



Lactic acid bacteria (*Leuconostoc mesenteroides*) as bioprotective agents against some pathogenic fungi in common bean

Mohamed DJELILATE,^{1,2} Lahouaria Mounia MANSOURI,⁴
Anas MAMI,^{1,3} Abdenour KHELOUFI^{*4}

¹Laboratory of Applied Microbiology, Faculty of Sciences, University of Oran, 31100 Oran, Algeria; e-mails: djelilatemohammed31@gmail.com; anas.mami@yahoo.fr

²Department of Biology, Faculty of Science and Technology, University of Relizane, 48000 Relizane, Algeria; e-mail: djelilatemohammed31@gmail.com

³Department of Life and Environment, University of Sciences and Technology of Oran, 31000 Oran, Algeria; e-mail: anas.mami@yahoo.fr

⁴Department of Ecology and Environment, Faculty of Natural and Life Sciences, University of Batna 2, 05078 Batna, Algeria; e-mails: l.mansouri@univ-batna2.dz; a.kheloufi@univ-batna2.dz

Manuscript received May 12, 2023; revised May 20, 2023, accepted June 12, 2023

Abstract. The common bean (*Phaseolus vulgaris* L.) is the most important edible food legume in the world. However, its cultivation encounters several phytopathogens. Our study aims to improve food quality and safety through more natural protection methods such as the use of microorganisms that allows us to avoid and minimize pesticide risks. For this purpose, we tested the antagonistic capacity of lactic acid bacteria isolated from raw goat's milk against four fungal strains isolated from *P. vulgaris* var. *djedida*. In this study, lactic acid bacteria (LAB) (*Leuconostoc mesenteroides*) were screened in vitro for antifungal activity at 28 °C against *Fusarium oxysporum*, *Botrytis cinerea*, *Aspergillus flavus*, and *Alternaria alternata*. The statistical analysis of the antifungal activity of LAB showed significant differences after a seven-day period. The results of the direct confrontation on the PDA (Potato Dextrose Agar) and MRS (de Man, Rogosa, and Sharpe) Agar medium showed better inhibition by the lactic acid strain on MRS Agar medium. *L. mesenteroides* gave the highest inhibition rate of 57.6% for the pathogen *B. cinerea* and 29.1%, 33.3%, and 26.7%, respectively, for the pathogens *A. alternata*, *B. cinerea*, *F. oxysporum*, and *A. flavus* on the solid PDA medium. However, on the MRS Agar medium, inhibition rates of 88.1% and 80.5% were observed for the pathogens *B. cinerea* and *A. flavus* and a total inhibition of 100% on *A. alternata* and *F. oxysporum* in the presence of the strain *L. mesenteroides*. This study led to suggest that food-derived LAB strains could be selected for biotechnological application to control phytopathogenic fungi.

Keywords: agriculture, antifungal activity, lactic bacteria, Fabaceae, phytopathology

1. Introduction

Agriculture is facing many challenges all across the globe, including climate change, loss of biodiversity, failure to control production techniques, diseases and pests that directly affect the already insufficient production in many developing countries [1]. Common bean (*Phaseolus vulgaris* L.) is the most important source of proteins for nearly five hundred million people in Africa, Latin America, and the Caribbean (LAC) [2]. The production of common beans still remains irregular and seems to be closely linked to a certain number of abiotic (poor cultivation practices, climate change, inadequate choice of land, and especially exhaustion of soil in a term of fertility) and biotic (genetic potential, the proliferation of diseases, pests, etc.) factors; hence the persistence of a significant deficit in production [3]. In Algeria, economically accessible food legumes occupy an important place in food security. These biotic and abiotic constraints are also due to the absence of tolerant or resistant varieties to these constraints; hence the need for varietal selection. Diseases are some of the major biotic factors affecting bean production. Most of the organisms causing these diseases are seed-borne arising from external contamination or being carried in the seed. Indeed, the rapid and insidious development of phytopathogenic agents on the bean crop often generates more or less serious yield losses. Sources of fungal contamination are numerous, and fruits can be infected with fungi during the growing season, as well as throughout the harvest process to storage [4].

The most harmful toxigenic fungi in agriculture belong to the genera *Alternaria*, *Botrytis*, *Fusarium*, and *Aspergillus* [5]. Transmission of these fungi on common bean can be as contaminants that attach or stick to the seed coat or infect the seed [6]. Fungal resistance poses great problems in terms of plant protection. Indeed, there are only a few antifungal products that are effective against certain multi-resistant agents [7]. However, the World Health Organization has banned the use of certain chemical fungicides due to their long-term adverse toxicological effects, including carcinogenicity [8]. In Algeria, common bean yields and disease resistance are significantly affected by variable weather and soil conditions. Global climate change is also an important consideration in agricultural production. Suboptimal water supply and temperatures exert adverse effects on common bean growth and yields [9].

On the other hand, pathogenic microorganisms are difficult to control because they can survive in the soil for long periods [10]. Therefore, it would be wise to search for microbial antagonists capable of ensuring the healthy bioprotection of the common bean within microorganisms, which are generally tolerated by

humans and animals. Among these microorganisms, lactic acid bacteria (LAB) have antimicrobial activities frequently used in developing fermented foods, and they could be promising agents [11]. In addition, they are devoid of any toxicity for the consumer, which is why they enjoy a GRAS (Generally Recognized as Safe) status [12]. LAB are, therefore, quite harmless microorganisms and appear to be beneficial for the health of consumers. A number of studies have shown that species belonging to the genus *Leuconostoc*, mainly *L. mesenteroides*, can inhibit the growth of several pathogenic bacteria due to their ability to produce organic acids, diacetyl, and bacteriocins, the best known of which being mesenterocin Y105 produced by *L. mesenteroides* [13], [14]. Our study focused mainly on LAB (*L. mesenteroides*) isolated from goat milk in order to study its effect in vitro against four phytopathogenic fungi. The fungal species chosen in this study were *Fusarium oxysporum*, *Botrytis cinerea*, *Aspergillus flavus*, and *Alternaria alternata*.

2. Materials and methods

2.1. Study area

The region of Relizane (Northwest Algeria) has a hot Mediterranean climate with dry summer, where the average temperature is 19.9 °C, and rainfall is on average 407.6 mm. The region of Relizane is characterized by an arid to semi-arid climate, especially at the level of the plain area. There are cold and rainy winters and hot summers with snowfall in some western regions exceeding 800 meters altitude and in the mountains of Ouarsenis, in the high mountains of Bourokba, and in the mountains of Beni-Chougrane, Mendes, Zemmora, and Dahra. The region of Relizane is characterized by a pronounced summer drought and a rainfall deficit, which makes irrigation compulsory. The low rainfall (279 mm/year) and the irregularity of annual rainfall (45% are recorded during the months of November and December) cause a water deficit estimated at 85 mm/year. The average temperatures vary between 11 °C and 30 °C for the months of January and August. The annual average temperature is 20 °C. The coldest months are January and December (11 °C), while the months of August and July are the hottest months with an average of 30 °C [15].

2.2. Biological material

2.2.1. Lactic acid bacteria (LAB)

The control strains of *Leuconostoc mesenteroides* were obtained from the collection of the Laboratory of Applied Microbiology of the Biology Department (Faculty of Sciences, University of Oran, Algeria).

2.2.2. Phytopathogenic fungi

Isolates of *A. alternata*, *B. cinerea*, *F. oxysporum*, and *A. flavus* were obtained from 70 plants of common bean (*Phaseolus vulgaris* var. *Djedida*). Their stems, pods, and leaves showed typical symptoms of the diseases caused by these pathogens. Samples were collected from commercial farms in Relizane (North-West Algeria) during the spring of 2020. Fragments were excised and placed aseptically on a PDA medium and incubated at 28 °C. The morphological identification of fungi mainly involves the cultural and morphological characteristics of the fungi isolated in the pure state [16]. We use pure cultures isolated from single conidial isolation (monospore cultures).

Identifications of the pathogen were based on a macroscopic study of the morphological characters of the culture. The distinction between pathogenic germs is expected to be made based on specific criteria *viz.* pigmentation, the appearance of mycelium, growth rate, and abundance of pycnidia [17]. Sometimes symptoms can be attributed to certain fungal species, such as discolouration, shrivelling, and cracks on common bean stems, pods, and leaves. In most cases, identification of a pathogen based only on such symptoms is not recommended, as these can be common to several fungi [18]. The predominant major foliage and pod pathogen was identified as *A. alternata*, while the common stem pathogens were identified as *F. oxysporum* and *B. cinerea*. Another important fungus that was isolated from leaves is *A. flavus*.

In a second step, a microscopic study was carried out on the evaluation of the morphological characters of the various organs of asexual reproduction and of the mycelium. Microscopic examination of a fungal culture was done under 40X magnification [19].

2.3. Confrontation method and evaluation of antifungal activity

In this study, we used the method of direct confrontation *in vitro*, which consists in inoculating the lactic strain on the PDA (Potato Dextrose Agar) or MRS (Man, Rogosa, and Sharpe) Agar medium in a Petri dish in two parallel streaks of 2 cm and incubating it at 30 °C for 48 hours [11]. Isolates on PDA medium were used as a control direct confrontation test. On the same medium and after 48 hours, a 0.5 cm agar disc of the 5-day-old fungal strain was deposited in the centre of the Petri dish (90 mm in diameter) and incubated at 28 °C for seven days. To determine the influence of the lactic acid strain, the radial growth of the fungal strains was measured daily in two perpendicular directions and compared with the controls [20]. The inhibition percentage (I) was calculated according to the method of [21].

$$\text{Inhibition percentage } (I)(\%) = \frac{C - T}{C} \times 100,$$

where C: Colony diameter in control (cm) and T: Colony diameter in treatment (cm).

2.4. Statistical analysis

The experiments were conducted with five replicates, and the results were expressed as means. All the data were subjected to one-way analysis of variance (ANOVA) and Tukey's HSD multiple comparisons test using SAS Version 9.0 (Statistical Analysis System) (2002) software. A value of $p < 0.05$ was considered as significant.

3. Results and discussions

3.1. Microscopic observation of *Leuconostoc mesenteroides*

Table 1 showed the characteristics of macroscopic and microscopic observation and physiological and biochemical tests. The study of these characteristics served as a basis for the identification of the genus and the species. The obtained characteristics made it possible to verify and confirm the relationship of the species that were isolated from the goat's milk of the region of Sig (Mascara, Algeria). The macroscopic appearance of the colonies of *L. mesenteroides* isolated and purified on MRS Agar medium showed that the colonies were small, round, and lenticular with a whitish colour (Figure 1).

According to Table 1, the microscopic appearance carried out after Gram staining revealed that our strains were Gram-positive, ovoid-shaped in pairs and in even, short, and curved chains. These results are similar to those of Ogier et al. [22]. The physiological and biochemical characteristics agree with the criteria specific to the genus *Leuconostoc*, and the results obtained are analogous to those obtained by [23].

Table 1. Microscopic and macroscopic observation and characteristics of some biochemical and physiological tests of the studied strain *L. mesenteroides*

| Gram | Fermentation type | | Microscopic observation | | | Macroscopic observation (Colony) | | | |
|---------------------------------------|-------------------|----|------------------------------------|----|----|---|------------------|----------------|---------------|
| + | Hetero | | Ovoid in pair/short, curved chains | | | Round, small, and lenticular shape, whitish in colour | | | |
| Physiological test | | | | | | | Biochemical test | | |
| Growth at different temperatures (°C) | | | | | | Growth pH | | Gas production | Catalase test |
| 4 | 37 | 40 | 45 | 55 | 63 | 4.8 | 6.5 | + | - |
| - | + | + | - | + | - | + | + | | |

Notes: (+): growth, (-): no growth.

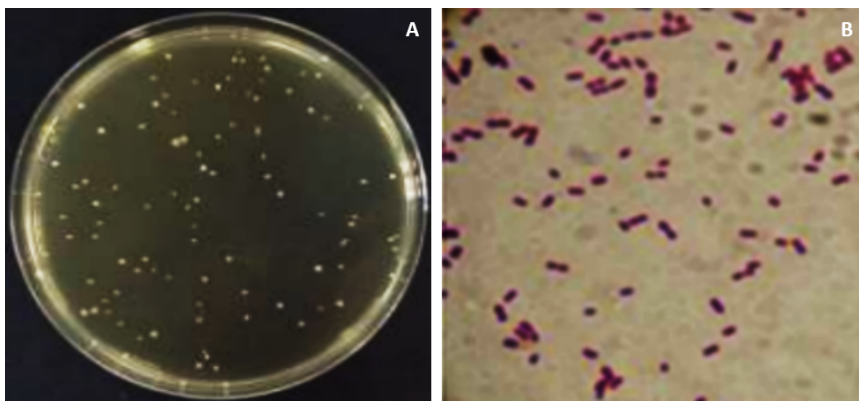


Figure 1. *L. mesenteroides* observation in a petri dish (A) and microscopic observation under 100X magnification (B).

3.2. Macroscopic and microscopic appearance of fungal pathogens

The predominant major foliage and pod pathogens were identified as *A. alternata*, while the common stem pathogens were identified as *F. oxysporum* and *B. cinerea*. Another important fungus that was isolated from leaves is *A. flavus*.

Botrytis cinerea: Colonies of *B. cinerea* on PDA medium were visually classified into two morphological types, without marked differences in sporulation. The identified mycelium was examined in the study, characterized by the absence of sclerotia formation and by a rather grazing mycelium. The pathogen *B. cinerea* produces a mycelium with articulated filaments, brownish or olive, sometimes cylindrical at the level of the median septum, the diameter of which varies considerably depending on the conditions of hyphal development. When the mycelium is in the fructification stage, it produces clumps of greyish, rounded-branched conidiophores containing clusters of conidia (Figure 1A).

Alternaria alternate: The colour of colonies ranged between light to dark olivaceous. The majority of the colonies have a fluffy or cottony appearance, and slight variations in mycelial growth with regular or irregular borders can be observed, with or without concentric zones. These observations are in agreement with those of [24]. Species of the genus *Alternaria* possessed septate conidia with transverse and longitudinal partitions in simple or branched chains, brown, irregular (20–80) x (9–18) μm (Figure 2B), more often with a short but well-differentiated apical rostrum [19].

Aspergillus flavus: The macroscopic study of *A. flavus* is performed with the naked eye after seven days of incubation, on PDA at optimal temperature (25 ± 2

°C). Indeed, *A. flavus* develops rapidly (2 to 3 days) on this medium. The shape of the colonies is downy to powdery, first white, then yellow, and then green-yellow. The microscopic examination of the strain under investigation revealed a segmented mycelium. The aspergillus head appeared as a spherical vesicle with phialides forming on metules (biserium head), each phialide producing globular conidia to subglobose, pale green, echinulate. *Aspergillus* heads are borne on hyaline conidiophores (Figure 2D). All of these observations – namely: macroscopic and microscopic – corroborate those of Tabuc [25], who indicates that they belong to *A. flavus*.

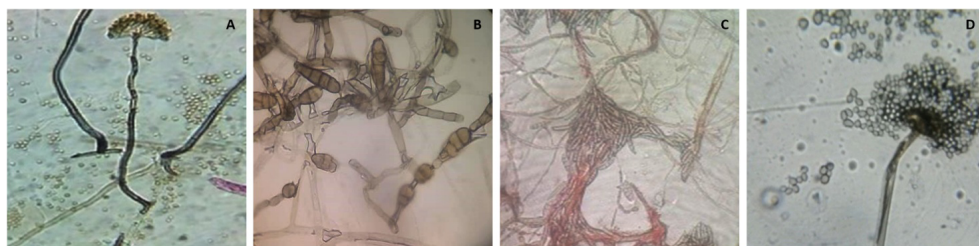


Figure 2. Microscopic observation of pathogens under 40X magnification [*B. cinerea* (A), *A. alternata* (B), *F. oxysporum* (C), *A. flavus* (D)]

Fusarium oxysporum: After purification of the isolates by monospore culture, several major morphological types were observed among the offspring. The aerial mycelium was fluffy in appearance, thick, dense, and relatively sparse. Microscopic observation showed the presence of a septate thallus with short monophialids, on which microconidia were found, as well as the presence of chlamydospores (Figure 2c).

3.3. Direct confrontation *in vitro*

The results of the method of the direct confrontation of the strain *L. mesenteroides* against the four fungal isolates (*A. alternata*, *B. cinerea*, *F. oxysporum*, and *A. flavus*) on PDA medium and MRS Agar are shown in Table 2 and figures 3–4. According to the results of the analysis of variance, the effect of the fungal strain was very significant ($p < 0.001$), just as the effect of the medium and the interaction of these two factors (strain x medium) were also very significant ($p < 0.001$).

Table 2. Inhibition percentage of *B. cinerea*, *A. alternata*, *F. oxysporum*, and *A. flavus* by *L. mesenteroides* after seven days of incubation at 28 °C in two different culture media

| Fungi isolates | Inhibition percentage (I) (%) | |
|---------------------|-------------------------------|-------------------|
| | MRS Agar Media | PDA Media |
| <i>B. cinerea</i> | 88.1 ^b | 57.6 ^a |
| <i>A. alternata</i> | 100 ^a | 29.1 ^c |
| <i>F. oxysporum</i> | 100 ^a | 33.3 ^b |
| <i>A. flavus</i> | 80.5 ^c | 26.7 ^d |
| <i>P-value</i> | < 0.001 | < 0.001 |

Note: Means with similar letters in each column with 5% probability are not statistically different.

3.4. Direct confrontation on the PDA medium

Direct confrontation of the *L. mesenteroides* strain isolated from goat's milk on a PDA medium gave a level of inhibition that varied from one pathogen to another. The highest rate of inhibition for *Botrytis cinerea* (57.6%) was observed on the 7th day of incubation. The inhibition of the 1st day for this isolate did not exceed 12.5%. On the other hand, for *F. oxysporum*, the highest inhibition rate was 33.3% on the 6th day of incubation compared to the 1st day and inhibition of 8.3%. The rate of inhibition of pathogenic strains *A. alternata* with *A. flavus* is very close (29.1% and 26.7% respectively). The highest rate of inhibition of these isolates was achieved on the 4th and 5th day of incubation (Figure 3). The results obtained showed that the radial growths of the tested fungal strains are much lower than those of the controls (Table 2).

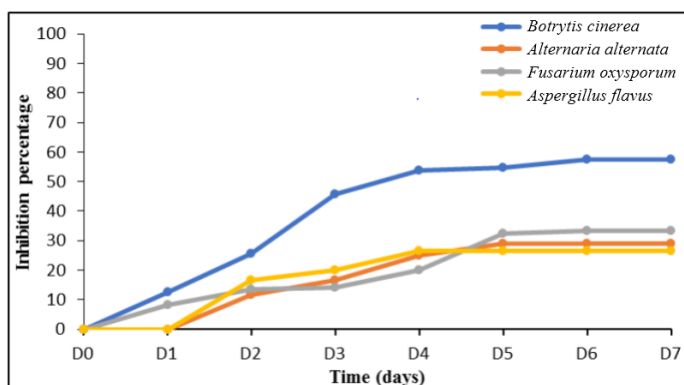


Figure 3. Kinetic of inhibition percentages of *B. cinerea*, *A. alternata*, *F. oxysporum*, and *A. flavus* by *L. mesenteroides* after 7 days of incubation at 28 °C in PDA media

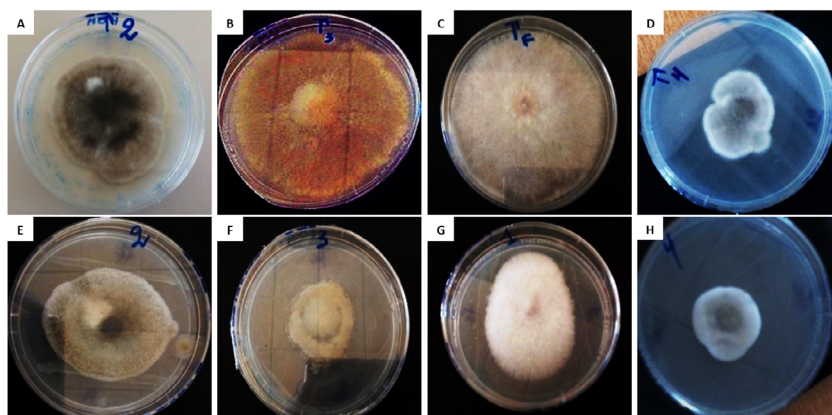


Figure 4. Antifungal activity of selected LAB strain (*L. mesenteroides*) evaluated by confrontation assay against *A. alternata* (A – control, E – confrontation assay), *B. cinerea* (B – control, F – confrontation assay), *F. oxysporum* (C – control, G – confrontation assay), and *A. flavus* (D – control, H – confrontation assay) after incubation at 28 °C for 7 days in PDA medium

3.5. Direct confrontation on the MRS Agar medium

The direct confrontation of the *L. mesenteroides* strain with the four fungal pathogens (*Botrytis*, *Fusarium*, *Aspergillus*, *Alternaria*) on the MRS Agar medium is illustrated in Figure 5. The LBA *L. mesenteroides* produced a strong inhibitory effect, which gives a percentage inhibition between 28.5% and 88.1% against *B. cinerea* (Figure 6B,F) and a moderate effect for the pathogen *A. flavus* at a percentage of 11.1% up to 80.5% (Figure 6D,H). On the other hand, for the pathogens *F. oxysporum* (Figure 6C, G) and *A. alternata* (Figure 6A,E), it was observed that there is no fungal growth on the MRS Agar medium in the presence of the *L. mesenteroides* strain, and this amounts to the fact that the lactic strain produces remarkable inhibitory substances on its specific medium (Figure 6).

Lactic acid bacteria (LAB), considered safe for human and animal health, are widely used for the fermentation and preservation of food. LAB are capable of producing various antifungal materials [11]. Research on the biological control of phytopathogenic fungi has intensified in recent years. The development of these methods would make it possible to define prevention strategies that are more respectful of the environment. Naturally occurring substances biosynthesised by bacteria, fungi, or higher plants have been shown to be important sources of molecules capable of inhibiting the growth of fungi [26]. Lactic acid bacteria exhibiting antifungal activities have also been isolated from other biological systems such as silages [27], raw milk, and sausages [28]. Antimicrobial compounds by lactic acid bacteria have attracted great attention due to their

application in biological control and are well known for their abilities to exhibit antifungal activity; they are currently used in dairy products for their antifungal properties [29].

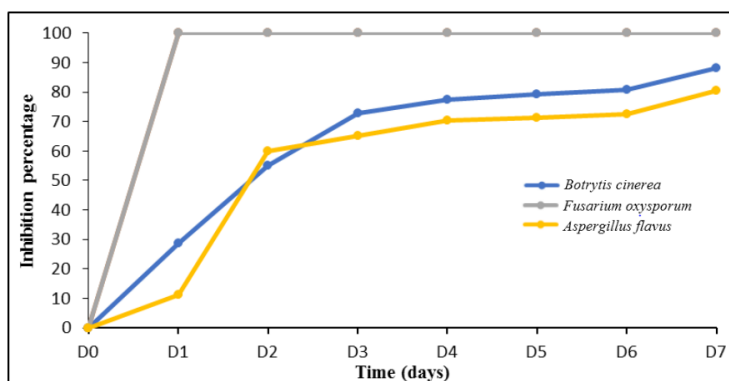


Figure 5. Kinetic of inhibition percentages of *B. cinerea*, *A. alternata*, *F. oxysporum*, and *A. flavus* by *L. mesenteroides* after 7 days of incubation at 28 °C in MRS Agar media

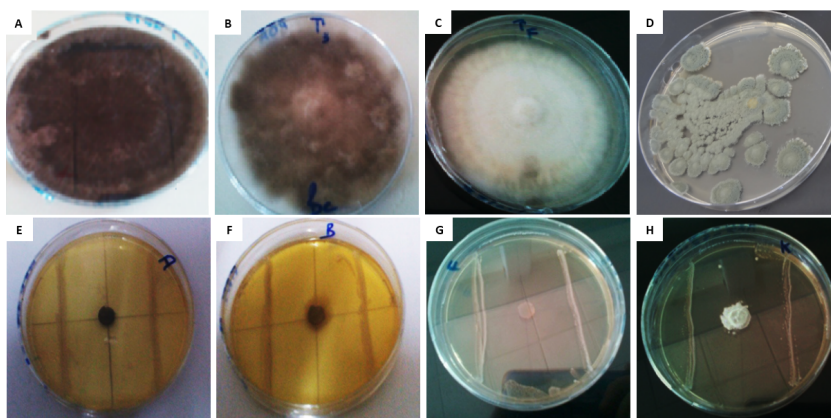


Figure 6. Antifungal activity of selected LAB strain (*L. mesenteroides*) evaluated by confrontation assay against *A. alternata* (A – control, E – confrontation assay), *B. cinerea* (B – control, F – confrontation assay), *F. oxysporum* (C – control, G – confrontation assay), and *A. flavus* (D – control, H – confrontation assay) after incubation at 28 °C for 7 days in MRS Agar medium

The results illustrated in this work present a comparison between the inhibition rates of the *L. mesenteroides* strain on the two culture media used for the comparison (PDA and MRS Agar) to see their antifungal influence on the isolated fungal strains. The inhibition rate of the *L. mesenteroides* strain is greater on the MRS

Agar medium than on the PDA medium since it is the specific medium for these lactic acid bacteria [30]. On the MRS Agar medium, the percentage inhibition is maximum and reaches 100% from the first day for the pathogens *A. alternata* and *F. oxysporum*. On the other hand, the inhibition reached 88% and 80.4% from the 7th day for *B. cinerea* and *A. flavus*. These results are close to those of Bianchini [31], who studied the effect of antifungal compounds produced by *Lactobacillus plantarum* on the growth of *Aspergillus spp.*

According to Dalie [11], the most convincing results are obtained when the bacteria were inoculated into the MRS Agar culture medium 48 hours before inoculation of the fungi. Significant activation of toxin production associated with a reduction in fungal growth is observed suggesting the existence in this medium of compounds, which would activate or act in synergy with the entities produced by the lactic acid strain. These hypotheses are in line with the conclusions of Schillinger and Villarreal (2010) [32], namely that certain compounds of the MRS Agar medium, viz. sodium acetate, influence the antifungal activity of lactic acid bacteria. In the light of the results found, we can conclude that the antifungal substances produced by the lactic acid bacteria of the genus *L. mesenteroides* are probably bacteriocins and exert a strong inhibitory effect on the growth of fungal strains isolated from beans.

4. Conclusions

During this work, the study of the antagonist influence of *L. mesenteroides* in vitro was based on the ability to inhibit the mycelial growth of phytopathogenic moulds, which affect common bean (*Phaseolus vulgaris*), their inhibition rate being higher on MRS Agar medium than on PDA medium since it is the specific medium for these lactic acid bacteria. The strain of *L. mesenteroides* is very interesting. The latter draws a conclusion that isolated bacteria are promising natural biocontrol agents and should be further studied and tested for the control of numerous plant diseases. Additional studies are required to definitively determine their mode of antifungal action, safety, and biocompatibility.

References

- [1] Amoabeng, B. W., Johnson, A. C., Gurr, G. M. (2019), Natural enemy enhancement and botanical insecticide source: A review of dual use companion plants. *Applied Entomology and Zoology* 54(1), 1–19.
- [2] Cortes, A. J., Monserrate, F. A., Ramírez-Villegas, J., Madriñán, S., Blair, M. (2013), Drought tolerance in wild plant populations: The case of common beans (*Phaseolus vulgaris* L.). *PLOS One* 8, e6289.

- [3] Muke, A., Asanzi, M. C., Kijana, R. (2019), Appropriate use of fertilizers in the production of common bean (*Phaseolus vulgaris*) in the humid tropics of eastern Democratic Republic of Congo. *Afrique Science* 15(3), 176–189.
- [4] Sanchez-Reinoso, A. D., Ávila-Pedraza, E. Á., Restrepo-Díaz, H. (2020), Use of biochar in agriculture. *Acta Biológica Colombiana* 25(2), 327–338.
- [5] El-Mabrok, A. S. W., Hassan, Z., Mokhtar, A. M., Hussain, K. M. A., F. K. S. B. A., K. (2012), Screening of lactic acid bacteria as biocontrol against (*Colletotrichum capsici*) on chilli Bangi. *Research Journal of Applied Sciences* 7(9–12), 466–473.
- [6] Guimarães, A., Venancio, A., Abrunhosa, L. (2018), Antifungal effect of organic acids from lactic acid bacteria on *Penicillium nordicum*. *Food Additives & Contaminants: Part A* 35(9), 1803–1818.
- [7] Šišić, A., Baćanović-Šišić, J., Karlovsky, P., Wittwer, R., Walder F., Campiglia, E., Radicetti, E., Friberg, H., Baresel, J. P., Finckh, M. R. (2018), Roots of symptom-free leguminous cover crop and living mulch species harbor diverse *Fusarium* communities that show highly variable aggressiveness on pea (*Pisum sativum*). *PLOS One* 13(2), e0191969.
- [8] Chahardehi, A. M., Ibrahim, D., Sulaiman, S. F. (2010), Antioxidant, antimicrobial activity and toxicity test of *Pilea Microphylla*. *International Journal of Microbiology* 6.
- [9] Mansouri, L. M., Kheloufi, A., Belatreche, R., Abdou, I., Boukhatem, Z. F., Nouzha, H. (2020), Effects of nitrogen fertilization on Mycorrhizal infection, nodulation and growth of *Phaseolus vulgaris* L. *Contemporary Agriculture* 69(3–4), 61–72.
- [10] Tschen, J. S. M., Kuo, W. L. (1985), Antibiotic inhibition and control of *Rhizoctonia solani* by *Bacillus subtilis*. *Plant Protection Bulletin* (Taiwan, R.O.C.) 27(2), 95–103.
- [11] Dalie, D. K. D. (2010), Biocontrol of fumonisin-producing molds of the genus *Fusarium* by selection of lactic acid bacteria indigenous to maize. [in French]. Ph.D. thesis, University of Bordeaux 1, France.
- [12] Gaspar, P., Carvalho, A. L., Vinga, S., Santos, H., Neves, A. R. (2013), From physiology to systems metabolic engineering for the production of biochemicals by lactic acid bacteria. *Biotechnology Advances* 31(6), 764–788.
- [13] Holland, R., Liu, Q. S. (2011), *Leuconostoc* sp, *Lactic acid bacteria*. Elsevier. 138–142.
- [14] He, Y., Zhu M., Huang, J., Hsiang, T., Zheng, L. (2019), Biocontrol potential of a *Bacillus subtilis* strain BJ-1 against the rice blast fungus *Magnaporthe oryzae*. *Canadian Journal of Plant Pathology* 41, 47–59.
- [15] Makrrougrass, Z. (2016), The use of satellite images in determining the degradation factors of agricultural ecosystems (factor of urban sprawl). [in French]. Abdelhamid Ibn Badis-Mostaganem University, Algeria.

- [16] Rappily, F. (1968): Les Techniques de mycologie en pathologie végétale. *Ann. Epiphytes* 19, 102. INRA, Paris.
- [17] Maufra, J. Y. (1996), Anthracnose [in French]. *Perspectives Agricoles* 22, 6–17.
- [18] Andersen, A. M., Leach, C. M. (1961), Testing seeds for seed borne organisms. In: Stefferud, A. (ed.), *Seeds: The Yearbook of Agriculture*. Washington, DC, USA: US Department of Agriculture. 453–457.
- [19] Botton, B., Breton, A., Fèvre, M., Gauthier, S., Guy, P., Larpent, J. P., Reymond, P., Sanglier, J. J., Vayssier, Y., Veau, P. (1990), *Useful and harmful molds. Industrial importance*. 2nd ed. [in French]. Masson, Paris.
- [20] Gerbaldo, G. A., Barberis, C., Pascual, L., Dalcero, A., Barberis, L. (2012), Antifungal activity of two *Lactobacillus* strains with potential probiotic properties. *FEMS Microbiology Letters* 332, 27–33.
- [21] Hammami, I., Trabelsi, H. D., El Gazzah, M. (2012), *In vitro* screening of soil bacteria for inhibiting phytopathogenic fungi. *African Journal of Biotechnology* 11(81), 14660–14670.
- [22] Ogier, J. C., Casalta, E., Farrokh, C., Saihi, A. (2008), Safety assessment of dairy microorganisms: The *Leuconostoc* genus. *International Journal of Microbiology* 126, 286–290.
- [23] Guiraud, J. P. (1998), *Microbiologie alimentaire*. Paris: DUNOD.
- [24] Rai, P. K., Kumari, L. (2009), Variability in *Alternaria alternata* infecting Periwinkle (*Catharanthus roseus*). *Progress in Agriculture* 9, 269–272.
- [25] Tabuc, C. (2007), *Flore Fongique de différents Substrats et Conditions Optimales de Production des Mycotoxines*. Thèse de Doctorat d'université: Pathologie, Mycologie, Genetique Et Nutrition. Toulouse: L'institut National Poly Technique et de l'université de Bucarest. France.
- [26] Czaczyk, K., Mysza, K. (2007), Biosynthesis of extracellular polymeric substances (EPS) and its role in microbial biofilm formation. *Polish Journal of Environmental Studies* 16(6), 799–806.
- [27] Magnusson, J., Schnurer, J. (2001), *Lactobacillus coryniformis* subsp. *Coryniformis* strain Si3 produces a broad-spectrum proteinaceous antifungal compound. *Applied and Environmental Microbiology* 67, 1–5.
- [28] Varsha, K. K., Nampoothiri, K. M. (2016), Appraisal of lactic acid bacteria as protective cultures. *Food Control* 69, 61–64.
- [29] Schwenninger, S. M., Ah, U., Niederer, B., Teuber, M., Meile, L. (2005), Detection of antifungal properties in *Lactobacillus paracasei* subsp. *Paracasei* SM20, SM29, and SM63 and molecular typing of the strains. *Journal of Food Protection* 68(1), 111–119.

- [30] Le Lay, C., Mounier, J., Vasseur, V., Weill, A., Le Blay, G., Barbier, G., Coton, E. (2016), *In vitro* and *in situ* screening of lactic acid bacteria and propionibacteria antifungal activities against bakery product spoilage molds. *Food Control* 60, 247–255.
- [31] Bianchini, A. (2010), Antifungal activity of lactic acid bacteria: Factors affecting production and stability of antifungal compounds of *Lactobacillus plantarum*, and effects of the antifungal compounds on growth and aflatoxin production by *Aspergillus spp.* Ph.D. thesis, Nebraska University.
- [32] Schillinger, U., Villarreal, J. V. (2010), Inhibition of *Penicillium nordicum* in MRS medium by lactic acid bacteria isolated from foods. *Food Control* 21: 107–111.