The academy contribution to society: Biotechnology Techniques on Teaching-Learning Process at Agricultural and Biological Engineering

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***Abstract*– In order to meet the world food demand, agricultural biotechnology techniques are oriented at the productivity of crops in an efficient and sustainable manner. By linking society's demands with academia's resources, this document aims to evaluate the skills acquired by students in the field of microbiology during the generation of basic and introductory knowledge of microorganisms. The methodology included: Application of biotechnology techniques and Disciplinary Learning Outcomes evaluation. Results from the biotechnological process highlighted the characterization of the three types of funguses, which are more commonly found in soils: Trichoderma spp, Aspergillus spp, Fusarium spp. The Disciplinary Learning Outcomes evaluation showed that students reached mainly the developing and developed levels and a minimal part stayed at the level of excellence, since the first phase of the process was mechanical with respect to the second phase where the students must name or distinguish the structures under a microscope by a specialized website. The biotechnological techniques acquired at the university promote among future professionals a new perspective on clean production and the Disciplinary Learning Outcomes evaluation demonstrated their usefulness both in the detection of weaknesses and as an input for the curricular revision.**

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I. Introduction

Recent studies on food demand and production have shown that the world's population will increase by more than a third (2,300 million people) between 2009 and 2050 [1], consequently, in order to meet the demand for food, by 2050 global agricultural and livestock production is expected to increase by 60% compared to 2006 [2], where 80 % of this increase would have to be a result of increased crop yields and a 10% increase in agricultural extension campaigns [3].

Therefore, the scientific community has developed alternative techniques aimed at improving yields and productivity of crops in an efficient and sustainable manner, such as agricultural biotechnology tools applied to the improvement of plants, animals and microorganisms [4], in particular oriented to plant protection through the use of microorganism as biofertilizers or biocontrol agents [5]. As the case of the fungus *Trichoderma* spp, used as an agent control pests and to stimulate the growth and development of plants [6].

The linking of society's demands and academia resources, it has generated mutually beneficial partnerships with ethical and social responsibility for students, academia, and community [7]. To this end, some mechanisms of rapprochement have been identified, such as the formation of advisory committees for university careers, with the objective of following up on academic and societal compliance based on the development of their professionals, which together with constant monitoring of skills and abilities acquired by students during their training generate inputs to improve the academic-society relationship. These mechanisms have the purpose of establishing what is desired in the professional field and the competencies associated with it by the students [8].

In this sense, the Escuela Superior Politécnica del Litoral (ESPOL) in its program of Agricultural and Biological Engineering (ABE) offers a package of subjects where the fundamental principles of biotechnology applied to agriculture are taught: Microorganisms in the soil, morphological identification, taxonomic codes, as well as different techniques of ecological management through the use of biocontrollers.

In this context, this document aims to evaluate the skills acquired by students in the field of microbiology during the generation of basic and introductory knowledge of microorganisms useful in biotechnological agricultural processes.

II. Methodology

The methodology enclosed the analysis of two components: the description of the microbiology techniques (biotechnological tools) and the evaluation of the students (eight) who attended the course, as detailed below:

A. *Biotechnology Techniques*

The laboratory practices (Fig. 1) were made at the Centro de Investigaciones Biotecnológicas del Ecuador (CIBE), the fungal and bacterial strains were isolated from soil samples of the Forest Protector Prosperina of Guayaquil around ESPOL,

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since it is an area with an huge biodiversity such as animals, plants and microorganism [9]. The samples were taken for an specific area near to the trees, walked in zic-zac around ESPOL forest, soil from 20 cm of deep was taken and store into a plastic bag [10].

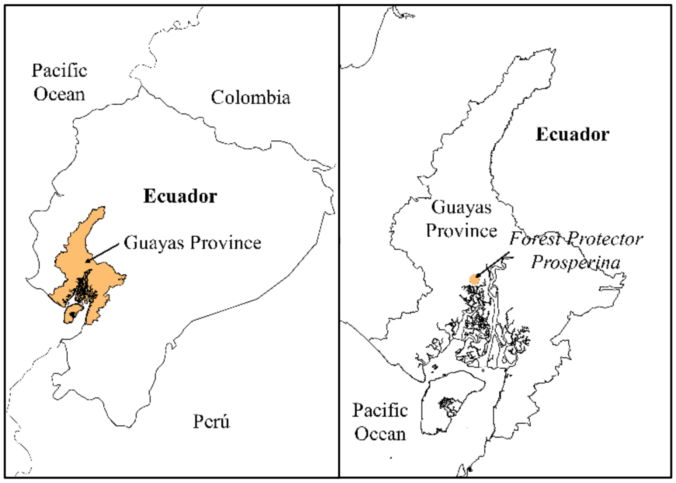

Fig. 1 Localization of Forest Protector Prosperina with respect to the Guayas province and Ecuador.

Regarding to the isolation process, the soil solution was made using sterile distillate water under sterile conditions. It was planting the soil solution in two ways: first serial dilution until 10^3 and 100 µl put into petri dish with 10 mL of solid papa dextrose agar (PDA) (Difco, Detroit, MI, USA); second, it was also planting 1 mL of the same soil solution with 9 mL of PDA at 40 °C, them individual strains were incubated at 28 °C (Fig. 2).

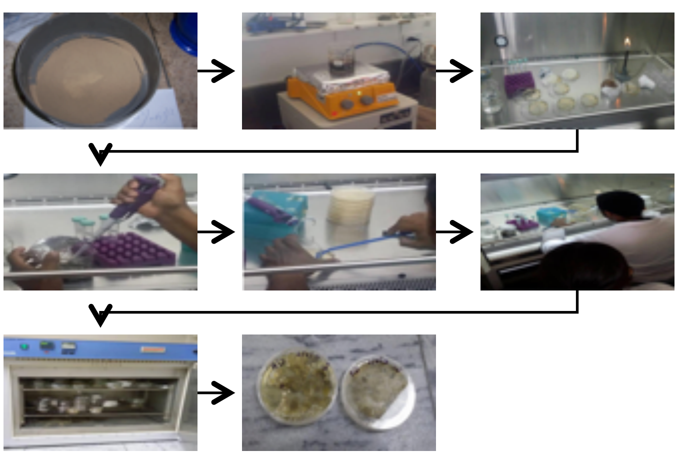
Fig. 2 Microorganism isolation process at the Centro de Investigaciones Biotecnológicas del Ecuador (CIBE).

Microscopic and taxonomic clades tools was made by morphological identification as described previously [11], [12]. Each strain growth usually at 28 °C from each strain the mycelium was taken under sterile conditions and using a slide, coverslips and methylene blue mycelium was distributed around slide. Morphological structure was observed through 40X objective with ZEISS Axio Scope microscopy. Characterization was made follow taxonomic clade described in the international website repositories for every kind of funguses into studio [13], [14], [15].

B. Disciplinary Learning Outcomes (DLOs)

The skills and abilities achieved by the students during the academic training were regulated through the career Disciplinary Learning Outcomes based on *knowledge, skills,* and *attitudes*. The evaluation of DLOs represents an effective tool in the diagnosis to improve and optimize the teaching-learning process [8].

The evaluation of the Disciplinary Learning Outcomes is done by using rubrics or matrices highlighted performance levels (Initial, developing, developed, excellence) versus performance criteria (Fig. 2) [8]. Based on this, it was evaluated the ability to apply skills, tools and techniques required in the agriculture engineering practice, according to following criteria:

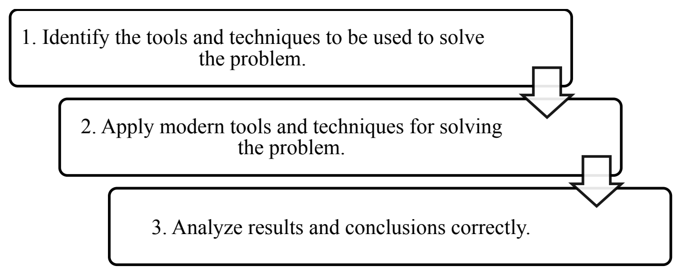

Fig. 3. Career performance criteria.

To emphasize the relationship between the students' abilities progress and the development of the course, it was proposed to carry out the evaluation in two moments of the academic semester to assess the next topics:

1) *Microorganism isolation*
2) *Morphological characterization*

III. Results and discussions

A. Biotechnology techniques

Mycoflora from ESPOL forest soil was analyzed via dilute plate technique into laboratory practice. Several strains were isolated, for the characterization process were selected three kinds of funguses based on growth rate and change of medium color. In order to characterize the funguses by morphological observation, the students recognized structures like hypha, phialides, conidiophores, conidia, chlamydospore, and vesicle.

Morphological identification of the potential *Trichoderma* strains was performed using the growth rate and morphological structure; the students search the genus of the funguses using different free online repositories with taxonomic clades.

From result three kinds of funguses were characterized. *Trichoderma* spp. (Fig. 4), different species of this genus can growth in all soil and decadent wood [16, 17]. *Trichoderma* genus is known as biocontroller against different plant pathogens like *Aspergillus* spp., *Fusarium* spp., *Alternaria* spp., *Rhizoctonia* spp. [18].

Taxonomic knowledge of the use of beneficial fungus in the agriculture is important based on different clades, as beneficial fungus, several species of this genus can make biocontrol action like *T. harzianum*, *T. viride*, *T. asperellum*. Furthermore, *Trichoderma* genus have mechanism of action like mycoparasitism [19], antibiosis and competition by nutrients and space [20]. But, morphological characterization is the first step to reach species level.

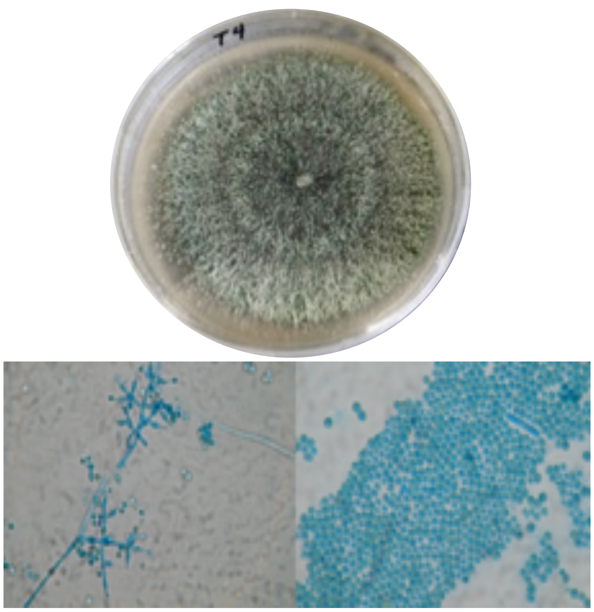

Fig. 4 Strain of *Trichoderma* spp.

On the other hand, *Aspergillus* spp. was characterized by microscopy observation follow typical morphological structure of this fungi (Fig. 5), this genus include 200 species [21]. Some species of *Aspergillus* are plant and human pathogen by mycotoxin production, some of this species are *Aspergillus niger* causal agent of black mold in fruit and vegetables [22], *Aspergillus fumigatus* associated with the seedling biodeterioration and human pathogen, *Aspergillus flavus* causal agent seedling store with mycotoxin production [23].

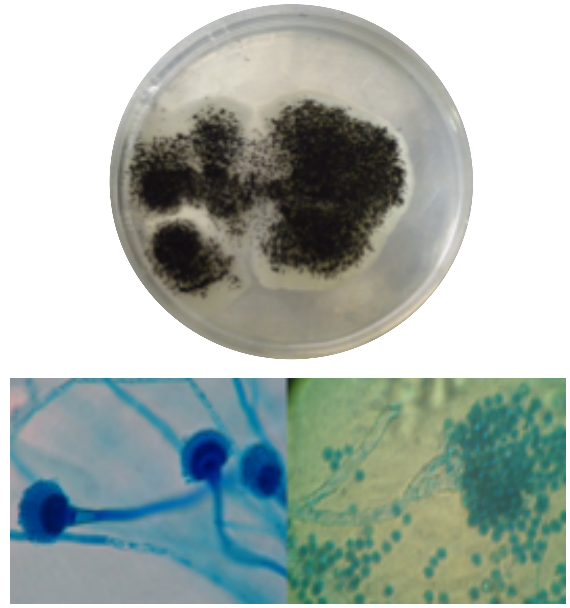

Fig. 5 Strain of *Aspergillus* spp.

Another known plant pathogen genus was isolated *Fusarium* spp., (Fig. 6) some species are agent causal of different diseases. *Fusarium oxysporum* f. sp. *cubense* is one of the most aggressive plant pathogen in banana crops being the causal agent of Panama disease [24]. Actually this is know as dangerous species because produces one of the most devastating diseases worldwide because it evolved into a new breed that is known as *F. oxysporum* f. sp. *cubense* R4. In the other hand, *Fusarium oxysporum* f. sp. *lycopersici* causal agent of tomato crow and root rot [25]. However, one specie of this genus can be used as biocontroller and it is knowed as *F. oxysporum* [26].

Fig. 6 Strain of *Fusarium* spp.

In order, to search any information of the microorganism, like *Trichoderma* species the information is available in (http://nt.ars-grin.gov/sbmlweb/fungi/keyData.cfm), in the case of *Aspergillus* spp., the special website is (http://www.aspergillus.org.uk), another important genus is *Fusarium* spp., (http://www.fusariumdb.org).

B. *Disciplinary Learning Outcomes*

The *microorganism isolation* phase evaluation (Fig. 8) showed for the criterion one that the 83 % of students reached the *developed* level, since they identified only an specific technique to be applied, and the 17 % of learners identified the techniques incorrectly placing them in the *developing* level. Criterion two revealed that the 8 % of the students were able to apply the techniques of biotechnology *excellently*, instead in the *developed* level the 75 % of student applied well the techniques but still requires constant guidance after the explanation and the 17 % of them make an inaccurate application of the techniques (*developing* level). At the same time criterion three registered that the 75 % of learners interpreted the results and conclusions partially (*developed* level) and the 25 % of them interpret the results and conclusions without connecting the result with the solution of the problem (*developing* level).

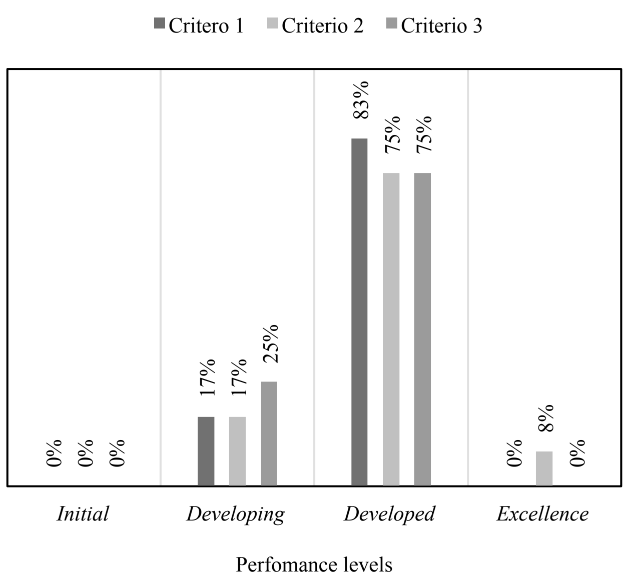

Fig. 7 Disciplinary Learning Outcomes evaluation for microorganism isolation

On the other hand, the evaluation at *morphological characterization of agents* (Fig. 9), it was observed that in criterion one the 25 % of the students are in the *developing* level and the 75 % in the *developed* level, in the first case students were not able to identified correctly the techniques for applying at the practice phase and in the second case they identified the techniques but in a partially way. While, in criterion two was recorded that the 25 % of the students are in the *developing* level, 37 % in the *developed* level and 8 % in *excellence,* due they known how to apply all the techniques. Finally, criterion three showed an increase in the population (33 %) with respect to criteria one and two in the *developing* level, and the difference (67 %) are in the *developed* level.

A more detailed analysis in both evaluations made visible that students who are in the *developing* level are those who attended the second semester of the program, hence, it can be inferred that in the first career semesters students are more tending to develop automatic mechanisms and with the advancement of the academic process they reinforce other types of skills such as autonomy in the teaching-learning process. In particular, the second evaluation presented that the students decreased their performance, since the first phase of the process was mechanical with respect to the second phase where the students must name or distinguish the structures under a microscope using the tools of free online repositories.

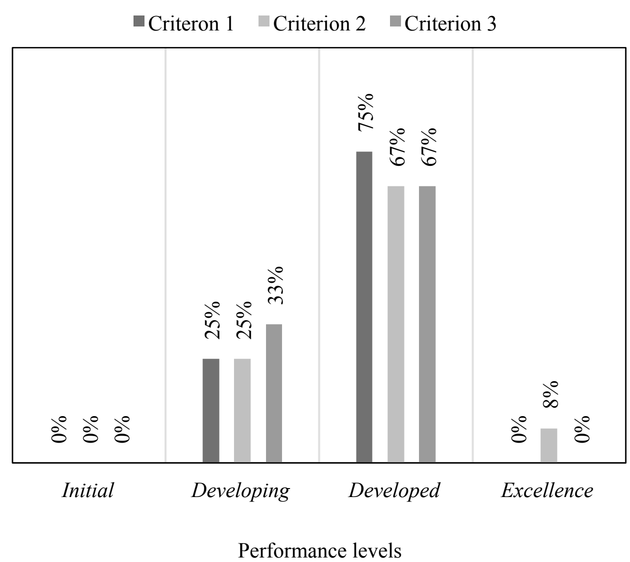

Fig. 8 Disciplinary Learning Outcomes evaluation for morphological characterization of agent.

A general overview indicated that students reached mainly the *developing* and *developed* levels and a minimal part stayed at the level of *excellence*. Furthermore, it is important to point out that the evaluation was carried out in students between the second and fourth semesters of the ABE program, however, the initial levels of technical knowledge students showed a high performance in terms of acquired skills.

Highlighting the importance of the Disciplinary Learning Outcomes evaluation in the continuous improvement of teaching-learning process, it was proposed in the restructuring of the curriculum program that the microbiology course should be move from the third to the fifth semester in order to promote an orderly continuation of the career by students and to respond to the *Consejo de Educación Superior* (CES) request of making a curricular revision every five years [27].

IV. Conclusions

The biotechnology tools enabled the identification of microorganism, as well as, the development of microscopy and its applications using online tools. Consequently, the morphological characterization was done by using specialized free online repositories of the most important microorganism, not only for the agriculture but also for pharmaceutical and food industry.

The morphological characterization highlighted the importance and feasibility of the beneficial microorganism's integration in the agricultural practices for a clean agriculture development with the use of *biocontrollers* like: antagonism microorganism as *Trichoderma* spp., natural controller of pathogenic strains of *Aspergillus* spp., and *Fusarium* spp. Furthermore, the utilization of secondary metabolites and enzymes produced by these beneficial fungi is other alternative, all inside of the biotechnology process.

The knowledge of the differentiating different funguses present in the soils can help to the students to the interpretation of the plant disease with the agent causal for each microorganism, for making better decisions to apply phytosanitary control through the use of biocontrollers agents.

These techniques acquired at the university promote among future professionals a new perspective on clean production, in response to society's demands for a sustainable production. And also the knowledge of these tools allows students to access to postgraduate programs at both national and international levels.

The local reality was a good scenario for students to acquire the knowledge of the profession, and develop their skills necessary for the academy-community relationship. Likewise, the enthusiasm shown by students in the practical application of what was acquired in class facilitated the learning process.

On the other hand, the continuous evaluation of learning outcomes in students demonstrates their usefulness both in the detection of weaknesses, where teachers can intervene reinforcing the activities carried out in each unit through autonomous work or participation at the laboratories practices; and as an input for the curricular revision.

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References

[1] FAO, "La agricultura mundial en la perspectiva del año 2050," in *Como alimentar al mundo 2050*, Roma, Italia, 2009.

[2] FAO, *El estado mundial de la agricultura y la alimentación. Cambio climático, agricultura y seguridad alimentaria*. 2016.

[3] N. Alexandratos and J. Bruinsma, "World agriculture towards 2030/2050: the 2012 revision." FAO, 2012.

[4] "What is Agricultural Biotechnology?" [Online]. Available: http://absp2.cornell.edu/resources/briefs/documents/warp_briefs_eng_scr.pdf. [Accessed: 08-Feb-2017].

[5] A. Russo, G. P. Carrozza, L. Vettori, C. Felici, F. Cinelli, and A. Toffanini, *Plant beneficial microbes and their application in plant biotechnology, Innovations in Biotechnology*. In Tech, 2012.

[6] G. Harman, C. Howell, A. Viterbo, I. Chet, and M. Lorito, "Trichoderma species —opportunistic, avirulent plant symbionts," *Nat. Rev. Microbiol.*, vol. 2, pp. 43–56, 2004.

[7] C. Pavel and A. Ţicău, "Role of university in relationship building between individual and community," *Procedia - Soc. Behav. Sci.*, vol. 142, pp. 118 – 122, 2014.

[8] M. F. Calderón *et al.*, "Uso de los Sistemas de Información Geográfica (SIG) como herramienta de aprendizaje aplicado en el proceso de integración Universidad-Comunidad," in *Proceedings of the Fourteenth LACCEI International Multi-Conference for Engineering, Education and Technology*, Costa Rica, 2016.

[9] ESPOL, "ESPOL - ESPOL Ecológico." [Online]. Available: http://www.espol.edu.ec/es/espol-ecol%C3%B3gico. [Accessed: 18-Feb-2017].

[10] L. L. Galarza Romero, "Aislamiento e identificación molecular de Trichoderma spp," Escuela Superior Politécnica del Litoral, Ecuador, 2011.

[11] Y. Elad, I. Chet, and Y. Henis, "Biological control of Rhizoctonia solani in strawberry fields by Trichoderma harzianum," *Plant Soil*, vol. 60, pp. 245–254, 1981.

[12] G. Samuel, P. Chaverri, D. Farr, and E. McCray, "Trichoderma online, systematic mycology and microbiology laboratory," 2014. [Online]. Available: http://nt.ars-grin.gov/taxadescriptions/keys/TrichodermaIndex.cfm.

[13] R. A. Samson *et al.*, "Phylogeny, identification and nomenclature of the genus Aspergillus," *Stud. Mycol.*, vol. 78, pp. 141–173, Jun. 2014.

[14] A. D. Pietro, M. P. Madrid, Z. Caracuel, J. Delgado-Jarana, and M. I. G. Roncero, "Fusarium oxysporum: exploring the molecular arsenal of a vascular wilt fungus," *Mol. Plant Pathol.*, vol. 4, no. 5, pp. 315–325, Sep. 2003.

[15] S. L. Dodd, E. Lieckfeldt, P. Chaverri, B. E. Overton, and G. J. Samuels, "Taxonomy and phylogenetic relationships of two species of Hypocrea with Trichoderma anamorphs," *Mycol. Prog.*, vol. 1, no. 4, pp. 409–428, 2002.

[16] L. Galarza *et al.*, "Characterization of Trichoderma species isolated in Ecuador and their antagonistic activities against phytopathogenic fungi from Ecuador and Japan," *J. Gen. Plant Pathol.*, vol. 81, no. 3, pp. 201–210, May 2015.

[17] L. Hjeljord and A. Tronsmo, *Trichoderma and Gliocladium in biological control: an overview. In: Harman GE, Kubicek CP (eds) Trichoderma and Gliocladium*, vol. 2. Boca Raton: Taylor and Francis, London; CRC Press, 1998.

[18] S. Woo and M. Lorito, *Exploiting the interactions between fungal antagonists, pathogens and the plant for biocontrol. In: Vurro M, Gressel J (eds) Novel biotechnologies for biocontrol agent enhancement and management*. Netherlands: Springer, 2007.

[19] L. Galarza, Y. Akagi, K. Takao, E. Peralta, E. Santos, and M. Kodama, "Involvement of ThSNF1 in the development and virulence of biocontrol agent Trichoderma harzianum," *J. Gen. Plant Pathol.*, vol. 81, no. 3, pp. 211–217, May 2015.

[20] L. Hoyos-Carvajal, G. Duque, and P. Orduz, "Antagonismo in vitro de Trichoderma spp. sobre aislamientos de Sclerotinia spp. y Rhizoctonia spp," *Rev. Colomb. Cienc. Hortic.*, vol. 2, pp. 76–86, 2008.

[21] H. Gugnani, "Ecology and taxonomy of pathogenic aspergilli," *Fronstiers Biosci.*, vol. 8, 357 346AD.

[22] S. Ruchi, "Pathogenecity of aspergillus niger in plants," *Cibtech J. Microbiol.*, vol. 1, no. 1, pp. 47–51, 2012.

[23] F. Tekaia and J.-P. Latgé, "Aspergillus fumigatus: saprophyte or pathogen?," *Curr. Opin. Microbiol.*, vol. 8, no. 4, pp. 385–392, Aug. 2005.

[24] J. Luo *et al.*, "DNA methylation patterns of banana leaves in response to Fusarium oxysporum f. sp. cubense tropical race 4," *J. Integr. Agric.*, vol. 15, no. 12, pp. 2736–2744, Dec. 2016.
[25] D. Manzo *et al.*, "Fusarium oxysporum f.sp. radicis-lycopersici induces distinct transcriptome reprogramming in resistant and susceptible isogenic tomato lines," *BMC Plant Biol.*, vol. 16, no. 1, Dec. 2016.
[26] M. K. Musyoki, G. Cadisch, J. Zimmermann, H. Wainwright, F. Beed, and F. Rasche, "Soil properties, seasonality and crop growth stage exert a stronger effect on rhizosphere prokaryotes than the fungal biocontrol agent Fusarium oxysporum f.sp. strigae," *Appl. Soil Ecol.*, vol. 105, pp. 126–136, Sep. 2016.
[27] Consejo de Educación Superior (CES), "Reglamento Régimen Académico." República del Ecuador, 2013.