International Journal of

Secondary Metabolite

Volume: 5 Number: 1
January 2018

ISSN-e: 2148-6905 Journal homepage: http://www.ijate.net/ http://dergipark.gov.tr/ijsm

Biodiversity of Fungi in Strawberry Fields in Anamur, TURKEY

Bahadır Törün, Mehmet Ali Yörükce, Fatma Yaman, Halil Bıyık

To cite this article: Törün, B., Yörükce, M.A., Yaman, F., & Bıyık, H. (2018). Biodiversity of Fungi in Strawberry Fields in Anamur, TURKEY. *International Journal of Secondary Metabolite*, 5(1), 20-26. DOI: 10.21448/ijsm.346209

To link to this article: http://www.ijate.net/index.php/ijsm/issue/archive
http://dergipark.gov.tr/ijsm

IJSM International Journal of Secondary Metabolite

Int. J. Sec. Metabolite, Vol. 5, Issue 1 (2018) pp. 20-26

Research Article

ISSN: 2148-6905 online Journal homepage: http://www.ijate.net/index.php/ijsm

Biodiversity of Fungi in Strawberry Fields in Anamur, TURKEY

Bahadır Törün[1*], Mehmet Ali Yörükce[1], Fatma Yaman[1], Halil Bıyık[1]

[1]Faculty of Science and Literature, Adnan Menderes University, Aydin, Turkey

Abstract: Strawberry is a delicious and aromatic fruit, which can be consumed as fresh and also is suitable for industry. However, strawberry is exposed to many fungal diseases that end with the loss of the product up to % 15 before harvest. The aim of this study is to determine the fungi that present in the field whether or not pathogenic. Samples were collected from different strawberry fields in Anamur in April 2016. Morphological identification was made according to the shape and color of the colonies, mycelium and spore structures. For molecular identification, ITS gene region was used. According to morphological and molecular methods, seven different fungal genera were found on strawberries.

ARTICLE HISTORY

Received: 5 September 2017
Revised: 3 October 2017
Accepted: 15 October 2017

KEYWORDS

Fungi,
Biodiversity,
Strawberry,
ITS,
Anamur,
Turkey

1. Introduction

Biodiversity is the establishment of environment administrations to which human prosperity is personally connected [1]. It is one of the essential parts of nature and it guarantees the survival of earth definitely.

Fruits are the comestible part of a mature ovary of flowering plants, which are normally eaten raw [2]. Strawberry is one of these fruits. However, fruits are easily spoilt and usually have active metabolism during the storage stage [3]. The importance of fruit in human nutrition cannot be overestimated as it provides essential growth factors such as vitamins and minerals necessary for proper body metabolism [4]. The high concentration of various sugars, minerals, vitamins, amino acids, and low pH also enhances the successful growth and survival of various parasitic and saprophytic forms of fungi [5]. Annual reports have shown that % 20 of fruits and vegetables produced are lost to spoilage [6].

Soil biodiversity impacts a gigantic scope of biological system forms that add to the maintainability of life on earth [7]. Biological activity is an essential factor in the physical and substance development of soils [8].

*Corresponding Author E-mail: bahadirtrn@yahoo.com.tr

ISSN: 2148-6905 online /© 2018 DOI: 10.21448/ijsm.346209

There are 110.000 defined fungi species were present in the World but it is estimated that 1.5 million fungi species exists [9]. The ITS region, one of the polymorphic DNA sequences among fungal species, is now considered to be a good candidate for accurate detection and can be largely separated from all other species by this application. It is important to determine the diversity of fungi, which cause diseases on strawberries and their ecological and genetic effects. Abdullah et al. (2016) studied fungal biodiversity of post-harvest rot of some fruits in Yemen. They found 16 fungal genera and 39 species [10]. Jensen et al. (2013) studied characterization of microbial communities on strawberries and found *Penicilium* spp were abundant [11].

In this study, fungi that cause disease in strawberry will be detected by morphological and molecular methods.

2. Material and Methods

Sample Collection: Samples were collected aseptically from the strawberry fields from Anamur in April 2016. Thirty rotten strawberry fruits were collected and kept in the portable refrigerator until brought to the laboratory.

Isolation of Fungal Species: One gram of strawberry fruits were weighted and homogenized in 9 ml of 0.85% Physiological Saline Water (PSW). 100 µL of these homogenised samples were inoculated on Rose Bengal Agar (RBA) and Potato Dextrose Agar (PDA). Samples than incubated at 27 °C for 5 days. After incubation, different fungi were selected and isolated from the mixed colony under the same incubation conditions.

Morphological Identification: Morphological identification of the fungi was made according to Samson [12]. Mycelium and spore structures smeared on a slide, dyed with lactophenol cotton blue and visualized under the microscope. Colonial shapes were determined and used in morphological identification.

Molecular Identification: Fungi samples were put in 1.5 ml Eppendorf tubes using a sterile toothpick. Then samples have reduced the powder using liquid nitrogen. DNA isolation of the samples was realized with 2X CTAB isolation protocol according to Doyle and Doyle [13]. Concentration and purity of the samples were measured with a Nanodrop Spectrophotometer (Thermo). ITS gene region was used to identify the species. Two universal ITS primers were used (ITS1:5'TCCGTAGGTGAACCTGCGG'3, ITS4: 5'TCCTCCGCTTATTGATATGC'3) [14]. PCR reactions were realized at initial denaturation 94 ºC 5 min, denaturation 94 ºC 30 sec, annealing 60 ºC 30 sec, extension 72 ºC 60 sec with 35 cycles and a final extension at 72 ºC 10 min. Reagents concentrations were 10X Taq Buffer, 0.5M dNTP mix, 10 pM from each primer, 7.5 mM MgCl2 and 1U Taq polymerase (GenMark) with the final volume of 25 µl. Agarose gel electrophoresis of the PCR products were observed with 1.4 % agarose concentration on 90 V 40 min. 100 bp DNA ladder was used for size comparison of the products. After PCR products were sent to DNA sequencing (Macrogen, Holland).

Data Analysis: Sequence results were aligned with the ones in GenBank using BLASTn software to find out the species of the samples. MEGA 7.0 was used to infer phylogenetic tree using maximum parsimony method.

3. Results

Morphological Identification: According to morphological methods seven different species were found (Table 1). Colony shape, mycelium and spore structures were investigated. Seven different species were spotted according to Samson [10].

Table 1. Morphological identification of the species.

No	Name
1	*Botrytis cinera*
2	*Mucor sp.*
3	*Fusarium sp.*
4	*Alternaria alternata*
5	*Aspergillus niger*
6	*Mucor circinelloides*
7	*Pestalotiopsis sp.*

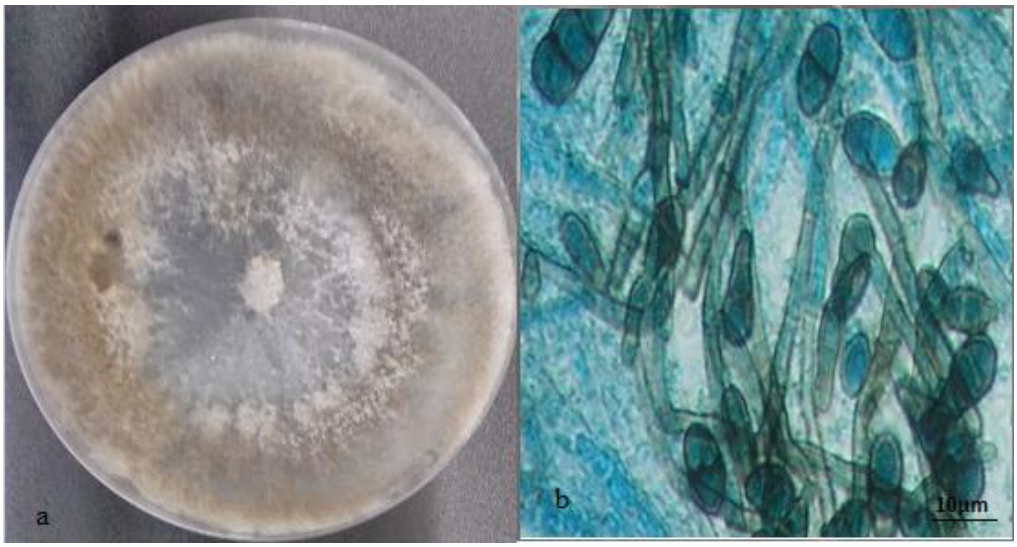

Figure 1. Morphological identification structures (*Botrytis cinera*). A) Colony image B) Mycelium image

Molecular Identification: ITS rDNA gene region was used to identify fungal species. After amplification PCR products were sent to sequencing to Macrogen (Holland). Molecular identification was made by comparing sequences with GenBank using BLASTn. Seven fungal species were found in accordance with morphological results (Table 2).

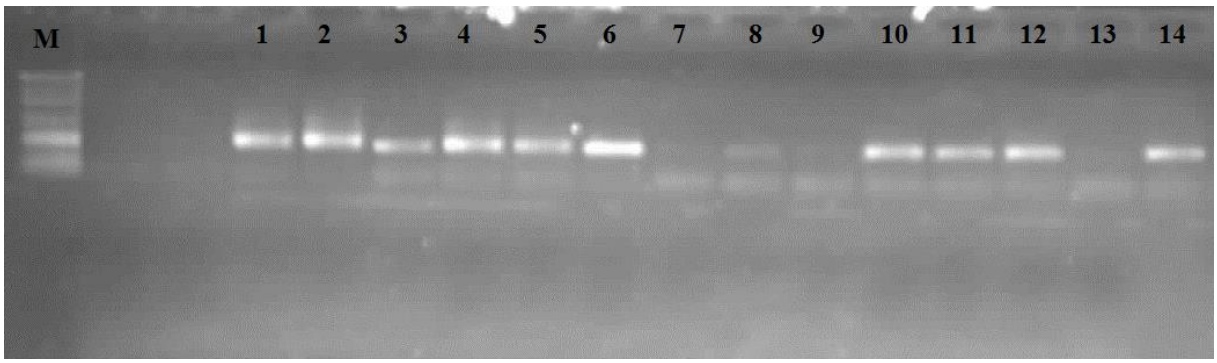

Figure 2. ITS PCR results of samples. (M: 100bp marker (GenMark), 1-14: Samples)

Table 2. Molecular Identification of species.

No	Name	Number of Isolates	Accession No
1	*Botrytis cinera*	10	KP151607.1
2	*Pestalotiopsis clavispora*	1	JF327826.1
3	*Mucor circinelloides*	4	KJ584557.1
4	*Mucor racemosus*	6	JN205991.1
5	*Alternaria alternata*	2	KP661568.1
6	*Fusarium oxysporum*	3	GQ121286.1
7	*Mucor fragilis*	4	JF327830.1

MEGA 7.0 was used to infer a phylogenetic tree. Maximum parsimony method was used to construct a tree (Figure 3). MP tree was obtained using the Subtree-Pruning-Regrafting (SPR) algorithm with search level 1 in which the initial trees were obtained by the random addition of sequences (10 replicates).All positions with less than 95% site coverage were eliminated.

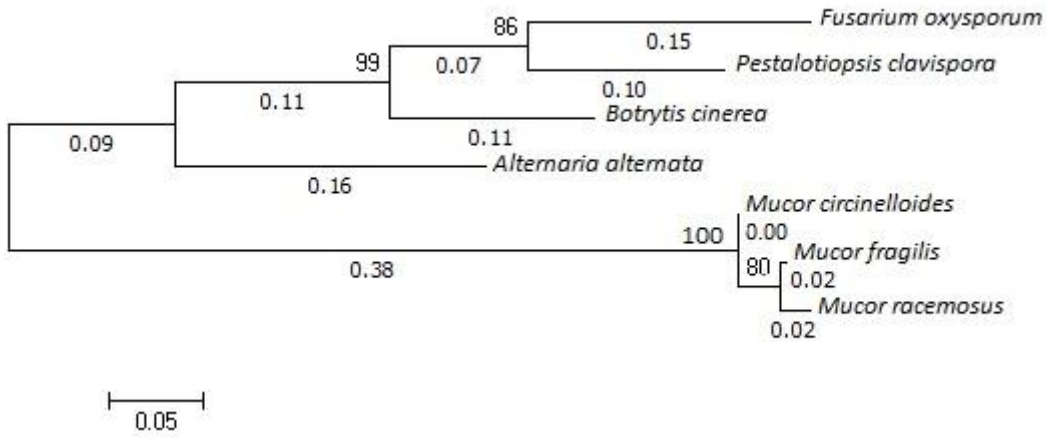

Figure 3. The evolutionary history was inferred using the Maximum Parsimony method. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (1000 replicates) is shown next to the branches. All positions containing gaps and missing data were eliminated. Evolutionary analyses were conducted in MEGA7.

4. Discussion

Because only spoiled fruits were used in this study all species found were associated with diseases. If other plant parts and soil were used more species can be found both pathogenic and non-pathogenic. Literature shows that procedures, such as gathering and transporting, natural products may experience physical damage that builds post-reap misfortune and the likelihood of contagious pollution [15, 16].

Kasiamdari et al. (2002), isolated *Rhizoctonia solani* CFM1 isolate from soil-grown cabbage, designed two primer sequences from the ITS gene region by Nested-PCR method and indicated that molecular methods would provide more advantages than microscopic methods [17]. Staats et al. (2004) used the DNA sequence of 3 nuclear protein-coding genes (RPB2, G3PDH and HSP60) to classify *Botrytis* spp. And compared them to conventional classifications. Phylogenetic analyses indicated that *Botrytis* spp. Separated from Sclerotiniaceae species, of the species had only 4 species, while line 2 contained 18 species

[18]. Khairnar et al. (2011) studied the soil-borne fungal biodiversity of some fruit crops in India and found 21 fungal species and suggested that all fungal species can be controlled with 500 ppm Moximate [19]. Abdelfattah et al. (2015) researched fungal biodiversity of olive and found 195 different Operational Taxonomic Units (OTUs). They found Ascomycota was the most abundant phyla that can be found in olives [20]. Mailafia et al. (2017) researched fungi associated with fruit species and identified six different fungi and one yeast species [6].

Pestalotiopsis clavispora causes crown rot and leaf spot on strawberries [21, 22]. Botrytis cinera is the cause of gray mold disease [23]. *Alternaria alternata* is the cause of leaf spot disease over 380 plant species [24]. *Mucor circinelloides* is both a plant and human pathogen [25]. *Mucor racemosus* is a plant pathogen that can cause allergic reactions in humans [26]. *Mucor fragilis* is reported as a growth promotor in plants [27]. *Fusarium oxysporum* is the cause of fusarium wilt disease [28].

5. Conclusion

This study was conducted in order to find fungal biodiversity on strawberries. As a result of this study seven fungal species were identified both by morphological and molecular methods. Spoiled fruits were used in study therefore all fungi identified were pathogenic. Although fungicides were used in the field fungal diseases, such as gray mold, can still be seen frequently. Further studies must be conducted to prevent these diseases.

Acknowledgements

This study was realized in Adnan Menderes University, Faculty of Science and Literature, Biology Department, Microbiology Laboratory with the support of Scientific Research Department (Project No: FEF-16022).

6. References

[1] Reddy, C.S., Ghai, R., Rashmi, K., Kalai, V.C. (2003). Polyhydroxyalkanoates: an overview. *Bioresource Technol*., 87(2), 137-146.

[2] Ikhiwili, O.M. (2012). Isolation and Characterisation of Microorganisms Associated with Rot Diseases of Fruit, Stem and Leaf of Carica papaya L. A Project Report Submitted to the Department of Biological Sciences, College of Science and Technology, Covenant University, Canaanland, Ota, Ogun state, Nigeria. 5-6.

[3] Singh, D., Sharma, R.R. (2007). Postharvest diseases of fruit and vegetables and their management. In: Prasad, D., editor. Sustainable Pest Management. Daya Publishing House, New Delhi, India.

[4] Al-Hindi, R.R., Al-Najada, A.R., & Mohamed, S.A. (2011). Isolation and identification of some fruit spoilage fungi: Screening of plant cell wall degrading enzymes. *Afr. J. Microbiol. Res.*, 5(4), 443-448.

[5] Droby, S. (2006). Improving quality and safety of fresh fruits and vegetables after harvest by the use of biocontrol agents and natural materials. *Acta Hortic*., 709, 45-51.

[6] Mailafia, S., Okoh, G.R., Olabode H.O.K, Osanupin, R. (2017). Isolation and identification of fungi associated with spoilt fruits vended in Gwagwalada market, Abuja, Nigeria. *Vet. World*, 10(4), 393-397.

[7] Hafez, E.E., Elbestawy, E. (2008). Molecular Chracterization of Soil Microorganisms: effect of industrial pollution on distribution and biodiversity. *World J Microbiol Biotechnol.* 25, 215-224.

[8] Bardgett, R.D. (2005). *The biology of soil: a community and ecosystem approach*. Oxford University Press Inc, New York.

[9] Hawksworth, D.L. (2001). The magnitude of fungal diversity: the 1.5 million species Estimate. *Mycol. Res.,* 105(12), 1422-1432.

[10] Abdullah, Q., Mahmoud, A., Al-harethi, A., (2016). Isolation and Identification of Fungal Post-harvest Rot of Some Fruits in Yemen. *PSM Microbiol*., 1(1), 36-44.

[11] Jensen, B., Knudsen, I.M., Andersen, B., Nielsen, K.F., Thrane, U., Jensen, D.F., & Larsen, J. (2013). Characterization of microbial communities and fungal metabolites on field grown strawberries from organic and conventional production. *Int. J. Food microbiol.*, 160(3), 313-322.

[12] Samson, R.A., Hoekstra, E.S. and Frisvad, J.C. (2004). Introduction to Food-and Airborne Fungi, *Laboratory Manual Series 2, Food and Indoor Fungi*, 389pp.

[13] Doyle J.J., Doyle J.L. (1987). Isolation of Plant DNA From Fresh Tissue. *Focus*, 12, 13-15.

[14] Boysen M., Skoube P., Frisvad, J., Rossen, L. (1996). Reclassification of the Penicillium roqueforti group into three species on the basis of molecular genetic and biochemical profiles. *Microbiol*., 142, 541-519.

[15] Baiyewu, R.A., Amusa, N.A., Ayoola, O.A., & Babalola, O.O. (2007). Survey of the postharvest diseases and aflatoxin contamination of marketed Pawpaw fruit (Carica papaya L.) in South Western Nigeria. *Afr. J. Agric. Res.*, 2(4), 178-181.

[16] Chukwuka, K.S., Okonko, I.O., & Adekunle, A.A. (2010). Microbial ecology of organisms causing pawpaw (Carica papaya L.) fruit decay in Oyo State, Nigeria. Am. *Eurasian J. Toxicol. Sci.*, 2(1), 43-50.

[17] Staats, M., Baarlen, P.V., Kan, J.A.V. (2004). Molecular Phylogeny of the Plant Pathogenic Genus Botrytis and the Evolution of Host Specificity. *Mol. Bio. Evol*., 22(2), 333-346.

[18] Kasiamdari, R.S., Smith, E.S., Scott, E.S., Smith, F.A. (2002). Identification of binucleate Rhizoctonia as a contaminant in pot cultures of arbuscular mycorrhizal fungi and development of a PCR-based method of detection. *Mycol. Res*. 106 (12), 1417-1426.

[19] Khairnar, D.N., Kelhe, A.S., Khairnar, A.B. (2011). Soil-borne Fungal Biodiversity of Some Fruit Crops of Nashik District and Control Measures. *Nat. Envir. Pollution Tech*., 10(1), 127-128.

[20] Abdelfattah, A, Li Destri Nicosia, M.G, Cacciola S.O., Droby, S, Schena, L. (2015). Metabarcoding Analysis of Fungal Diversity in the Phyllosphere and Carposphere of Olive (Olea europaea). *PLoS ONE*, 10(7), e0131069.

[21] Van Hemelrijck, W., Ceustermans, A., Van Campenhout, J., Lieten, P., Bylemans, D. (2017). Crown rot in strawberry caused by Pestalotiopsis. *Acta Horticul*., 1156, 781-786.

[22] Zhao, J.N., Ma,, Z., Liu, Z.P. (2016). Pestalotiopsis clavispora causing leaf spot on strawberry. *Mycosystema*, 35(1), 114-120.

[23] Adrian, M., Jeandet, P., Veneau, J., Weston, L.A., & Bessis, R. (1997). Biological activity of resveratrol, a stilbenic compound from grapevines, against Botrytis cinerea, the causal agent for gray mold. *J. Chem. Eco*., 23(7), 1689-1702.

[24] Lagopodi, A.L., & Thanassoulopoulos, C.C. (1998). Effect of a leaf spot disease caused by Alternaria alternata on yield of sunflower in Greece. *Plant Disease*, 82(1), 41-44.

[25] Johnson, G. I., Sangchote, S., & Cooke, A. W. (1990). Control of stem end rot (Dothiorella dominicana) and other postharvest diseases of mangoes (cv. Kensington Pride) during short-and long-term storage. *Tropic. Agricult. Trinidad and Tobago*, 67(2), 183-187.

[26] Narasimhan, M.L., Damsz, B., Coca, M.A., Ibeas, J.I., Yun, D.J., Pardo, J.M., & Bressan, R.A. (2001). A plant defense response effector induces microbial apoptosis. *Mol. Cell*, 8(4), 921-930.

[27] Menkis, A., Vasiliauskas, R., Taylor, A.F.S., Stenström, E., Stenlid, J., & Finlay, R. (2006). Fungi in decayed roots of conifer seedlings in forest nurseries, afforested clear-cuts and abandoned farmland. *Plant Patho*., 55(1), 117-129.

[28] Di Pietro, A., García-Maceira, F. I., Meglecz, E., & Roncero, M.I.G. (2001). A MAP kinase of the vascular wilt fungus Fusarium oxysporum is essential for root penetration and pathogenesis. *Mol. Microbiology*, 39 (5), 1140-1152.