INTERNATIONAL JOURNAL OF CONSERVATION SCIENCE
ISSN: 2067-533X Volume 13, Issue 3, July-September 2022: 855-864 www.ijcs.ro

ASSESSMENT OF GROWTH REQUIREMENTS OF BIOLOGICAL DEGRADATION ON THE COATING GELATIN LAYER ON HERITAGE PHOTOGRAPHS

Rasha SHAHEEN[1*], Abdelrahman M. ELSEROGY[2], Shaaban Abd ELAA[2], Mona ALI [3], Abdelrahman METWALY[4], Mohamed Eida[5], Ahmed Gad[6]

[1] Egyptian Museum, Conservation Dept., Tahrir Sq., Cairo, Egypt.
[2] Conservation Dept., Faculty of Archaeology, Fayoum University, Fayoum, Egypt.
[3] Conservation Dept., Faculty of Archaeology, Cairo University, Cairo, Egypt.
[4] The Central Lab for Date Palm Research & Development, Agricultural Research Center, Ministry of Agriculture, Cairo, Egypt.

Abstract

Photographic archives in museums, libraries, and private collections are exposed to many environmental factors that are threatening their sustainability. Microorganisms are considered one of the most threatening factors of the extinction of gelatin photograph prints, due to the nature of the components of the photographs. This paper presents the identification of micro-entities growing on gelatin photograph prints as a first step to study the effect of using Nanomaterials to treat microbiological damage on gelatin photographs, which will be explained in part II. UV photography was used to identify the extent of biological damage on the image and where the spores were present. USB microscope 60X with UV-LED 400nm was used to study the appearance of biological damage on the surface of the coating gelatin layer and paper. Samples were grown in a suitable nutrient medium. Czapek's medium was used to isolate and grow fungal isolates. The resulting organisms were grown in specialized environments, which are cellulose agar and gelatin environment agar. Microorganisms were defined. Growth requirements were determined. It was recommended to control the temperature and humidity in the conservation environment to be between 15 - 20°C and 30% - 50% humidity. The potential daily change is 3°C for temperature and 10% for humidity. By identifying the growth requirements of these organisms, the surrounding environment was controlled to stop the growth of these organisms, in preparation for treating those using Nanomaterials in the second part of the study.

Keywords*: Coating gelatin layer; Photography prints; Biodeterioration; Biodegradation; Microorganisms; Growth requirements.*

Introduction

Gelatin silver black and white photograph print-out appeared in the late 1880s and were used until the twenties in the nineteenth century. It generally consists of several basic layers which are coating gelatin layer, baryta, and supporting paper. Baryta layer has been added to the sheets of matte and glossy gelatin, which represents the thick surface layer that can hide the paper's fibers even under an electron microscope [1]. Gelatin is the medium in which light-sensitive silver salts are deposited. Gelatin performs the required purpose in the manufacture of sensitive emulsions, as it covers and encapsulates light-sensitive silver salts and is distinguished by its ability to absorb water and then swelling. It also allows disposal of water when necessary.

* Corresponding author: rashashaheen55@yahoo.com

It is compatible because it melts in the water at 100 degrees Fahrenheit, but it can withstand higher temperatures when it is strengthened with special materials, then it melts at 170°F (49°C). Also, its melting allows the materials to be deposited in it, such as silver salts, and then returns to the freezing state when it cools. All these characteristics, which are swelling, melting, solidifying, and drying, make it an ideal medium in the manufacture of sensitive layers because the swelling property would allow light-sensitive silver salts to penetrate and diffuse in it [2, 3].

The relative humidity is considered one of the most important factors of atmospheric damage that affects photographic prints since photographs are composed of organic materials represented in gelatin and paper, which are hygroscopic materials that are affected by high and low humidity. Relative humidity plays an important role in the deterioration of photographs, and the process of deterioration in photographs is accelerated with the high level of humidity. The gradual increase in the percentage of humidity leads to the spread of fungi and bacteria in the images, thus lead to severe damage to photographs, prints, damage, and the collapse of their physical and mechanical properties [4]. The change in temperature is considered one of the destructive factors of photographs, as heat represents a form of energy that increases the speed of chemical reactions and leads to damage of the photographs. The high and low temperature factor affects the material of the photographs, so the images dry out and lack their elasticity due to the frequency between heat, cold, and the difference of the expansion coefficient [5, 6]. The decrease in the temperature means the transformation of water vapour into drops that condense, encourage biological growth, and work to attract dust and pollutants from the surrounding atmosphere, in addition to raising the water content of the photographic components and thus changing the physical and mechanical properties of the images. There is an inverse relationship between humidity and temperature: chemists state that each rise of 10°C in temperature is matched by doubling in the rate of chemical reaction "decomposition" [7-12].

Experimental part

Materials and Methods

The sample used is black and white gelatin photograph prints dating back to the 1950s, with biological damage due to poor storage (Fig.1).

Fig. 1. Shows black and white gelatin photograph prints dating back to the 1950s:
A: presents the photo in the wooden frame before treatment, B: presents the back of the frame

It is part of Mrs Alia Ali's collection. Swabs was taken from the surface of the supported paper and the coating gelatin surface of the image. The swabs were taken in the Regional Center for mycology and biotechnology, Al-Azhar University. Fungi have been defined in the microbiology laboratory at the Research and Preservation Center of the Ministry of Antiquities. Bacteria were defined in the Central Lab of Armed Forces. The swabs were placed in an activation environment for 48 hours in the incubation with the fungi dish placed at 26°C and the bacteria dish at 24°C. Specialized environments are established to isolate and identify fungi and bacteria. USB microscope 60X with UV-LEDS 400nm was used to identify the form of biological damage and its destructive effect on the coating gelatin layer and the supported paper. The photograph is contained in a wooden frame and has a glass cover, in order to identify the type of biological damage on the layers of the photograph. It was necessary to separate the image from the frame (Fig. 1).

Result and Discussion

Ultraviolt Photogrphy

The Fujifilm FinePix IS Pro digital SLR camera was used for ultraviolet photography with a frequency response rated from 295 to 325nm to study the deterioration and structures that are not apparent under visible light as evidence of infection (Fig. 2).

Fig. 2. Shows UV photography: A. the photo before UV photography;
B. the photo after UV photography; Note the fluorescence of Microorganisms' places

USB Microscope 60X with UV-LED

The USB microscope 60X with UV-LED 400nm was used to recognise the damage and study the appearance of biological damage on the surface of the coating gelatin layer and paper. Using UV-LED 400nm to show the appearance of biological infection, the extent, and the size of the damage by the growth of these microbial organisms on the surface (Fig. 3).

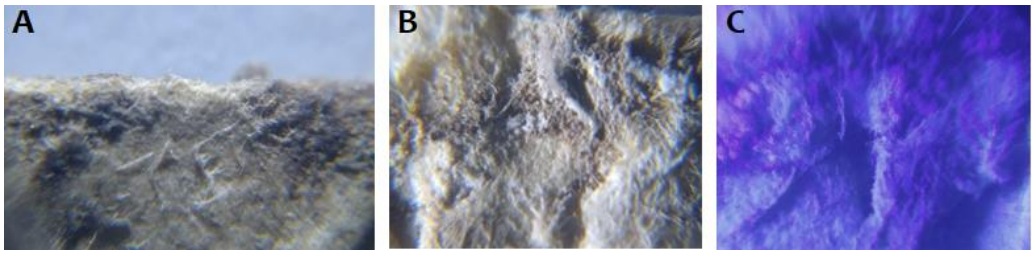


Fig. 3. Shows the forms of damage on supported paper (secondary support):
A. Damage without magnification; B. The shape of biological damage at 60X magnification;
C. The biological damage form at 60X magnification using UV fluorescence

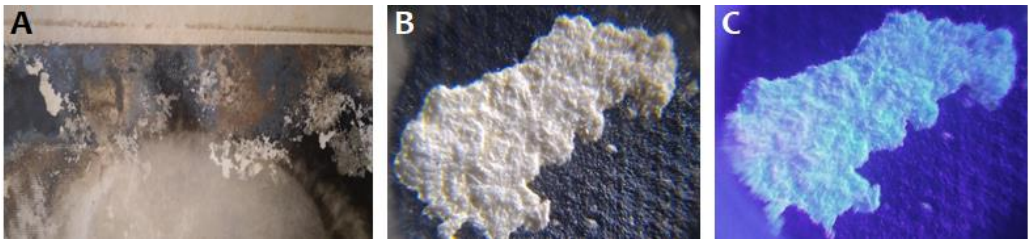


Fig. 4. Shows the forms of damage on coating gelatin layer:
A. Damage without magnification; B. the shape of biological damage at 60X magnification;
B. The biological damage form at 60X magnification using UV fluorescence, that shows fungal spore

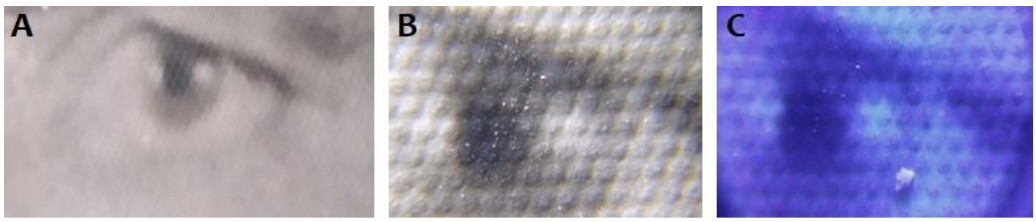


Fig. 5. Shows the forms of the coating gelatin layer without damage:
A. Damage without magnification; B. The shape of biological damage at 60X magnification;
C. The coating gelatin layer at 60X magnification using UV fluorescence, note that there is no fungal spore

Microorganism identification

Cultivation of samples was done on appropriate food media. The samples were suitable for the cultivation of microbial growth and so as to know the amount and types of microbial load on the gelatin photographs specimen's environments. Czapek's medium was used for isolation, maintenance, and growth of fungal isolates (Table 1).

Table 1. Sucrose Agar Environment (Czapek's medium)

Component	**g/L^{-1}**	**Component**	**g/L^{-1}**
Sucrose	30.0	**KCL**	0.5
$NaNo_3$	2.0	**$FeSO_4 \cdot 7H_2O$**	0.01
K_2HPO_4	1.0	**Agar**	15.0
$MgSO_4 \cdot 7H_2O$	0.5	**Distilled water (mL)**	1000

Isolation and Purification

The resulting organisms were grown in specialized environments that stimulate the image material, which is cellulose environment agar and gelatin environment agar (Tables 2 and 3).

Table 1. Cellulose environment agar

Component	g/L-1
Cellulose	30.0
$NaNo_3$	2.0
K_2HPO_4	1.0
$MgSO_4 \cdot 7H_2O$	0.5
KCl	0.5
$FeSO_4 \cdot 7H_2O$	0.01
Agar	15.0
Distilled water (mL)	1000

Table 2. Gelatin environment agar

Component	g/L-1
Gelatin	30.0
$NaNo_3$	2.0
K_2HPO_4	1.0
$MgSO_4 \cdot 7H_2O$	0.5
KCl	0.5
$FeSO_4 \cdot 7H_2O$	0.01
Agar	15.0
Distilled water (mL)	1000

Definition of biological organisms

The objects that have been purified was grown on private food environments by definition. The work of microbial sliced them to know the morphological characteristics and to compare the qualities of the standard morphological existing books and specialized scientific references the definition of micro-organisms Domsch et al definition, (1980) [13], and Samson (1995) [14].

Species Description

a. **Fungi.** Five kinds of fungi have been identified, and they are shown in table 4.

Penicillium spp is usually found in soil, in decaying and dried organic matter. Also, in fresh vegetables and fruits. As well As, it is found on the surfaces of water-damaged building materials, and in house dust. Penicillium is mesophilic fungi, growing between 5-37°C (optimal, 20 – 30°C) at pH = 3-4.5. Maximum growth in vitro is obtained at 23°C with pH = 3-4.5. Water Activity: 0.78 - 0.88. Penicillium has been found in up to 53% of contaminated homes before treatment in great amounts [15].

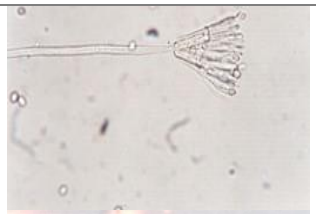
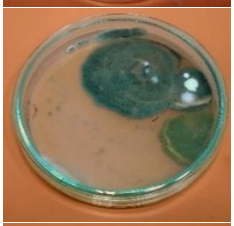
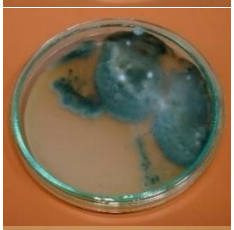
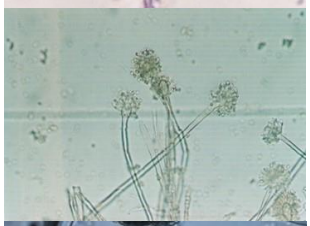
Aspergillus Niger (*A. Niger*) is a common occurrence. It grows aerobically on organic matter. This species is a common contaminant on various substrates, found in soil and litter, in compost, and on decaying plant material. It can even be found in icy environments and marine environments, but usually prefers dry and warm soils. A. Niger is a mesophilic fungus: its optimal growth temperature is 20-40°C, with good growth at 37°C. It can survive at 60°C, can be killed by exposure at 63 °C for 25 minutes. This species is xerophilic and requires a minimal Aw (available water) of 0.77. The species can, however, grow very well within an environment of 90-100% relative humidity. It can also grow at a very low pH (2.0) [16].

Aspergillus Flavus (*A. flavus*) is imperfect filamentous fungi. It is having a worldwide distribution, mostly growing as a saprophyte in the soil. It is an opportunistic pathogen causing invasive and non-invasive aspergillosis in humans, animals, and insects. This Aspergillus also infects agricultural crops and contaminates stored grains while producing the most toxic and potent carcinogenic metabolites such as aflatoxins and other mycotoxins. A. flavus is a mesophilic fungus; it can grow between 17-19 and 47-48°C, with optimal growth between 25

and 42°C. The optimum growth pH is 7.5. Optimal growth is obtained with a relative humidity of 80 to 85%. A. flavus is known to be able to grow on paper, wood, painted building materials, textiles, and leather; it has even been found on synthetic materials, varnishes, and waxes as well as on electronic parts and photographic glass plates [17].

Aspergillus Sulphureus (*A. sulphureus*) are a ubiquitous and species-rich genus, currently containing more than 300 filamentous fungi. The genus covers a wide range of phenotypes and has a substantial economic footprint, as it includes fermenters of foodstuffs, key cell factories for production of enzymes and organic acids, plant pathogens, model organisms for cell biology, human opportunistic pathogens, producers of animal and human mycotoxins, and degraders of a wide range of organic biomass relevant for bioenergy conversion [18].

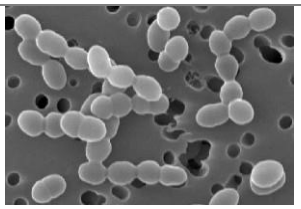
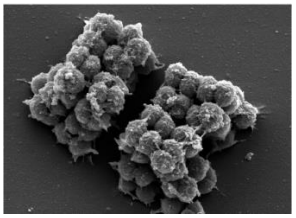
Table 3. The purified isolated fungi from the coating gelatin layer and the paper

No	*Plate Photo*	*Microscope Photo*	*Molecular Identification*	*Sources*
1			*Penicillium spp*	*https://www.uniprot.org/taxonomy/104259*
2			*Aspergillus niger*	*https://www.uniprot.org/taxonomy/2747892*
3			*Asp. flavus*	*https://www.uniprot.org/taxonomy/691689*
4			*Aspergillus sulphoreus*	*https://www.uniprot.org/taxonomy/138284*
5			Asp Fumigatus	https://www.uniprot.org/taxonomy/330879

Aspergillus Fumigatus (*A. Fumigatus*) is a heat-resistant and thermophilic species that is able to grow between 12 and 57°C (on average at a temperature between 37 and 43°C). Its maximum growth is achieved in vitro when incubated at 37°C, at a pH of 3 to 8. The minimum amount of free water (Aw), between 0.82 and 0.97 (Aw = 0, 90), is necessary for the species to germinate. This fungus remains viable at temperatures up to 70°C and can tolerate pasteurization for 25 minutes. Fumigatus grows easily in the indoor environment on damp building materials (drywall, wood, chipboard, hardboard ceiling panels, insulation materials), on these materials, this fungus typically produces a medium-sized growth area from gray-to-gray green. The Fumigatus also grows well on organic substrates indoors such as house dust and on any building materials that contain cellulose, it is also developing in humidifiers, air conditioning systems, ventilation ducts, and air filtration systems. These fungi can also contaminate everyday items made of cloth, leather, or paper [19].

b. **Bacteria.** Two kinds of bacteria have been identified, and they are shown in table 5.

Table 4. The purified isolated bacterial from the gelatin layer and the paper

No	*Plate Photo*	*Microscope Photo*	*Molecular Identification*	*Sources*
1		 After Shin, 2015	Leuconostoc mesenteroides	https://bacteria.ensembl.org/Multi/Search/Results?species=all;idx=;q=Leuconostoc%20mesenteroides;site=ensemblunit
2		 After Sims, 2009	Kytococcus sedentarius	https://bacteria.ensembl.org/Multi/Search/Results?species=all;idx=;q=Kytococcus%20sedentarius;site=ensemblunit

Leuconostoc mesenteroides (*L. mesenteroides*) are non-porous and non-motile species. It is anaerobic and a member of the family of lactic acid bacteria. Species appear as cocci, and they form long or double chains as they grow. L. mesenteroides are found on the surface of fruits and vegetables. Under anaerobic conditions, L. mesenteroides are responsible for initiating fermentation processes in many different foods, such as fermentation of dairy products and fermentation of bread types. The complete fermentation process cannot be completed without the presence of L. mesenteroides. This demonstrates the importance it enjoys in shaping its own environment as a specialized builder. During the fermentation of meat and dairy, it produces the protein bacterioicin, which prevents the growth of other bacterial species, indicating that it is feeding on its environment [20, 21].

Kytococcus sedentarius (*K. sedentarius*) is known for the production of polyketide antibiotics as well as for its role as an opportunistic pathogen. It is strictly aerobic and can only grow when amino acids are provided. Optimum growth temperature is 25-37°C. It is primarily isolated from human skin and is one of the major causes of pitted keratolysis [22].

Conclusion

The previous results show that the fungi and bacteria that were caused damage to the gelatin photograph subject of the study are damaging and harmful organisms, as they fed on the gelatin layer and silver halides and it also caused damage to the paper supporting the photograph. By identifying the growth requirements of these organisms, the surrounding environment was controlled to stop the growth of these organisms, in preparation for treating those using Nanomaterials in the second part of the study.

Acknowledgements

We would like to gratefully acknowledge the help of Dr. Ahmed Gad Taha (MD Microbiology and Immunology) for his great co-operation in identify the bio-organisms. This work was performed under the auspices of the Central Lab of Armed Forces, Egypt.

References

[1] G. Weaver, **A Guide to Fiber-Base Gelatin Silver Print Condition and Deterioration,** Image Permanence Institute, Rochester Institute of Technology, 2008.

[2] S. Calixto, N. Ganzherli, S. Gulyaev, S. Figueroa-Gerstenmaier, *Gelatin as a Photosensitive Material*, Molecules, 23(8), 2018, Article Number: 2064, DOI: 10.3390/molecules23082064.

[3] S. Burgi, *The conservation of silver gelatin prints: research on the applicability of the methylene blue method for measuring residual thiosulfate and polythionates in aged photographs*, **Master Theses**, Rochester Institute of Technology, RIT Scholar Works, 1984.

[4] A. Sherring, S. Lewincamp, *Conservation treatment of a silver gelatin photograph*, **University of Melbourne Collections**, Issue 10, 2012, pp. 36-37.

[5] H. Alten, *Temperature and Relative Humidity, How temperature and relative humidity affect collection deterioration rates*, **Collections Caretaker,** Northern States Conservation Center, Vol. 2, No. 2, 1999.

[6] M. Roosa, **CARE, Handling, and Storage of Photographs,** International Preservation Issues, Number Five, 2004.

[7] A. Yosri, A. Yosri, N. Mohamed, A. Atef, O. Atef, R. Attia, M. Ali, *Analytical Study on the Effects of Pollutants on Silver Gelatin Prints*, **International Journal of Conservation Science, 11**(1), 2020, pp. 39-50.

[8] M.E. Osman, S. Ismael, E. Ciliberto, S. Stefani, Y.M. Elsaba, *Evaluation of Microbial Deterioration of Silver Gelatin Photographs Stored in an Old Photographic Archive*, **International Journal of Conservation Science, 13**(2), 2022, pp. 527-540.

[9] M. Ali, M. Fawzy, *The Effect of The Vapors of Clove and Lavender Oils on the Chemical and Optical Properties of Silver Gelatin Prints in Wood Frames*, **International Journal of Conservation Science, 12**(3), 2021, pp. 961-976.

[10] O. Abdel-Aziz, M. Mahmoud, Y. Abo-Elfath, S. Abdel-Aziz, M. Samir, M. Ali, *Evaluation of Conventional Treatments for Mirrored Silver Gelatin Prints: Experimental and Applied Study*, **International Journal of Conservation Science, 10**(1), 2019, pp. 81-96.

[11] R. Shaheen, M. Fouad, O. Saqr, S. Reda, A. Labeeb, *Assessment of the Photo-Chemical Degradation of Silver Gelatin Photograph Print-Out*, **International Journal of Conservation Science, 11**(4), 2020, pp. 1093-1102.

[12] M. Yosri, M. Ali, F. Stanco, K. Talaat, *Restoration of Silver Gelatin Prints in the Digital Era: An Innovative Approach*, **International Journal of Conservation Science, 9**(3), 2018, pp. 375-388.

[13] K.H. Domsch, et al, Compendium of Soil Fungi. Academic Press, New York, USA, 1980, pp. 1156.

[14] R.A. Samson, J.I. Pitt, **Modern Concepts in Penicillium and Aspergillus Classification,** Ed., NATO ASI Series, Series A, Vol. 185. Plenum Press, New York, USA, 1989.

[15] F.J. Lichtner, V.L. Gaskins, K.D. Cox, W.M. Jurick, *Global transcriptomic responses orchestrate difenoconazole resistance in Penicillium spp. causing blue mold of stored apple fruit*, **BMC Genomics, 21**(1), 2020, Article Number: 574, DOI: 10.1186/s12864-020-06987-z.

[16] A.M. Abdel-Azeem, F. Mahmoud Salem, M. Abdel Azeem, M. Allam Nafady, Biodiversity of the Genus Aspergillus in Different Habitats (Chapter), **New and Future Developments in Microbial Biotechnology and Bioengineering,** Elsevier, 2016, pp. 3-28, DOI:10.1016/B978-0-444-63505-1.00001-4.

[17] M.T. Hedayati, A.C. Pasqualotto, P.A. Warn, P. Bowyer, D.W. Denning, *Aspergillus flavus: human pathogen, allergen and mycotoxin producer*, **Microbiology-SGM**, **153**, 2007, pp. 1677-1692.Part 6, DOI: 10.1099/mic.0.2007/007641-0.

[18] H. Al-Sheikh, H.M.A. Abdelzaher, *Isolation of Asperagillus sulphureus, Penicillium islandicum and Paecilomyces variotii from Agricultural Soil and their Biological Activity Against Pythium spinosum, the Damping-Off Organism of Soybean*, **Journal of Biological Sciences, 10**(3), 2010, pp. 178-189, DOI: 10.3923/jbs.2010.178.189.

[19] A. Lonvaud-Funel, **LEUCONOSTOC, Encyclopedia of Food Microbiology**, Elsevier, 1999, pp. 1183-1194.

[20] T.M. Hohl, M. Fedmesser, *Aspergillus fumigatus: Principles of Pathogenesis and Host Defense*, **Eukaryotic Cell, 6**(11), 2007, pp. 1953-1963, DOI: 10.1128/EC.00274-07.

[21] S. Shin, N.S. Han, *Leuconostoc spp. as Starters and Their Beneficial Roles in Fermented Foods*, **Microbiology Monographs**, Vol. 27, Springer, pp. 111-132.

[22] D. Sims, T. Brettin, J.C. Detter, C. Han, A. Lapidus, A. Copeland, T.G. Del Rio, M. Nolan, F. Chen, S. Lucas, H. Tice, J.F. Cheng, D. Bruce, L. Goodwin, S. Pitluck, G. Ovchinnikova, A. Pati, N. Ivanova, K. Mavromatis, A. Chen, K. Palaniappan, P. D'haeseleer, P. Chain, J. Bristow, J.A. Eisen, V. Markowitz, P. Hugenholtz, S. Schneider, M. Goker, R. Pukall, N.C. Kyrpides, H.P. Klenk, *Complete genome sequence of*

Kytococcus sedentarius type strain (541T), **Standards in Genomic Science**, **1**(1), 2009, pp. 12-U4, DOI:10.4056/sigs.761.

Received: July 07, 2021
Accepted: August 2, 2022