Chapter 8

Endophytic Yeast and Hosts: A Mutualistic Association Friendly to the Environment

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Additional information is available at the end of the chapter

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Abstract

Recent studies have shown that endophytic yeasts benefit their host, which has stimulated their use in different applications in agribusiness. The research has focused on evaluating the effectiveness of handling these yeasts to solve problems such as biocontrol of pathogens, plant growth and/or improvements in the quality of fruits and vegetables. However, in order to obtain information that contributes to the selection and the implementation of a yeast able to interact with a broader spectrum of hosts and to help solve postharvest problems, it is necessary to deepen the knowledge on the association of these symbionts and to establish possible changes in the host, the issues that are covered in this chapter. The results show that the endophytic yeasts can generate structural changes in the host as a starting point for further applied research and to propose other mechanisms of action.

Keywords: biocontrol, endophytic yeast, mode of action, mutualism, postharvest

1. Introduction

The relationship between plants and microorganisms has been classified as a symbiosis; however, when referring to endophytic yeasts, this association takes a mutualistic character. While the plant is providing the yeast a propitious space to live, the yeast offers benefits to the plant, which are mainly related to the biological control of pathogens, encouraging their use as an alternative method for the management of postharvest diseases of fruits and vegetables [1].

Nevertheless, the knowledge regarding the dynamics of host colonization by the endophyte to understand this mutualistic relationship as well as the evaluation of the inoculated host is still limited. Isaeva et al. [2] state that the research on endophytic yeasts has not been carried out in a systematic way, so the existing information is incomplete. They also identify the need to know the distributional patterns and biological properties of endophytic yeast, in order to understand the ecological characteristics of these yeasts and propose solutions to various postharvest problems.

The fact that endophyte yeasts can live in the host involves studying the dynamics of colonization within the host and establishing whether it is affected by providing a habitat for the yeast surviving, so it is necessary to use alternative methodologies that allow visualizing both the yeast and the host, as well as changes inside it.

Accordingly, the results obtained by implementing techniques of microscopy and magnetic resonance imaging (MRI) in order to evaluate the interaction between a host and an endophyte yeast are explained below. These pieces of evidence allow to deepen the knowledge of this mutualistic relationship and to propose another mode of action of the yeasts in which these indirectly contribute to prolonging the useful life of the host.

2. Endophytic yeasts and plants: a mutualistic action

Etymologically, the word endophyte means "within the plant." This definition encompasses a wide variety of residents and hosts, this last including bacteria, fungi, insects and algae among others [3]. Among the definitions proposed for the term endophyte is "Fungus that colonizes plant tissue without causing any immediate negative effect" [4]. Even so, some authors consider that this definition excludes other microorganisms such as bacteria and algae. In this context, Stone et al. [5] argue that a more wide-ranging definition should emphasize the asymptomatic nature of the infection without taking into account a particular group of organisms. That is why Petrini [6] explains endophyte from a topographical perspective: "An endophyte colonizes and can live inside the living tissues of it is host without causing damage."

Xin et al. [7] ponder all these aspects and characterize endophytic yeast as: "Unicellular fungi that reproduce asexually by budding—without a hyphal phase or with a reduced hyphal phase—and can live in their host without generating apparent harm." Pieces of research show that these yeasts can be isolated from different parts of plants (see **Table 1**).

Yeast	Isolated from	References
Williopsis saturnus	Maize (*Zea mays* L.) roots	Nassar et al. [10]
Wild poplar strain 1 (WP1)	Wild cottonwood (*Populus trichocarpa*)	Xin et al. [7]
PTD 2	Stems of hybrid poplar (*Populus trichocarpa* x *Populus deltoides*)	Xin et al. [7]
Candida guilliermondii	Heterograft tomato crop (HGTC)	Celis et al. [24]

Table 1. Some endophytic yeast reported.

In recent years, there has been an increase in research on how endophytic yeast benefits the host; it has been established that in some cases, it contributes to the protection against pathogens. Therefore, it is possible to use them successfully as agents for biological control [8, 9]. Also, some studies have shown that these yeasts foster the growth of plants by means of bringing out auxins, as reported by Nassar et al. [10], who isolated the endophytic yeast *Williopsis saturnus* and found that it is capable of producing indole-3-acetic acid (IAA), a growth hormone. In addition, Zhao et al. [11] discovered that exogenous administration of *Pichia guilliermondii* improved the postharvest lifetime and the quality of cherry tomato fruits stored.

This association between plant and microorganisms is denominated symbiosis, a term coined by Anton De Bary as: "The association, at least for part of its life cycle, between two or more specifically different organisms" [12]. For the host plant, this relationship can be positive (mutualism); neutral (neutralism), or negative (parasitism or competition). For the symbiotic microorganism, the association can be positive (mutualism, commensalism, or parasitism), neutral (neutralism), or negative when there is competition. A symbiosis is successful provided that it involves at least the following three events: (i) the symbiont's entrance into the tissues; (ii) their colonization and (iii) the expression of one of the symbiotic relationships mentioned above. The symbiont must be able to have a relationship with the host to establishing a compatible interaction, which implies that it overcomes or manipulates the host defense system [13].

It has been verified that in the case of endophytic yeast, the association is closer to mutualism than to parasitism [2] since yeast can bring to the plant several of the above benefits mentioned. On the other hand, yeasts as copiotroph organisms find in the host plant the nutrients and the suitable environment for their development. Here the question is: how do you experimentally identify whether an endophytic yeast is related in a mutualistic way to its host? It could be answered if we adapt Sieber's proposal [14] of using the Koch's four postulates, modified as follows:

1. The appearance of an endophyte should be associated with a benefit to the host.
2. The endophyte should be isolated from the tissue in which the benefit was observed and grown in a culture medium.
3. The endophyte that has grown in the culture medium should generate the same benefit when it is reintroduced into a host free of the endophyte.
4. Then, again the endophyte should be isolated from the experimentally inoculated host.

In order to identify new endophytic yeast, it is possible to apply these postulates experimentally.

Concerning asymptomatic colonization, characteristic of endophytic yeasts, Schulz et al. [3] suggested a hypothesis in which the absence of negative symptoms is associated with a balance of antagonists: host and endophyte. The endophytes have mechanisms to infect and colonize the host; this, in turn, responds with its defense system. The balance between the "infection system" and the "defense system" generates an asymptomatic interaction;

if the balance is broken, diseases can occur for the host or death of the symbiont. However, the verification of this balance, which is an experimental challenge, is not solved in the study of endophytes yet.

3. Endophytic yeasts and their projection in agro-industry

During the postharvest period, the quality of fruits and vegetables is deteriorated due to different factors: manipulation and improper storage, metabolic events, and phytopathogen attacks generating economic losses of more than 25% of the total production in industrialized countries and more than 50% in developing countries [15, 16].

In the case of fruits, most of these losses are caused by the attack of several fungal pathogens, controlled mainly with synthetic fungicides, which has generated concern regarding possible health risks derived from the consumption of food treated with agrochemicals [17], as a consequence, the demand of organic fruits and vegetables has increased. To deal with this need, healthier and environmentally friendly strategies have been evaluated to control the attack of plant pathogens and to maintain the quality of fruits and vegetables, in that context, microbial antagonists, such as yeasts have emerged as a viable option [18].

To understand how the yeast can be used to solve this problem, we can identify different interactions with the host and with the phytopathogen. In relation to the host, the yeast can colonize the fruit surface for long periods; some of them produce extracellular polysaccharides that contribute to the fruit survival and to restrict the growth of pathogens; they can use nutrients from the environment and proliferate at a high rate. In addition, their activity does not involve the production of toxic metabolites and are less affected by fungicides [1, 19]. When a yeast colonizes the internal tissues of the host without generating damage or is in the interior contributing to lengthen its useful life, this kind can be classified as an endophyte. These aspects make yeast a potential microbial agent able to control postharvest diseases.

In the interaction, the yeast with the phytopathogens is possible to determine different kinds of interactions such as nutrients and space competition, mycoparasitism, secretion of antibiotics, lytic enzymes, and other antifungal compounds. The importance of any one mode of action can vary between biocontrol systems (pathogen, yeast, and host).

Among all the yeasts' modes of action identified, the competition for nutrients and space is considered the most common because yeasts have the ability to grow and survive faster in the environment (host) than pathogens; thus, the bio-controlling activity is associated with an increase in the concentration of the antagonist and a decrease in the concentration of the pathogen [20]. In other cases, yeasts have the ability to adhere to fungal hyphae by restricting pathogen proliferation [21, 22], which is called parasitism and, in some cases, occurs with the production of lytic enzymes, which help bring about degradation of the cell wall of the pathogen. Other yeasts produce antibiotic compounds, case in which the control mechanism is associated with the production of secondary metabolites that inhibit the growth of pathogens [23, 24].

When studying the problem focusing on the host, it has been established that plants have the capacity to defend themselves against pathogen attacks by triggering their defense system, which can be activated by some yeasts; as a result, it is another way of action in which the yeast helps indirectly to reduce the growth and development of the pathogen.

Punja and Utkhede [25] have stated that this process can take place through the production of elicitors (signal compounds) or because of tissue colonization reducing the development of the pathogen. They have pointed out what has been reported by some researchers that the internal colonization of the tissues without causing apparent damage to the cells—characteristic associated with the endophytic yeasts—triggers the defense system of the host.

The entomologists define biocontrol like "the control of the organism by other organism," but when we talk about control of plant's diseases by yeast, the definition of biocontrol is wider because the plant's diseases are a process that involves three elements: pathogen, host, and micro environment. Then, studying the use of yeast in this context implies studying the host to and how this can change by the yeast action.

Therefore, in the case of studies on endophyte yeasts, it is necessary to characterize the host surface and its inner for establishing if it is modified and if so, define the relationship between the changes and the benefits. In regard to the production of elicitors, as a mode of action in biocontrol, this can be understood like a process in which the yeast helps the plant to activate its defense system against the attack of pathogens, however, the association between the induction of the defense system and the endophyte yeasts is not fully understood.

These aspects should also be taken into account when evaluating situations in which an endophyte yeast colonizes its host, generating in this one a different benefit from biological control. In approaching the problem from this perspective, it is possible to obtain additional information from this mutualistic relationship, which allows proposing solutions to practical problems associated with the postharvest period.

Recent investigations on the yeast *Candida guilliermondii* isolated from a heterograft tomato crop (HGTC) in Sogamoso (Boyacá, Colombia) have shown that it is able to colonize its host without generating damage to the cell walls; on the contrary, it delays loss of water; in addition, its effectiveness in biological control against *Rhizopus stolonifer* was determined [24]. These results, together with the definition of endophyte, allow us to classify this yeast as an endophyte yeast of interest in agro-industry, due to the possibility of using it in a promising way to prolong the useful life of its host.

Indeed, this endophytic yeast contributes to lengthening the useful life of its host and also can be used as an antagonist offers the possibility of using it to study this mutualistic relationship and obtain information that allows solving problems associated with the postharvest period, such as fruit quality, storage, and phytopathogen biocontrol.

However, the following questions arise: is it possible that as a result of the endophytic yeast-host interaction, changes will occur in the host? What can these changes be? Are there new modes of action of these yeasts in activating the plant defense system?

Searching for answers to these questions is possible to implement alternative methodologies that allow researchers to assess the dynamics of yeast colonization, identifying and quantifying changes in the host, and to propose another mode of action of the endophytic yeast.

4. Evaluating the action of an endophyte yeast on its host

Traditionally, to check the efficiency of a microbial antagonist and/or to evaluate a colonization process, the researchers quantify the number of microorganisms present in a plant in terms of colony forming units (CFUs). To get such measurements, it is necessary to dilute the sample, take an aliquot of it and, finally, transfer it to an appropriate medium that allows the microorganisms to grow in visible colonies [26–28].

Other investigations have proposed the direct observation of endophytic yeasts inside the plant tissues using microscopy techniques. For instance, Isaeva et al. [29] studied the distribution and species diversity of yeast in the storage tissues of fruits, seeds, and roots and found that the yeast cells were most often located in the intercellular space or in cells with intact membranes. These results suggest that internal storage tissues of plants are usually habitats of yeast and can be used as a model for studies of coevolving plant-microbe associations.

Nassar et al. [10] used light and transmission electron microscopy to observe maize root inoculated with *W. saturnus* and stained with 0.1% toluidine blue. The images show the distribution of yeast cells within the root cortex, intercellular spaces, and xylem vessels.

On the other hand, it is possible to characterize, with a vertical resolution of 10^{-9} m, the topography of fruits and vegetables from the observation of tissue samples using the atomic force microscope (AFM) [30, 31]. This methodology has also been used to evaluate the formation of antimicrobial films [32]. For their part, Isaacson et al. [33] evaluated the biomechanical properties as well as the resistance to microbial infections of tomato fruit cuticles. Because of its resolution, this microscope can be used to visualize the cell surface topography and to determine cell wall nanomechanical properties of yeast mutants [34].

In addition, evaluating the interaction of endophytic yeasts with their hosts—and taking into consideration the definition of endophyte—implicates characterizing both the surface and the interior of the host, yet it is necessary to use different methodologies from the traditional ones. From this perspective, MRI offers a non-destructive and non-invasive technique that can be used to obtain two-dimensional images of fruits and/or vegetables from which it is possible to evaluate *in vivo* changes inside, changes that take place as a result of own metabolic processes during the development and/or maturation, or associated with modifications by external agents [35–38].

According to the preceding points, the use of microscopy and MRI makes it possible to characterize qualitatively and quantitatively the host changes by the endophytic yeast action, providing information that can contribute to understanding this mutualistic relationship and to think about other conceivable action modes. Below are some of the results found when using these methodologies; for its implementation, and according to Koch's postulates, the tomato fruit was used as a host, and it was inoculated with the endophytic yeast *C. guilliermondii*.

4.1. Formation of endophytic yeast biofilms

Atomic force microscopy (AFM) enables researchers to study at a nanometric scale the distribution of endophytic yeast on the host surface as well as the topographic changes in it. Although plant tissue samples are commonly used for the implementation of this methodology, surface modifications are not only brought about by the external agent action (endophyte) but also come from the different tissues that make up the host's interior. Because of that, whole tomato fruits were used to evaluate the topography and to analyze before and after inoculation by being sprinkled with yeast *C. guilliernondii*. This methodology allows the researchers to study *in vivo* the time-related evolution of the colonization process evaluating images—taken both in contact mode and in intermittent contact mode—of the host surface.

The 3D images of the uninoculated whole fruit (zero time) surface, taken in contact mode, show that its topography is not homogeneous since it has ridges and valleys whose average value is 700 nm from the center line. It is also possible to observe bright areas associated with the epicuticular waxes, as shown in **Figure 1a**. From these images, it was determined that the average surface roughness was 240 nm.

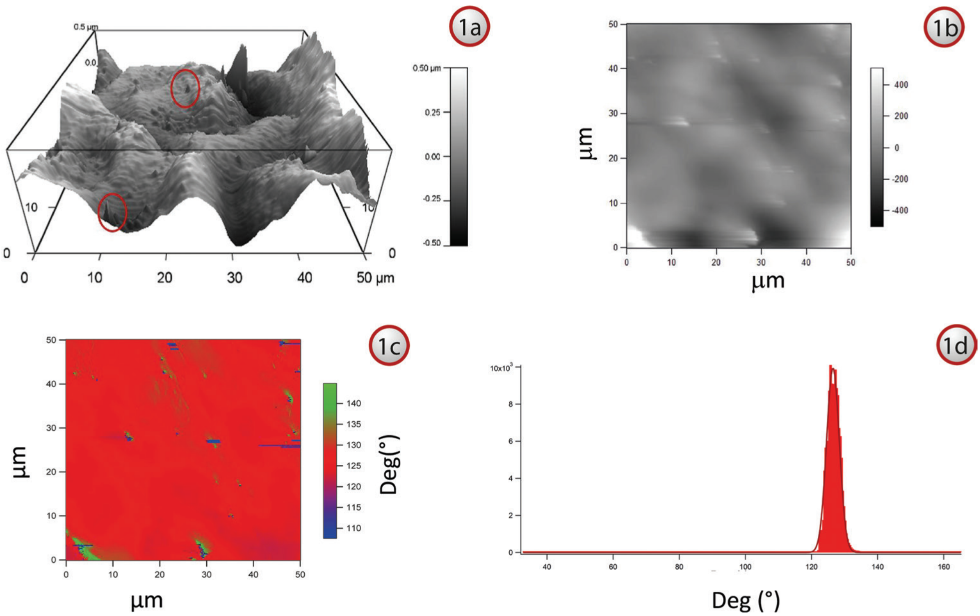

Figure 1. Images of the host surface (uninoculated fruit) obtained by AFM. (1a) 3D image taken in contact mode; the epicuticular waxes are shown in red, the vertical scale corresponds to ±0.5 μm. (1b) 2D image of the surface taken in tapping mode. (1c) Phase map. (1d) In the histogram phase for the surface of the host, there is only one phase whose value is between 120 and 135°.

The topographic characterization of the host obtained from the images taken in contact mode plus the images of the surface taken in tapping mode or intermittent contact (measuring the phase difference between the signal received when the microscope tip does not interact with the sample and the one received when the tip interacts with the sample—tap), allow to obtain information about changes in the local properties of the surface.

Figure 1b shows the two-dimensional image of the surface of the uninoculated fruit taken in tapping mode; **Figure 1c**, its corresponding map, and **Figure 1d**, its histogram phase. The results indicate that the surface has only one phase corresponding to host surface.

From the topographic images obtained 5 hours after inoculating the fruit with the yeast, it is determined that on the surface some areas associated with yeast clusters randomly appears, whose average height to the midline is 1600 nm (see **Figure 2a**). In the images of the host surface taken in tapping mode, areas of similar characteristics are observed, both in the 2D image and in the phase map (see areas surrounded by circles in **Figure 2c**).

It should be noted that the value of the phase for the yeast clusters is between 80 and 90°, a result that differs from host surface before inoculation. Additionally, the histogram phase reveals two different phases on the surface: one associated with the yeast and another associated with the surface of the fruit.

Finally, 72 hours after inoculation, the surface of the host does not present clusters as the ones described above; on the contrary, less roughness is seen, suggesting that the yeast has been colonizing and homogenizing the surface of the host (see **Figure 3a**). When calculating the roughness parameter, it is found that it has decreased to a value of 120 nm.

Concerning the map and the histogram phase, only one phase appears again, but now the value of this parameter is between 80 and 95°, for the same as the yeast clusters. This indicates that the endophyte adhered to its host formed a biofilm.

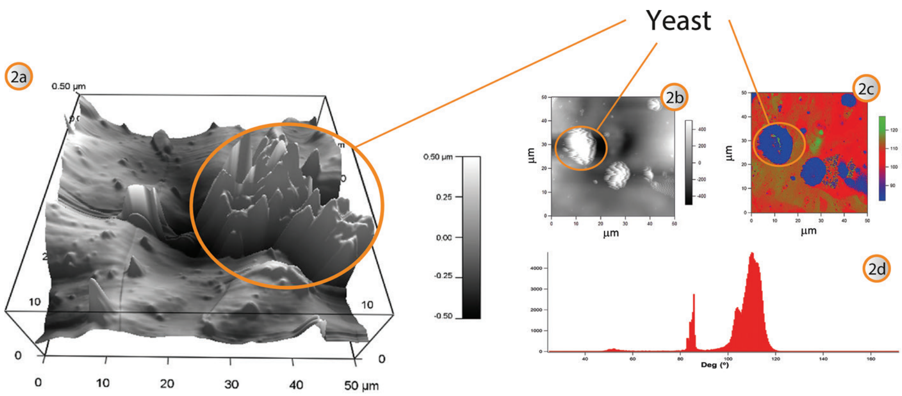

Figure 2. Host surface images taken 5 hours after inoculation with *C. guilliermondii* endophytic yeast. (2a) 3D Image taken in contact mode, the vertical scale corresponds to ±0.5 µm. (2b) 2D Image of the surface taken in tapping mode. (2c) Phase map. (2d) In the histogram phase, the two peaks confirm that the surface of the host has two phases.

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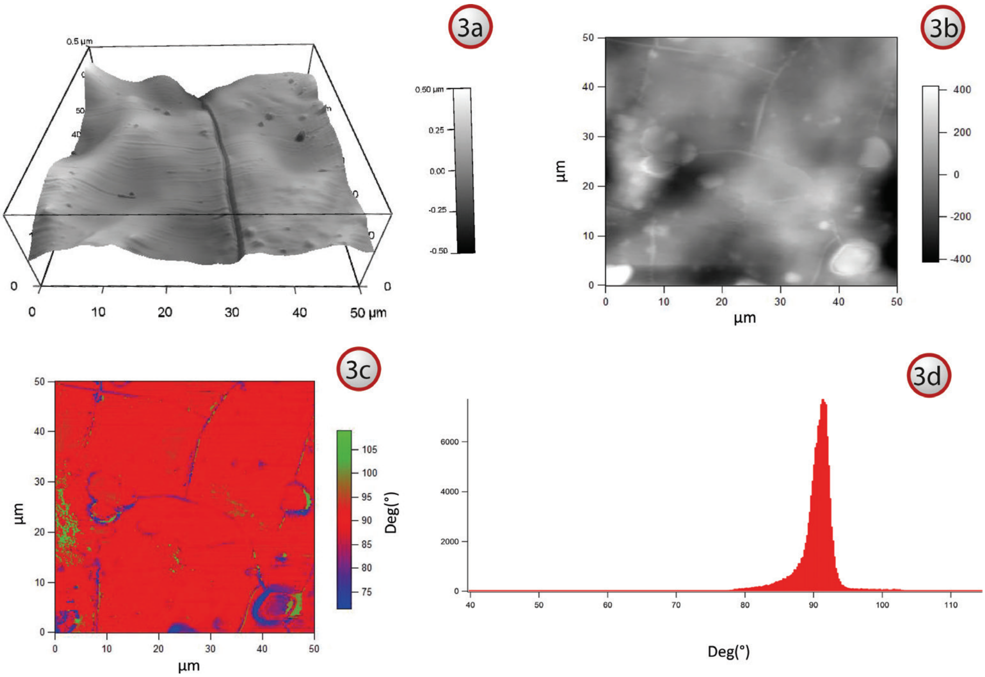

Figure 3. Host surface images obtained 72 hours after inoculation. (3a) 3D Image taken in contact mode, the vertical scale corresponds to ±0.5 μm. (3b) 2D Image of the surface taken in tapping mode. (3c) Phase map. (3d) In the histogram phase, only one phase associated with the yeast is detected.

The assessment of the host's topography allows asserting that the endophytic yeast modifies its host, reducing its surface roughness, which implies a lower adhesion of phytopathogens. In relation to the images captured in tapping mode, the results are visible how the endophytic yeast adheres to its host forming a biofilm that contributes to water retention inside the host.

4.2. Dynamics of colonization within the host

As stated by the Petrini's definition [6] "An endophyte colonizes and can live inside the living tissues of its host without causing damage," the evaluation of optical microscopy images of transverse sections of the host inoculated with the yeast enables researchers to establish if a yeast effectively is included in this definition.

In addition, this methodology allows assessing the colonization dynamics with the purpose to determine the pathways of the yeast and its average speed of migration into the host's, as well as to identify possible damage in the plant tissue and/or modifications in its structures by the endophytic action. Following the methodology proposed by Infante, Marquinez, and Moreno [39], cross-sectional images of the host can be obtained for each time after inoculation, in which the plant tissue and the yeast are simultaneously visualized, making it possible to determine the aforesaid parameters.

Figure 4 shows transverse cuts of the fruit rind inoculated with the yeast at different times after inoculation. In the control samples, the presence of endophytes is not observed. In contrast, in the inoculated samples, an increase in the number of yeasts found on the surface of the host is observed over time: in the epidermis, yeasts are observed 8 hours after the inoculation, and in the parenchyma, after 22 hours.

The images display the absence of lesions in the tissue both in the outer cuticular layer and in the cells of the epidermis and parenchyma. In relation to the yeast's pathway into the host, it is possible to establish that this endophyte, after entering, moves along the cuticular layer and then travels via apoplast, in a linear order, occupying the intercellular spaces of both the epidermis and the parenchyma as well (see **Figure 4d**).

The presence of yeast inside the host 72 hours after inoculation proves that it provides the yeast with nutrients and adequate conditions to survive, which confirms the notion of a mutualistic relationship between the endophytic yeast and the plant.

Additionally, changes by the action of the endophyte yeast in the host structures were evaluated. The results reveal an average decrease of 3 μm in the thickness of the outer cuticular layer of the bark of the tomato fruit inoculated in comparison with that of the control fruits. The outcomes are shown in **Figure 5**. The decrease in the cuticular layer thickness implies an upsurge in density, which favors the retention of water inside the fruit.

Simultaneous observation of inoculated tissues and endophyte yeasts looks into a new approach to assessing this mutual symbiosis identifying the benefits for the symbionts involved, taking into account the structural changes in the host as well as the yeasts paths and distribution patterns.

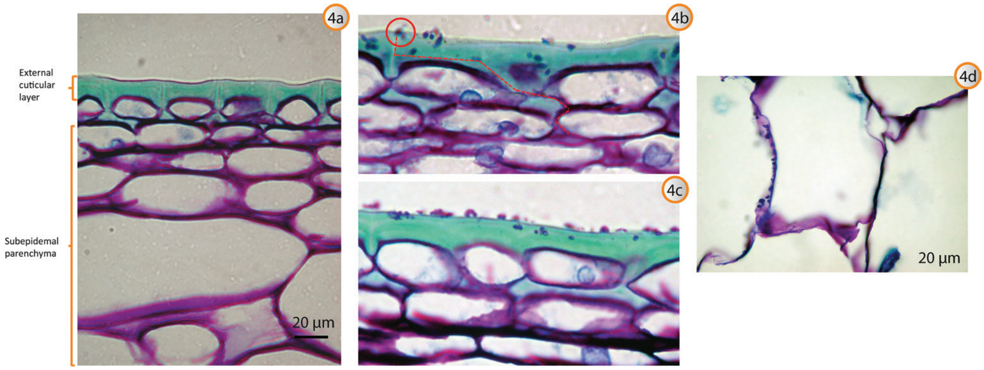

Figure 4. Cross-sectional images of tomato fruit stained with Toluidine blue, different times postinoculation. (4a) Control sample; (4b) 22 hours; (4c) 48 hours; (4d) 48 hours.

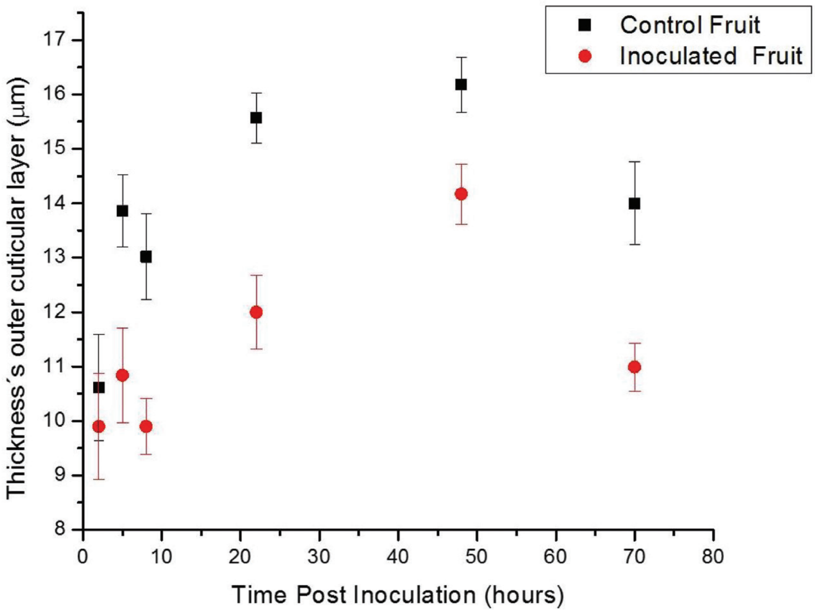

Figure 5. It measured the thickness of the outer cuticular layer of the tomato fruit rind to different times postinoculation. The differences in thickness between the control fruits and the inoculated ones are statistically significant.

4.3. How an endophytic yeast modifies the interior of its host

The results reported in relation to the yeast *C. guilliermondii* have shown that it adheres to the host forming a biofilm and colonizes its interior without causing damage to the cell walls. Instead, it contributes to decreasing both the phytopathogens attacks and the water loss. As this is an endophyte yeast, it is interesting to identify changes in the internal structures of the host and its relation to the benefits that it receives, with the intention of deepening the knowledge of this symbiosis.

To study these alterations, it is advisable to use magnetic resonance imaging (MRI)—a non-invasive technique—which enables investigators to see changes *in vivo* inside the host triggered by the endophyte's action, as in the case of the modifications that happened in the tomato fruit inoculated with *C. guilliermondii*. On the minus side, MRI does not permit researchers to observe simultaneously the host and the yeast—unlike the techniques of MRI microscopy—since in this case, the scale resolution is the tenth of a millimeter.

The main advantage of this technique is the possibility to obtain images weighted by different parameters—relaxation times (T2), proton density, and diffusion, among others—which correspond to the characteristics of the evaluated system. With the aim to see the temporal evolution of the host, images of tomato fruits inoculated by sprinkling with the yeast *C. guilliermondii* were taken at different times after inoculation.

Changes in the dimensions of the host were evaluated. The results obtained indicate that the most affected fruit region by the yeast is the pericarp; also, the diameter of the inoculated fruits decreases more slowly; however, the pericarp thickness diminishes more in comparison with the control fruits (**Figure 6**). This suggests that there are structural changes by the action of the endophyte in this region of the fruit, which can contribute to water retention and, as a consequence, delay the loss of turgor. This is the reason why the decrease of its size is slower compared with the control fruits. Nevertheless, it is necessary to evaluate parameters such as relaxation time (T2) and mobility to confirm these assertions.

With the propose of establishing the biochemical changes within the host, T2-weighted images were taken; the results indicate differences in the values associated with this parameter for the different regions of the fruit (see **Figure 7**). It was also found that T2 decreases in both control and inoculated fruits, signifying molecular variations associated with postharvest processes. However, this decrease occurs in the inoculated fruits more rapidly, which evidences lessening of mobility due to molecular modifications inside the fruit.

Finally, the diffusion-weighted images allow establishing changes in the mobility of molecules, which is a fundamental aspect in this case because the yeast helps to retain water inside the host. From the obtained images, the apparent diffusion coefficient (ADC) was calculated. It is lower in the pericarp region of the inoculated fruits than for the control ones, which indicates that the host is modified by the action of the endophyte, reducing the movement of the water molecules inside. This result, combined with that reported for the T2 parameter, allows to state that in the fruits inoculated with the yeast the water molecules present in the pericarp region are surrounded by different molecules that limit their mobility.

Evaluating the images obtained by MRI, it is possible to sustain that the endophytic yeast modifies the interior of the host; in the case of the inoculated tomato fruit, a decrease in its thickness was observed for the pericarp region in comparison with the control fruits, fact that

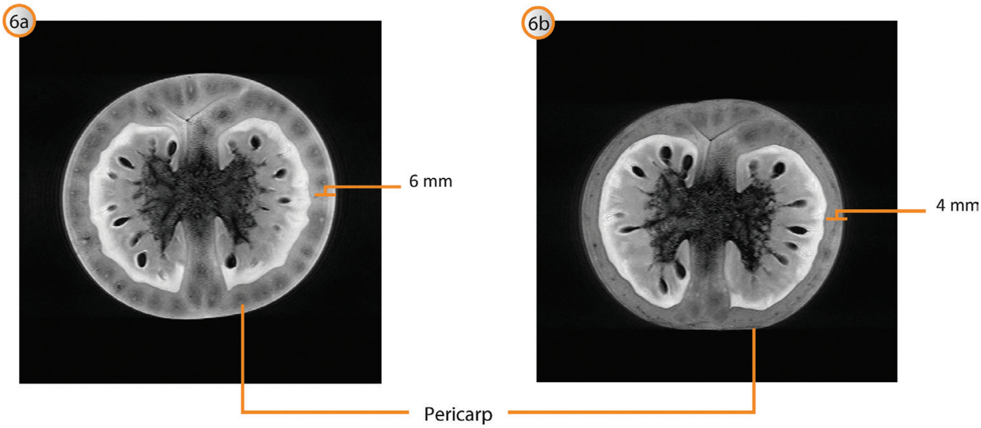

Figure 6. High-resolution images of a cross-section of the inoculated tomato fruit. (6a) Zero time. (6b) 14 days postinoculation.

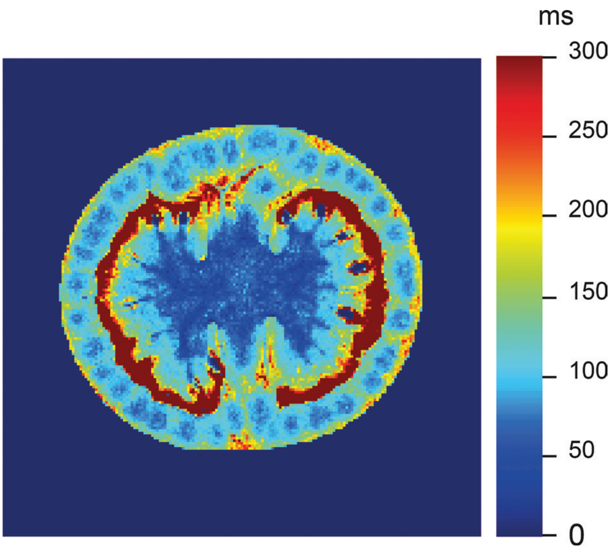

Figure 7. T2 map in a cross-section of the fruit. High values of T2 (more than 250 ms) specify zones with water molecules that can move easily; on the contrary, low values (70 ms) indicate the presence of different molecules.

correlates with biochemical changes that help to reduce the mobility of the molecules in this region. These aspects together favor the retention of water inside the host contributing to maintaining the quality of the fruit.

5. Another approach on endophytic yeasts' action

Reported research has shown that endophytic yeasts can be used in different agro-industrial applications contributing to host and/or pathogen control improvements, however, some aspects remain unclear. For instance, the way the yeast triggers the defense system in the host, where the relationship between the elicitors and the antagonist provides a field to be explored. Another aspect that has drawn attention is the formation of biofilms and how these can be used to improve biological control [16]; additionally, it is necessary to evaluate the changes produced in the host by the yeast's action and its incidence. All of them are topics that to date have been little explored.

The relationships established between yeast, pathogen, host, and metabolic changes that occur in the host during the postharvest period allow to understand the plant-endophyte mutualistic association and define other modes of action.

Evaluating these relationships focusing on the host, it was found that the metabolic processes associated with the postharvest period—such as starch degradation, water loss, and disassembly of cell walls—lead to changes that affect the quality of the product. Concerning its interactions with pathogens, these colonize the host generating various diseases, to which the host can respond by activating its defense system and producing antifungal compounds. On the contrary, their relation with the endophytic yeasts is of mutualistic character, since these generate a benefit for the host while it offers to them optimal conditions for their survival.

The relationships described above are shown in **Figure 8**; the arrows indicate direct interactions; however, when it comes to endophytic yeasts, it is necessary to consider indirect relationships, in which the yeast can modify its host generating benefits in it, helping solve some of the postharvest period problems.

In the previous section, the results obtained when evaluating changes in the host (tomato fruit) by the action of the endophyte yeast (*C. guilliermondii*) were presented. Using atomic force microscopy (AFM), it could be established that the surface roughness of the inoculated host diminishes when a yeast biofilm is formed, besides it contributes to retaining the water inside the host prolonging its useful life.

On the other hand, when the samples inoculated with the yeast were evaluated by optical microscopy (OM), it was determined that the thickness of the outer cuticle layer showed an average decrease of 3 μm in comparison with the control samples, suggesting an increase in the density of the same and, therefore, changes in its permeability.

It should be noted that in relation to cuticle evaluation and its function in resistance to phytopathogens, Curvers et al. [40] studied a mutant of tomato (*Solanum lycopersicum*) with reduced abscisic acid (ABA) production, and established that it presents increased resistance to the necrotrophic fungus *Botrytis cinerea*. They further compared the thickness of the cuticle of the mutant with that of other evaluated tomato fruits, identifying that the cuticular layer of the first one presents a decrease in the thickness, which favors the signaling processes.

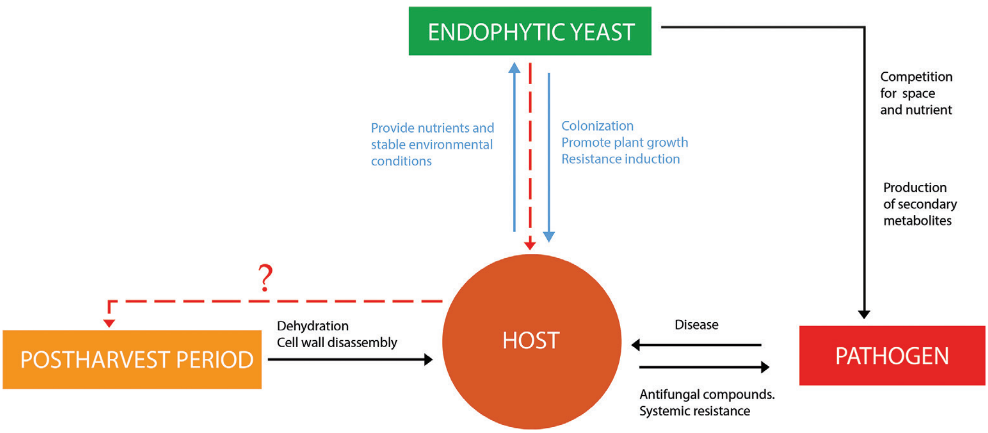

Figure 8. Interactions between host, yeast, pathogen, and postharvest processes. The blue arrow indicates a yeast–host mutualistic relationship; the question mark points to a possible indirect relationship between the yeast and postharvest period.

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Previous studies about yeast *C. guilliermondii* determined its effectiveness in the control of *Rhizopus stolonifer* and its production of secondary metabolites; this outcome together with the results found by means of microscopy techniques allow to affirm that the effectiveness of an endophyte yeast in the biological control could be associated with more than one mode of action, one of which may be related to structural changes in the host by action of the endophyte.

Lastly, from MRI, it was determined that with respect to the control fruits, in the fruits inoculated with the endophytic yeast appear a decrease both in the thickness of the pericarp and in the mobility of the molecules present in this region of the fruit; changes that favor the retention of water inside. **Figure 9** shows the modifications generated in the different structures of the host by the action of yeast and its relation to the observed benefits.

According to the abovementioned determination, it is possible to highlight several aspects that contribute to deepening the knowledge of endophyte yeasts and their use in the search for solutions to problems typical of the postharvest period.

The first one refers to the fact that the endophyte yeast colonizes not only the surface of the host but also enters into it and remains inside it without causing damage: evidence of the mutualistic relationship between the symbionts.

In addition, from the results found, it is possible to propose another mode of action of the endophytic yeasts: they generate propitious structural changes in the surface and the interior of the host, which reduce phytopathogen attacks and loss of water. Therefore, it can be said that the endophytic yeasts could be used to help solve some of the problems relevant to agro-industry.

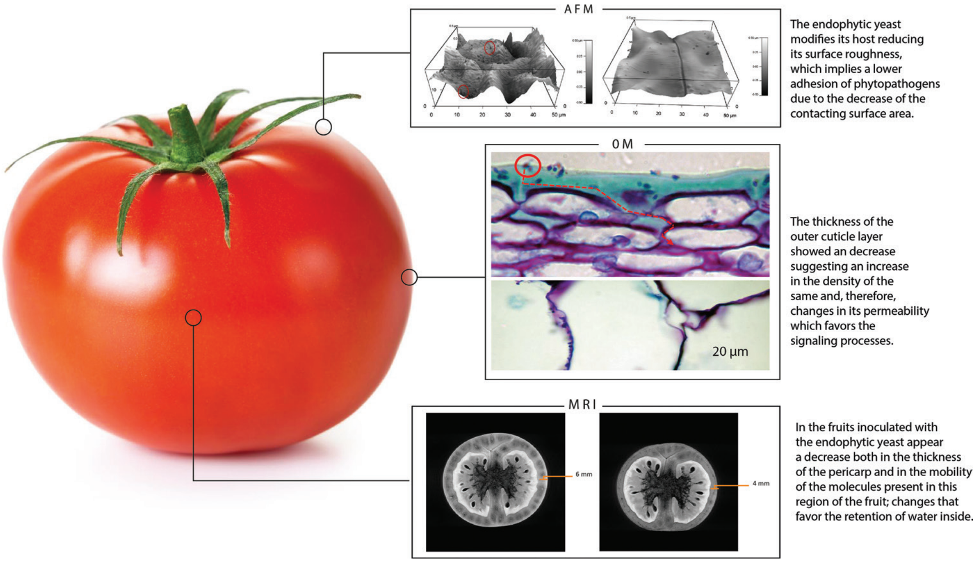

Figure 9. Physical modifications in the host (tomato fruit) by the action of the endophyte yeast (*C. guilliermondii*).

It is noteworthy that this mutualistic coexistence of plant-endophytic yeast can be applied to develop healthy and friendly alternatives that are advantageous to the environment, offering organic food to the consumers and avoiding the use of agrochemicals and genetic engineering intended to enhance the quality of fruits and vegetables.

6. Conclusion

This chapter shows a new way to understand the endophytic yeasts, analyzing variations in their host looked through microscopy and the magnetic resonance imaging. The results confirmed the Petrini's definition: "An endophyte colonizes and can live inside the living tissues of its host without causing damage" additionally —observing the inoculated host— it is thinkable to propose a new mode of yeast action in which the physical characteristics of the surface and the inside of the host change by the action of the yeast, contributing to improve their quality during the postharvest period, without causing health problems to the humans beings, because by this way the use of chemicals to control phytopathogens is avoided.

The new information about endophytic yeast opens the possibility to new researches: how the host "understand" that this microorganism is good for it?; how is the process in the host that allows the entry of the endophytic yeast?; how can this kind of yeast be used to obtain organic products in order to improve the health?; how does the biochemical environment of the host changes by the yeast?

I hope that these new methodologies and information about the endophytic yeast contribute to solve these questions.

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References

[1] Sharma RR, Singh D, Singh R. Biological control of postharvest diseases of fruits and vegetables by microbial antagonists: A review. Biological Control. September 2009;**50**(3):205-221. [Internet]. Available from: http://linkinghub.elsevier.com/retrieve/pii/S1049964409001236 [Accessed: November 6, 2012]

[2] Isaeva OV, Glushakova AM, Garbuz SA, Kachalkin AV, Chernov IY. Endophytic yeast fungi in plant storage tissues. Biology Bulletin. February 9, 2010;**37**(1):26-34. [Internet]

Available from: http://www.springerlink.com/index/10.1134/S1062359010010048 [Accessed: January 20, 2013]

[3] Schulz B, Boyle C, Sieber TN, editors. Microbial Root Endophytes. Berlin: Springer-Verlag; 2006. p. 367

[4] Hirsch G, Braun U 1992. Communities of parasitic microfungi. In: Winterhoff W, ed. Handbook of vegetative science, Volume 19. Fungi in vegetation science. Dordrecht, the Netherlands: Kluwer Academic Publishers, 225-250

[5] Stone J, Bacon C, White J. An overview of endophytic microbes: Endophytism defined. In: Bacon C, White J, editors. Microbial Endophytes. New York, USA: Marcel Der; 2000

[6] Petrini O. Fungal endophytes of tree leaves. In: Andrews JH, Hirane SS, editors. Micorbial Ecology of Leaves. New York, USA: Springer; 1991. pp. 179-197

[7] Xin G, Glawe D, Doty S. Characterization of three endophytic, indole-3-acetic acid producing yeast ocurring in populus trees. Mycological Research. 2009;**113**:973-980

[8] Dal Bello G, Mónaco C, Rollan MC, Lampugnani G, Arteta N, Abramoff C, et al. Biocontrol of postharvest grey mould on tomato by yeasts. Journal of Phytopathology. May 22, 2008;**156**(5):257-263. [Internet] Available from: http://doi.wiley.com/10.1111/j.1439-0434.2007.01351.x [Accessed: March 1, 2014]

[9] Bautista-Rosales PU, Calderon-Santoyo M, Servín-Villegas R, Ochoa-Álvarez NA, Ragazzo-Sánchez JA. Action mechanisms of the yeast *Meyerozyma caribbica* for the control of the phytopathogen *Colletotrichum gloeosporioides* in mangoes. Biological Control. Jun 2013;**65**(3):293-301. [Internet] Available from: http://linkinghub.elsevier.com/retrieve/pii/S1049964413000595 [Accessed: September 14, 2014]

[10] Nassar AH, El-Tarabily KA, Sivasithamparam K. Promotion of plant growth by an auxin-producing isolate of the yeast *Williopsis saturnus* endophytic in maize (Zea mays L.) roots. Biology and Fertility of Soils. July 1, 2005;**42**(2):97-108. [Internet] Available from: http://www.springerlink.com/index/10.1007/s00374-005-0008-y [Accessed: November 30, 2012]

[11] Zhao Y, Wang R, Tu K, Liu K. Efficacy of preharvest spraying with *Pichia guilliermondii* on postharvest decay and quality of cherry tomato fruit during storage. African Journal of Biotechnology. 2011;**10**(47):9613-9622

[12] De Bary A. 1879. Die Erscheinung der Symbiose. In: Trubner KJ, ed. Vortrag auf der Versammlung der Naturforscher und Ärtze zu Cassel. Strassburg, Germany: Verlag, 1-30

[13] Singh LP, Gill SS, Tuteja N. Unraveling the role of fungal symbionts in plant abiotic stress tolerance. Plant Signaling & Behavior. February 2011;**6**(2):175-191

[14] Sieber T. Endophytic fungi in forest trees: Are they mutualists? Fungal Biology Reviews. 2007;**21**:75-89

[15] Nunes CA. Biological control of postharvest diseases of fruit. European Journal of Plant Pathology. December 21, 2012;**133**(1):181-196. [Internet] Available from: http://link.springer.com/10.1007/s10658-011-9919-7 [Accessed: October 14, 2014]

[16] Liu J, Sui Y, Wisniewski M, Droby S, Liu Y. Review: Utilization of antagonistic yeasts to manage postharvest fungal diseases of fruit. International Journal of Food Microbiology. October 15, 2013;**167**(2):153-160. [Internet] Available from: http://www.ncbi.nlm.nih.gov/pubmed/24135671 [Accessed: October 14, 2014]

[17] Robiglio A, Sosa MC, Lutz MC, Lopes CA, Sangorrín MP. Yeast biocontrol of fungal spoilage of pears stored at low temperature. International Journal of Food Microbiology. June 30, 2011;**147**(3):211-216. [Internet] Available from: http://www.ncbi.nlm.nih.gov/pubmed/21546110 [Accessed: September 23, 2014]

[18] Lai T, Wang Y, Li B, Qin G, Tian S. Defense responses of tomato fruit to exogenous nitric oxide during postharvest storage. Postharvest Biology and Technology. November 2011;**62**(2):127-132. [Internet] Available from: http://linkinghub.elsevier.com/retrieve/pii/S0925521411001281 [Accessed: July 22, 2014]

[19] Jiang F, Chen J, Miao Y, Krupinska K, Zheng X. Identification of differentially expressed genes from cherry tomato fruit (*Lycopersicon esculentum*) after application of the biological control yeast *Cryptococcus laurentii*. Postharvest Biology and Technology. September 2009;**53**(3):131-137. [Internet] Available from: http://linkinghub.elsevier.com/retrieve/pii/S0925521409000660 [Accessed: January 20, 2013]

[20] Wang Y, Yu T, Xia J, Yu D, Wang J, Zheng X. Biocontrol of postharvest gray mold of cherry tomatoes with the marine yeast *Rhodosporidium paludigenum*. Biological Control. May 2010;**53**(2):178-182. [Internet] Available from: http://linkinghub.elsevier.com/retrieve/pii/S1049964410000101 [Accessed: January 20, 2013]

[21] Chan Z, Tian S. Interaction of antagonistic yeasts against postharvest pathogens of apple fruit and possible mode of action. Postharvest Biology and Technology. May 2005;**36**(2):215-223. [Internet] Available from: http://linkinghub.elsevier.com/retrieve/pii/S0925521405000128 [Accessed: January 20, 2013]

[22] Lima JR, Gondim DMF, Oliveira JTA., Oliveira FSA, Gonçalves LRB, Viana FMP. Use of killer yeast in the management of postharvest papaya anthracnose. Postharvest Biology and Technology. September 2013;**83**:58-64. [Internet] Available from: http://linkinghub.elsevier.com/retrieve/pii/S0925521413000884 [Accessed: July 22, 2014]

[23] Mari M, Martini C, Spadoni A, Rouissi W, Bertolini P. Biocontrol of apple postharvest decay by *Aureobasidium pullulans*. Postharvest Biology and Technology. November 2012;**73**:56-62. [Internet] Available from: http://linkinghub.elsevier.com/retrieve/pii/S0925521412001287 [Accessed: October 14, 2014]

[24] Celis ZC, Moreno DG, Sequeda-castañeda LG, García A, Albarracín DM, Charry B, Claudia L. Determining the effectiveness of *Candida guilliermondii* in the biological control of *Rhizopus stolonifer* in postharvest tomatoes. Universitas Scientiarum. 2014;**19**(1):51-62

[25] Punja ZK, Utkhede RS. Using fungi and yeasts to manage vegetable crop diseases. Trends in Biotechnology. September 2003;**21**(9):400-407. [Internet] Available from: http://www.ncbi.nlm.nih.gov/pubmed/12948673 [Accessed: October 4, 2014]

[26] Ahmed L, Martin-Diana AB, Rico D, Barry-Ryan C. Effect of delactosed whey permeate treatment on physico-chemical, sensorial, nutritional and microbial properties of whole tomatoes during postharvest storage. LWT – Food Science and Technology. April 2013;**51**(1):367-374. [Internet] Available from: http://linkinghub.elsevier.com/retrieve/pii/S002364381200429X [Accessed: March 1, 2014]

[27] Mahmoud BSM. The effects of X-ray radiation on Escherichia coli O157:H7, *Listeria monocytogenes*, *Salmonella enterica* and *Shigella flexneri* inoculated on whole Roma tomatoes. Food Microbiology. December 2010;**27**(8):1057-1063. [Internet] Available from: http://www.ncbi.nlm.nih.gov/pubmed/20832685 [Accessed: March 1, 2014]

[28] Adekunte A, Tiwari B, Cullen P, Scannell AG, O'Donnell C. Effect of sonication on colour, ascorbic acid and yeast inactivation in tomato juice. Food Chemistry. October 2010;**122**(3):500-507. [Internet] Available from: http://linkinghub.elsevier.com/retrieve/pii/S0308814610001068 [Accessed: March 1, 2014]

[29] Isaeva OV, Glushakova AM, Yurkov AM, Chernov IY. The yeast *Candida railenensis* in the fruits of English oak (*Quercus robur* L.). Microbiology. June 7, 2009;**78**(3):355-359. [Internet] Available from: http://www.springerlink.com/index/10.1134/S002626170903014X [Accessed: January 20, 2013]

[30] Wiśniewska SK, Nalaskowski J, Witka-Jeżewska E, Hupka J, Miller JD. Surface properties of barley straw. Colloids and Surfaces B: Biointerfaces. June 2003;**29**(2-3):131-142. [Internet] Available from: http://linkinghub.elsevier.com/retrieve/pii/S0927776502001789 [Accessed: May 9, 2013]

[31] Xin Y, Chen F, Yang H, Zhang P, Deng Y, Yang B. Morphology, profile and role of chelate-soluble pectin on tomato properties during ripening. Food Chemistry. July 2010;**121**(2):372-380. [Internet] Available from: http://linkinghub.elsevier.com/retrieve/pii/S0308814609014587 [Accessed: December 6, 2012]

[32] La Storia A, Ercolini D, Marinello F, Mauriello G. Characterization of bacteriocin-coated antimicrobial polyethylene films by atomic force microscopy. Journal of Food Science. May 2008;**73**(4):T48-T54. [Internet] Available from: http://www.ncbi.nlm.nih.gov/pubmed/18460145 [Accessed: March 1, 2014]

[33] Isaacson T, Kosma DK, Matas AJ, Buda GJ, He Y, Yu B, et al. Cutin deficiency in the tomato fruit cuticle consistently affects resistance to microbial infection and biomechanical properties, but not transpirational water loss. The Plant Journal. October 2009;**60**(2):363-377. [Internet] Available from: http://www.ncbi.nlm.nih.gov/pubmed/19594708 [Accessed: January 7, 2013]

[34] Dague E, Bitar R, Ranchon H, Durand F, Fran JM. An atomic force microscopy analysis of yeast mutants defective in cell wall architecture. Yeast. 2010;**27**:673-684

[35] Galed G, Fernández-Valle ME, Martínez A, Heras A. Application of MRI to monitor the process of ripening and decay in citrus treated with chitosan solutions. Magnetic Resonance Imaging. January 2004;**22**(1):127-137. [Internet] Available from: http://www.ncbi.nlm.nih.gov/pubmed/14972402 [Accessed: September 23, 2014]

[36] Musse M, Quellec S, Devaux M-F, Cambert M, Lahaye M, Mariette F. An investigation of the structural aspects of the tomato fruit by means of quantitative nuclear magnetic resonance imaging. Magnetic Resonance Imaging. June 2009;**27**(5):709-719. [Internet] Available from: http://www.ncbi.nlm.nih.gov/pubmed/19144488 [Accessed: January 20, 2013]

[37] Zhang L, McCarthy MJ. Measurement and evaluation of tomato maturity using magnetic resonance imaging. Postharvest Biology and Technology. May 2012;**67**:37-43. [Internet] Available from: http://linkinghub.elsevier.com/retrieve/pii/S0925521411002912 [Accessed: September 5, 2014]

[38] Defraeye T, Lehmann V, Gross D, Holat C, Herremans E, Verboven P, et al. Application of MRI for tissue characterisation of "Braeburn" apple. Postharvest Biology and Technology. January 2013;**75**:96-105. [Internet] Available from: http://linkinghub.elsevier.com/retrieve/pii/S0925521412001913 [Accessed: September 29, 2014]

[39] Infante E, Marquinez X, Moreno G. Tomato peel (*Solanum lycopersicum* L.) colonization by the endophyte yeast *Candida guilliermondii* (Castellani) Langeron et Guerra. Agronomía Colombiana 2012;**30**(3):388-394

[40] Curvers K, Seifi H, Mouille G, de Rycke R, Asselbergh B, Van Hecke A, et al. Abscisic acid deficiency causes changes in cuticle permeability and pectin composition that influence tomato resistance to *Botrytis cinerea*. Plant Physiology. October 2010;**154**(2):847-860. [Internet] Available from: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=2949027&tool=pmcentrez&rendertype=abstract [Accessed: July 22, 2014]