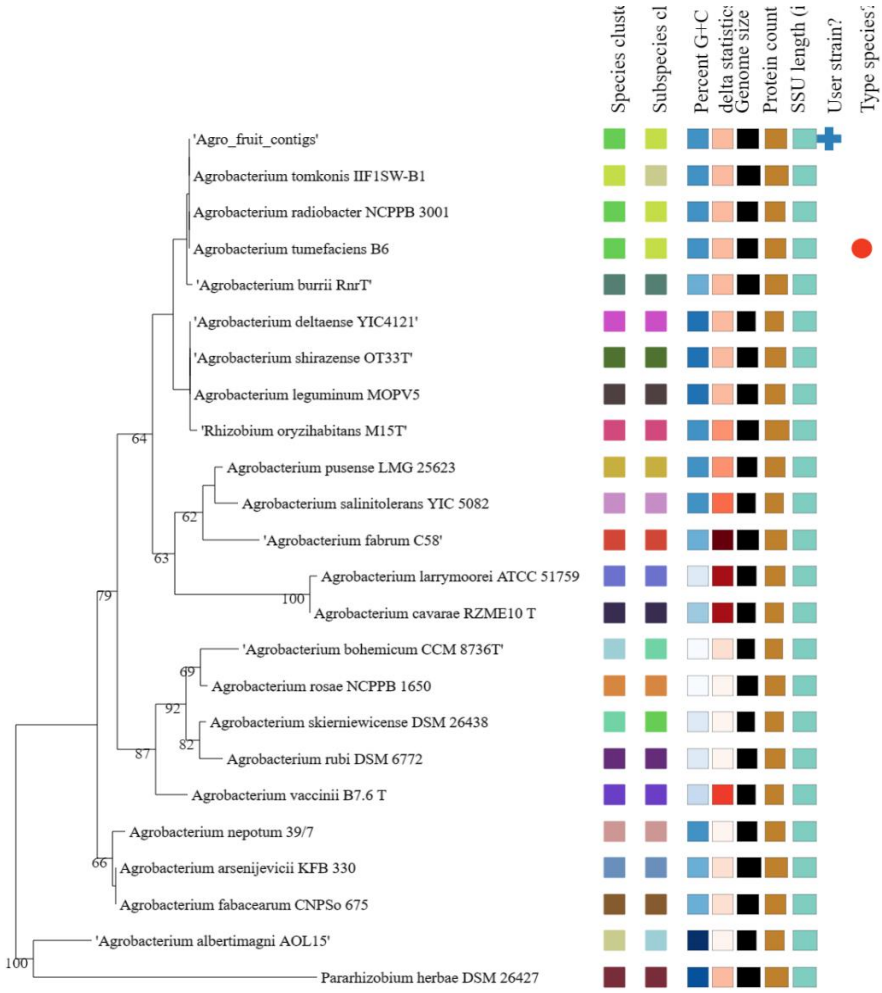

Fig. 3. The tree was inferred with FastME v. 2.1.6.1 [25 2798 –2800 p] from Genome Blast Distance Phylogeny (GBDP) distances calculated from genome sequences using the TYGS server **(**https://tygs.dsmz.de**)** [26 p. 2182] and rooted with Pararhizobium herbae DSM 26427 [27 p. 104, 141–148] as an outgroup. The branch lengths are scaled in terms of the GBDP distance formula d5. The numbers above the branches are GBDP pseudobootstrap support values of ≥68% from 100 replications. The tree was rooted at the midpoint [28 p 645– 668].

Conclusion. Based on the results obtained, it can be concluded that the farm Dekhkan farm "Vatan", Yangibog site, Tursunzade, Tajikistan contains the pathogen *A. radiobacter*, which can lead to the development of venous bile in the vine. It is worth noting that for three years, the laboratory of the Center for Bio-

technology of the Tajik National University studies of pathogenic plant bacteria isolated from plant and soil samples collected from a grape farm in Tajikistan.

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REFERENCES

1. Zhang, LS, Li XY, Zhang F, Wang GJ. 2014. Genomic analysis of *Agrobacterium radiobacter* DSM 30147(T) and emended description of *A. radiobacter* (Beijerinck and van Delden 1902) Conn 1942 (Approved Lists 1980) emend. Sawada et al. 1993. Standards in Genomic Sciences 9:574–584.

2. Kado CI. 2014. Historical account on gaining insights on the mechanism of crown gall tumorigenesis induced by *Agrobacterium tumefaciens*. Front Microbiol 5:340.

3. Vain P (2007) Thirty years of plant transformation technology development. Plant Biotechnol J 5:221-229.

4. Idnurm A, Bailey AM, Cairns TC et al (2017) A silver bullet in a golden age of functional genomics: the impact of *Agrobacterium*-mediated transformation of fungi. Fungal Biol Biotechnol 4:6. https://doi.org/10.1186/s40694-017-0035-0.

5. Mousavi SA, Osterman J, Wahlberg N et al (2014) Phylogeny of the *Rhizobium-Allorhizobium- Agrobacterium* clade supports the delineation of *Neorhizobium* gen. nov. Syst Appl Microbiol 37:208-215.

6. Kuzmanovic N, Puiawska J, Prokic A et al (2015) *Agrobacterium arsenijevicii* sp. nov., isolated from crown gall tumors on raspberry and cherry plum. Syst Appl Microbiol 38:373-378.

7. De Lajudie PM, Young JPW (2017) International Committee on Systematics of Prokaryotes Subcommittee for the Taxonomy of Rhizobium and Agrobacterium–minutes of the meeting, Budapest, 25 August 2016. Int J Syst Evol Microbiol. https://doi.org/10.1099/ijsem.0.002144.

8. Bouzar H, Moore LW (1987) Isolation of different *Agrobacterium* biovars from a natural oak savanna and tallgrass prairie. Appl Environ Microbiol 53:717-721.

9. Nflsslein K, Tiedje JM (1998) Characterization of the dominant and rare members of a young Hawaiian soil bacterial community with small-subunit ribosomal DNA amplified from DNA fractionated on the basis of its guanine and cytosine composition. Appl Environ Microbiol 64:1283-1289.

10. Teixeira LC, Peixoto RS, Cury JC et al (2010) Bacterial diversity in rhizosphere soil from Antarctic vascular plants of Admiralty Bay, maritime Antarctica. ISME J 4(2010):989-1001.

11. Inceoglu O, Al-Soud WA, Salles JF et al (2011) Comparative analysis of bacterial communities in a potato field as determined by pyrosequencing. PLoS One 63(2):460-470.

12. Lundberg DS, Lebeis SL, Paredes SH et al (2012) Defining the core *Arabidopsis thaliana* root microbiome. Nature 488:86-90.

13. Bulgarelli D, Rott M, Schlaeppi K et al (2012) Revealing structure and assembly cues for Arabidopsis root-inhabiting bacterial microbiota. Nature 48:91-95.

14. Burr TJ, Katz BH, Bishop AL (1987) Populations of *Agrobacterium* in vineyard and non vineyard soils and grape roots in vineyards and nurseries. Plant Dis 71:617-620.

15. Bouzar H, Ouadah D, Krimi K et al (1993) Correlative association between resident plasmids and the host chromosome in a diverse *Agrobacterium* soil population. Appl Environ Microbiol 59:1310-1317.

16. Krimi Z, Petit A, Mougel C et al (2002) Seasonal fluctuations and long-term persistence of pathogenic populations of *Agrobacterium* spp. in soils. Appl Environ Microbiol 68:3358-3365.

17. Hwang HH, Gelvin SB, Lai EM. 2015. Editorial: "*Agrobacterium* biology and its application to transgenic plant production". Front Plant Sci 6:265.

18. El-Sehemy I.A., Haroun B.M., El-Diwany A.I., El-Shahed K.Y., Awad H.M., Tolba I.H. Isolation and identification of native Egyptian curdlan producing *Agrobacterium* isolate 1Az. J. Pharm Sci. Vol. 45, March 2012. 399-412.

19. Dzhuraeva M, Shokirova M, Azaryan A, Panosyan H, Bobodzhanova K, Birkeland NK. 2020. Draft Genome Sequence of *Escherichia coli* Strain Tj, Isolated from the Varzob River in Tajikistan. Microbiol Resour Announc 9.

20. Aziz RK, Bartels D, Best AA, DeJongh M, Disz T, Edwards RA, Formsma K, Gerdes S, Glass EM, Kubal M, Meyer F, Olsen GJ, Olson R, Osterman AL, Overbeek RA, McNeil LK, Paarmann D, Paczian T, Parrello B, Pusch GD, Reich C, Stevens R, Vassieva O, Vonstein V, Wilke A, Zagnitko O. 2008. The RAST Server: rapid annotations using subsystems technology. BMC Genomics 9:75.

21. Li W, O'Neill KR, Haft DH, DiCuccio M, Chetvernin V, Badretdin A, Coulouris G, Chitsaz F, Derbyshire MK, Durkin AS, Gonzales NR, Gwadz M, Lanczycki CJ, Song JS, Thanki N, Wang J, Yamashita RA, Yang M, Zheng C, Marchler-Bauer A, Thibaud-Nissen F. RefSeq: expanding the Prokaryotic Genome Annotation Pipeline reach with protein family model curation. Nucleic Acids Res. 2021 Jan 8;49(D1): D1020-D1028. doi: 10.1093/nar/gkaa1105. PMID: 33270901.

22. Haft DH, DiCuccio M, Badretdin A, Brover V, Chetvernin V, O'Neill K, Li W, Chitsaz F, Derbyshire MK, Gonzales NR, Gwadz M, Lu F, Marchler GH, Song JS, Thanki N, Yamashita RA, Zheng C, Thibaud-Nissen F, Geer LY, Marchler-Bauer A, Pruitt KD. RefSeq: an update on prokaryotic genome annotation and curation. Nucleic Acids Res. 2018 Jan 4;46(D1): D851-D860. doi: 10.1093/nar/gkx1068. PubMed PMID: 29112715.

23. Tatusova T, DiCuccio M, Badretdin A, Chetvernin V, Nawrocki EP, Zaslavsky L, Lomsadze A, Pruitt KD, Borodovsky M, Ostell J. NCBI prokaryotic genome annotation pipeline. Nucleic Acids Res. 2016 Aug 19;44(14):6614-24. doi: 10.1093/nar/gkw569. PMID: 27342282.

24. Parks DH, Imelfort M, Skennerton CT, Hugenholtz P, Tyson GW. 2015. CheckM: assessing the quality of microbial genomes recovered from isolates, single cells, and metagenomes. Genome Res 25:1043-55.

25. Lefort V, Desper R, Gascuel O. 2015. FastME 2.0: a comprehensive, accurate, and fast distance-based phylogeny inference program. Mol Biol Evol 32:2798 –2800. https://doi.org/10.1093/molbev/msv150.

26. Meier-Kolthoff JP, Goker M. 2019. TYGS is an automated high throughput platform for state-of-the-art genome-based taxonomy. Nat Commun 10:2182. https://doi.org/10.1038/s41467-019-10210-13.

27. Bai, J., Yao, T., Lan, X., Yang, Y., Wang, Z., Wang, X., 2022. Isolation of *Agrobacterium tumefaciens*/ biovar 1 from the crown gall of Cerasus humilis in China. Journal of Plant Pathology 104, 141–148. doi:10.1007/s42161-021-00947-6.

28. Farris JS. 1972. Estimating phylogenetic trees from distance matrices. Am Nat 106:645– 668. https://doi.org/10.1086/282802.