Sensory quality of coffee beverrage produced thereof linked to the inhibition of molds growth and ochratoxin A removal from coffee cherries using Lactobacillus plantarum strains.

Runing title : Lactobacillus plantarum against mold growth and ochratoxin A production from coffee cherry to beverage.

ABSTRACT

Aims : Mold contamination of foods especially by mycotoxin producing fungi is not only a global food quality concern for food manufacturers, but it also constitutes a high risk for human and animal health resulting in massive economic losses globally. This study investigated the effect of Lactic Acid Bacteria (LAB) on the growth of *Aspergillus carbonarius* strains and their production of ochratoxin A (OTA).

Methodology: Seven fresh coffee cherry and 9 dry coffee cherry samples were collected from Man, Daloa and Akoupé 3 main coffee producing regions in Côte d'Ivoire. LAB were isolated from fresh coffee cherries while mold strains were from both fresh and dry coffee cherry. The inhibitory effect against mold growth and the ability for OTA removal of selected LAB strains were tested successively in vitro and then during coffee cherry primary postharvest processing before evaluating their influence on sensory quality of beverage. OTA production ability of molds strains are studied using both solid (CYA) and liquid (CYB) Cazapeck Yeast medium.

Results: About 34 fungal isolates belonging to *Aspergillus* and *Penicillium* genus were studied for their OTA production using the agar plug technique and HPLC-FD. Five *A. carbonarius* strains were capable of OTA production between 15.9 and 83mg.kg^{-1}.Out of seven isolates of *Lactobacillus plantarum*, two were successful in inhibition of mycelial growthproduced fungicidal activity; five were successful in retarding it produced fungistatical activity All of *L. plantarum* isolates exhibited OTA reduction ability at about 99 %. The

inoculation of two highest anti-ochratoxigenic LAB to fermenting coffee cherries resulted in great inhibition of mold growth and OTA contents reduction varying from 63.2 to 82.2%. The addition of LAB to coffee cherries did not influence the sensory attributes of the beverages produced thereof.

Conclusion: This study highlighted that LAB are very promising biological candidates for reduction of mold contamination and removal OTA from coffee cherry during primary postharvest processing.

Keywords: Coffee sherry, Biocontrol, Lactic acid bacteria, mold growth, ochratoxin A, sensory quality.

I. Introduction

Coffee grows in over 85 countries through Latino America, Asia and Africa [1]. Various foodstuffs and beverages including coffee cherry and their products are commonly exposed to ochratoxin A (OTA) [2]. Coffee has been reported as a crop currently contaminated by OTA[3]at different stages: immature, mature and overripe cherries from trees, overripe cherries from the ground and beans during drying and storage on the farm [4]. OTA is a secondary metabolite produced mainly by molds belonging to *Penicillium*[5] and *Aspergillus*[6] genus. Unfortunately, removal of OTA from foodstuffs is particularly difficult [7] because OTA has been reported to be resistant to acidity and high temperatures. The destruction of OTA was not complete when exposed 3 hours to high pressure steam sterilization of 121°C and even at 250°C [8]. Some studies highlighted that roasting decreased partially OTA content in coffee beans [9].OTA is still considered as nephrotoxic, carcinogenic, embryotoxic and teratogenic metabolite[10].Many countries have statutory limits for OTA, and concentrations need to be reduced to as low as technologically possible in foodstuffs. The most important measures which have been taken to control OTA were preventive in order to avoid fungal growth and OTA production. However, these measures

including chemical and physical methods are difficult to implement in all cases with the consequence of OTA remaining in crops [11]. Nowadays, biological control is more and more recommended as a prophylactic control on several foodstuffs [12]. Antagonist microorganisms or their products can inhibit or destroy undesired microorganisms in food and agricultural products, particularly mycotoxinogenic molds [13]. Among the microorganisms, lactic acid bacteria (LAB) have been considered to be promising natural biological antagonists for mycotoxigenic mold growth in various agricultural commodities [14]. The main mechanism involved in antimicrobial efficiency of LAB is the production of organic acids, antagonistic compounds and competition for nutrients [15].LAB have been reported to have a reliable ability to inhibit mycelia growth of different species [16]. So they could be used as the best alternative for reduction of pre/post-harvest mold infections [17].*A. carbonarius* is reported to be a greater OTA-producer in coffee cherry [18]. Although Côte d'Ivoire is one of the greatest coffee producing countries, coffee cherry sourced from this country are currently exposed to the high OTA level [19]. In addition, the ability of the LAB strains to inhibit the growth of *A. carbonarius* and to reduce OTA content in coffee cherry and the influence of the addition of these microorganisms on the sensory quality of coffee beverage produced thereof have not yet been study. This work aimed to investigate the ability of LABs for reduction of mold growth and elimination of ochratoxin A in coffee cherries during the primary post-harvest processing and to evaluate the effect of inoculation of LAB to the raw coffee beeans on the sensory quality of coffee beverage produced thereof.

II. Material and methods

1. Sampling of coffee cherries

Nine samples of 1-5 kg fresh coffee cherries (robusta *Coffea canephora*) were harvested directly from coffee trees and seven samples of dry cherries stocks were collected from

Akoupé, Daloa, Man; 3 main coffee producing region of Côte d'Ivoire in January 2018. All coffee cherry samples were stored 5 hours at -20°C until further use.

2. Isolation and identification of microorganisms

LAB were isolated from 10 g of fresh coffee fruit per sample blended with 90 mL of sterilized peptone water diluted to 10^{-6}[20].Molds were isolated by direct plating of five coffee cherries per sample on PDA medium, pH 3.5by addition of tartric acid solution (0.1N) in order to inhibit the bacterial growth and then incubated (25°C, 3 days). The morphological characteristics of the mycelia and conidia were used [20] to identify *Aspergillus* and *Penicillium*. Bacterial DNA was extracted using thermal shock heating to 100°C for 10 min, then rapid cooling to - 80°C for 10 min. The 16S rRNA genes of DNA presumptive LAB strain were amplified using specific primers as indicated by Sebastian et al. [21]. The genomic DNA of presumptive OTA producing mold was extracted as described previously by Atoui et al. [22]. The β-tubulin gene of DNA was amplified with specific primers as described previously [23].

3. Ochratoxin A (OTA) production

Molds identified as belonging to *Aspergillus* section *Nigri* wereinvestigated for OTA production. Conidia suspensions (10^5 conidia.mL^{-1}) were prepared from sporulating fungal cultures [24].This suspension (1mL) was spread on former Czapeck yeast Agar (CYA)mediumor added to 25 mL of Czapeck yeast broth (CYB).Both cultures were incubated at 25°C, 3 days but liquid cultures were done with shaking. OTA contents were evaluated from 4 agar plugs of about 5 mm diameter taken around of fungal [25] or from 5mL of CYA.OTA was extracted for 20 min using an ultrasonic bath and filiterated by appropriated method [26]. Detection of OTA was performed by HPLC (Shimadzu LC-10 ADVP, Japan) using fluorimetric detector (Shimadzu RF20A, Japan) according to the method previsouly described by Kedjebo et al. [6].

100 **4. Inoculation of antifungal LAB to coffee cherries**

101 Fresh coffee cherries sample was divided into 14 fractions of 2.5 kg. The first fraction
102 inoculated with 200 mL of sterile distilled water was considered as the negative control. Two
103 hundred milliliters of LAB D12 ($4\times7\ 10^{8}$ UFC.g^{-1}) and LAB D13 ($3\times7\ 10^{8}$ UFC.g^{-1}) were
104 applied individually to the coffee fruits of fractions 2 and 3. The coffee cherries of fractions 4
105 and 5 were inoculated with a mixture of 100 mL of conidial suspension (4×10^{7} conidia.g^{-1}) of
106 OTA producing mold strain (AcA41) and 100 mL of LAB D12 and LAB D13 cells
107 suspension respectively. Fraction 5 was inoculated with a mixture of 100 mL of conidial
108 suspension of OTA producing mold (AcA41) and 100 mL of LAB D13 cells suspension.
109 Fraction 6 was inoculated with 200 mL of only conidial suspension of mold strain AcA41.
110 Fraction 7 was not inoculated (paysant control). All experiments of inoculation were
111 duplicated. The different inoculated coffee cherries were incubated for 16 hours (overnight) at
112 ambient temperature and sun-dried on a plastic tarpaulin for 11 days.

113 **5. Determination of OTA contents of inoculated coffee cherries**

114 Dried coffee cherries (1 kg) of each fraction were weighted and then dehusked. Green coffee
115 beans (100 g) per fraction were frozen at -80 °C for 2 hours for grinding. Ground coffee (10
116 g) were collected from each sample and added to 100 mL of specific solvent (methanol + 3%
117 sodium bicarbonate solution, 50+50, v/v). The different suspensions obtained were
118 mechanically shaken (300 rpm, 30 min) and then centrifuged (6000 rpm, 10 min and 25°C).
119 Each extract (25 mL) was purified for extraction of OTA with an immuno-affinity column
120 (Ochraprep®, R-Biopharm, France). Final different eluates were added to 1 mL of the OTA
121 mobile phase (purified water + methanol + glacial acetic acid, 30:69:1, v/v/v) for the OTA
122 quantification using previous HPLC-FLD method [6].

123 **6. Analysis of beverage sensory quality**

Three green coffee fractions containing OTA amiount below 8 µg.kg^{-1} were sampled for sensory analysis. The coffee beverage was prepared by brewing 50 g of roasted coffee in 1 L of water for 5 min as previously described by Sanchez and Chambers [27]. The beverages were prepared using 50 g of roasted coffee beans, which ground in 1000 mL of filtered water (pH 7). The cup quality of the coffee beans samples was assessed twice by 8 expert tasters using 7 sensory criteria: aroma (intensity and quality), acidity, sourness, body, astringency, bitterness and global quality [28]. A hedonic assessment was carried out when the beverage temperature reached 55 °C. Scoring was on a scale of 0 to 10, where a score of 0 corresponded to the total absence of the criterion in the coffee[29].

7. Statistical analyses

Statistica software (XLSTAT, USA 2022) was used to perform all statistical analyses. Data were expressed as mean ± standard deviation. Following ANOVA, the sensory and volatile compound values were compared by Tukey test ($p< 0.05$) [28].For the sensory analysis of the coffee drinks, the results were analysed with the XLSTAT 2022.1.2.1274 software (Fisher LSD test at the 5%). Statistical differences with a probability of less than 0.001 ($p < 0.001$) are considered significant and those with a probability of more than 0.001 ($p > 0.001$) are not significant.

III. RESULTS

1. Isolation and identification of detected microorganisms

Sixteen LAB isolates presented common morphological and biochemical characteristics of LAB (results not showed). Molecular identification showed that eleven isolates (75 %) were *Lactobacillus plantarum*, 2 isolates were *Weissella paramesenteroides* (12.5 %), 1 isolate was *W. confusa* (6.3 %) and 1 unidentified isolate (Table 1). Mycological study revealed that 34 molds strains dominated by isolates belonging to genus *Aspergillus* were found in coffee cherries samples. Twenty-three mold strains (67.6 %) belonged to *Aspergillus* section *Nigri*

149 while 4 isolates (11.8 %) were *Aspergillus* section *Fumigati*, 4 isolates were *Rhizopussp* (11.8

150 %) and 2 isolates were *Penicilliumsp.* strains (5.9 %).

151 **Table 1**. LAB isolates from fresh coffee fruits identified using molecular technique

Codes of isolates	Bacterial species	% Similarity	Query coverage	Accession number
D12	*Lactobacillus plantarum*	100	100	MT322914
D13				
D20				CP017066
D23				MN636335
A11				MN602940
A12				CP046262
D24				LC512751
M20		99.5	99.5	CP021929
M24		99.6	100	CP046262
D31		99.6	100	EF439684
A10		99.6	99.6	MN602939
D10		99.6	99.3	MN700260
M31	*Weissella paramesenteroides*	100	100	MH845061
M33				
M21	*W. confusa*	100	100	LC506181
D32	Non identified LAB	-	-	-

152

153 **2. OTA production ability of *Aspergillus* section *Nigri* isolates**

154 Mycotoxin analysis revealed that all 5 mold isolates produced OTA greater than other isolated

155 mold strains belonged to *Aspergillus* section *Nigri*. These mold isolates wereidentified as *A.*

156 *carbonarius* strains. They produced OTA quantities ranging from 15.9 to 83.0mg.kg^{-1} of CYA

157 medium and from 4.9 to 75.8 ng.mL^{-1} in CYB medium (Table 2).

158 **Table 2.** OTA amount produced by *A. carbonarius* isolates from dry coffee cherries on

159 different culture media. Data points are mean values of two replicates ± SE

Code of *A. carbonarius* isolates	Similarity (%)	Query Coverage (%)	Accession number	OTA quantity	
				PDA (mg.kg^{-1})	*CYB (ng.mL^{-1})*
AcA41	100	100	GU296700	83.1 ± 0,9^{a}	25.5 ± 0,8^{b}

AcA42	100	100	KC520549	78.8 ± 7,6[a]	26.0 ± 2,7[b]
AcD61	99,6	100	KP259287	15.9 ± 0,5[c]	4.9 ± 0,2[c]
AcD63	100	97	KC520550	54.0 ± 3,6[b]	75.8 ± 1,7[a]
AcD64	100	98	MG701891	17.4 ± 1,2[c]	6.4 ± 0,1[c]

In a column, the values of OTA quantity assigned to the same alphabetical letter indicated no significate difference at $p \leq 0.05$.

3. Inhibition of *A. carbonarius* growth by cells of LAB strain

The results of assay showed that 10 LAB strains exhibited antifungal activities. Among them, 3 *L. plantarum*coded M24, D20 and D23 had low rate inhibition against mold growth less than 20 %. Four *L. plantarum* coded D13, D31, D32 and D10 showed antifungal activity ranged between 20 to 40 %. Two *L. plantarum* (D24 and D12) and one *Weissella confusa* (M21) showed high rates inhibition over than 70 % against mold growth. However, 2 *Weissella paramesenteroides* (M31 and M33) and four *L. plantarum* (A11, M20, A10 and A12) had no inhibitor effect on *A. carbonarius* AcD64 growth (Table 3).

Table 3. Comparative antifungal acitivity of 16 LAB strains against *Aspergillus carbonarius* growth PDA medium for 48h at 30°C.

LAB strains	Reduction in growth *of A. carbonarius* growth (%)
L. plantarum (D12)	76.4 ± 2.6[a]
L. plantarum (D13)	2.6 ± 1.0[bc]
L. plantarum (D24)	78.7 ± 3.5[a]
Weissella paramesenteroides (M33)	0.0
L. plantarum (M20)	0.0
L. plantarum (M24)	16.9 ± 0.6[c]
W. confusa (M21)	76.4 ± 2.0[a]
L. plantarum (D31)	29.6 ± 0.6[b]
L. plantarum (D23)	17.6 ± 0.6[c]
L. plantarum (A10)	0.0
L. plantarum (D10)	28.5 ± 0.6[b]
L. plantarum (D20)	17.6 ± 0.6[c]
W. paramesenteroides (M31)	0.0
L. plantarum (A11)	0.0
L. plantarum (A12)	0.0
Not identified (D32)	29.1 ± 1.0[b]

Values with the same alphabetical letter do not differ significantly at the 5% level.

4. Effect of antifungal *LAB* strains on OTA of postharvest processed coffee cherries

The results about the effect of LAB strains D12 and D13 addition to coffee cherries showed that OTA contents were reduced from 6.46 (control) to 1.15 and to 2.38 $\mu g.kg^{-1}$ in green coffee beans respectively. The OTA reduction rates were 82.2 and 63.2 % for LAB strains D12 and D13 respectively. In addition, inoculation of OTA producing *A. carbonarius* AcA41 promoted the production of OTA content reached about 20 $\mu g.kg^{-1}$. However, LAB strain D12 and LAB strain D13 reduced OTA content three times and stimulated OTA production from 19.95 to 21.6 $\mu g.kg^{-1}$ in green coffee beans respectively when they were individually co-inoculated with *A. carbonarius* AcA41 (Figure 1).

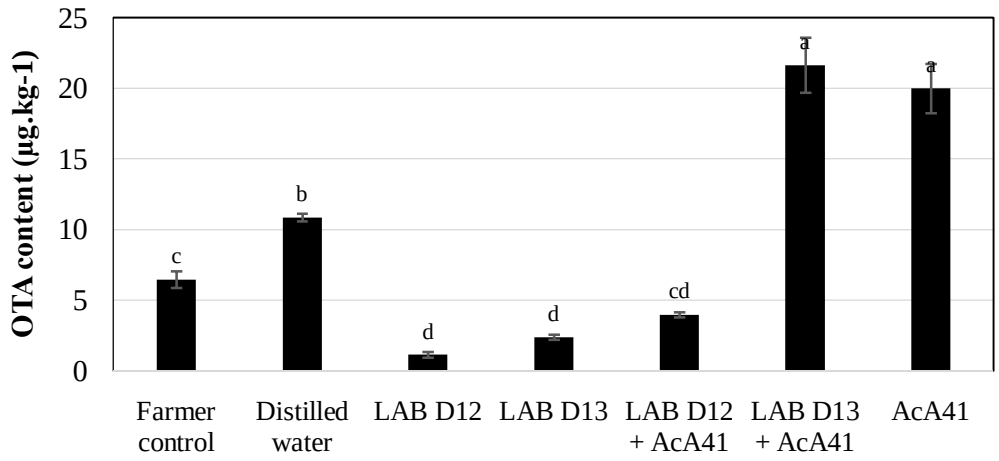

Figure 1. Microbial inoculation tests

Changes in average OTA content measured in coffee cherries inoculated by antifungal LAB strains D12 and D13 co-inoculated with *A. carbonarius* AcA41 during 11 days on the farm. Data points are mean values of two replicates ± SE.Data with different letters are significantly different (One-way ANOVA, Tukey Test, p-value < 0.05).

7. Sensory attributes of coffee beverage linked to the inoculation of OTA reducer LAB strains

Figure 2 presents the sensory attributes of the coffee beverages made from the detoxified coffee beans samples in OTA. The results showed that the coffee beverage from the coffee cherries (farmer's control) recorded most intense coffee flavour with the score of 6.37. Both the beverages made from coffee cherries inoculated with LAB strains D12 or D13 recorded the score about 5. However, no significant difference ($p < 0.05$) was observed at the 5% level about the attributes such as "acidity", "bitterness", "astringency", "body in the cup", "sourness" and "overall quality" between all analyzed coffee beverages.

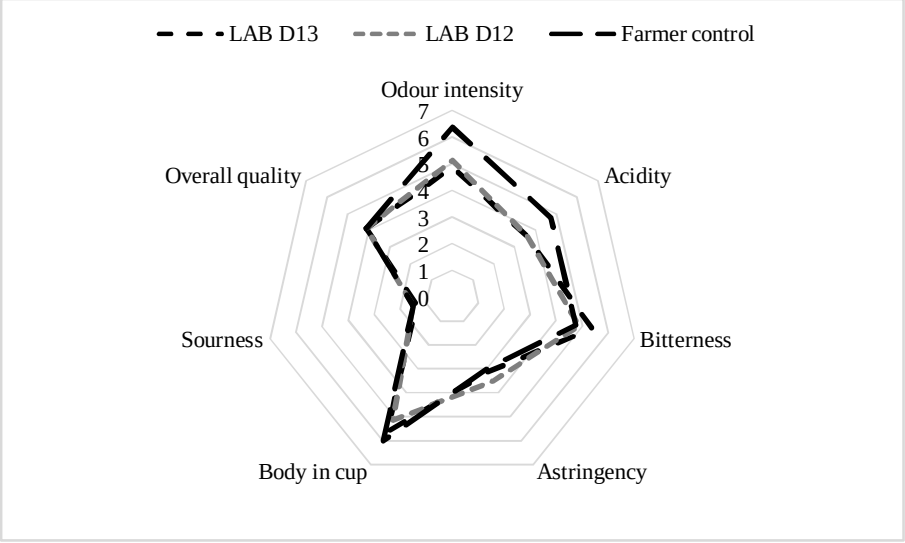

Figure 2. Effect of the *L. plantarum* strains addition to coffee cherries for detoxification in OTA on the sensory attributes of beverages produced

IV. DISCUSSION

Sixteen LAB and 34 fungal strains were isolated from coffee cherries collected from different areas of Côte d'Ivoire. *Lactobacillus* species dominated the bacterial microbiota with 75% of isolates. LAB isolates were lower than those found in Taiwanese coffee cherries [30]. These differences could be ascribed to the impact of various factors including climatic factors, altitude where the coffee farm located, genotype of coffee and post-harvest storage dry coffee cherries. Also, the aerobic conditions as well as the low moisture content could cause the low

208 contamination level of coffee cherries by LAB [31].Some *Lactobacillus plantarum* strains
209 were frequently reported to be predominant species among LAB microbiota found in coffee
210 cherries source fromTaïwan. The fungal strains isolated from our tested coffee cherry
211 belonged mainly to *Aspergillus*, *Penicillium* and *Rhyzopus* genera. Our results are similar to
212 those found in coffee beans[30]. The presence of these fungi could be due to the conditions
213 promoting spoilage of coffee cherries before harvesting and to the fungal contamination
214 during primary postharvest processing [32]. Pre-harvest fungal invasions were mainly due to
215 the interactions between coffee plants and other organisms such as insects. While post-harvest
216 fungal invasions were caused by nutrient availability, temperature, humidity and biotic factors
217 [33]. Proliferation of mold in coffee cherry could be also due to bad post-harvest practices
218 [34]. Our results are different from those found by Vale et al. [31]. indicated that *Aspergillus*,
219 *Penicillium*, *Fusarium*, *Cladosporium* as main genera and by Martinez et al. [35] concluded
220 that *Aspergillus*, *Penicillium*, *Fusarium* and *Rhizopus* genera were dominated. However,
221 *Aspergillus*, *Penicillium*, *Fusarium* and *Cladosporium* were the most common fungal genera
222 contaminated Ivoirian coffee cherries [19, 34]. Our results showed that molds species
223 belonged to *Aspergillus* section *Nigri* were predominant. These results are in agreement with
224 those found by Lu et al. [32]and highlighted some risks for OTA production. Most of our *L.*
225 *plantarum* isolates (9) were exhibited ability varying from 18 to 78% for the reduction of
226 fungal growth. So, *L. plantarum* had a greater negative impact on the fungal growth than
227 other LAB species. Our observations are in agreement with those made by Møller et al. [36].
228 Moreover, Shehata et al. [37]. have concluded that this may be due to the production of
229 metabolites as well as toxicity of the compounds. Furthermore, Møller et al. [36] evoked
230 bacteriocin-forming ability of the LAB strains to explain the inhibition or reduction of molds
231 growth. Dong et al. [38]reported that the antifungal activity of LAB is expressed either
232 directly through competition of live bacterial cells for growth nutrients. The interactions

between LAB and *Aspergillus carbonarius* can cause over-fermentation of coffee cherries, induce undesirable flavours and produce OTA [39].*A. carbonarius* strains found in coffee cherries were able to produce OTA at concentration between 15.9 and 83.0mg.kg^{-1} of solid medium and between 4.9 and 75.8 ng.mL^{-1} in liquid medium respectively. Our isolated *A. carbonarius* strains produced OTA in contrary to Martins et al. [40] who have observed that all *A. carbonarius* strains did not produce OTA. Changes in OTA production abilities of *A. carbonarius* could be explained by many factors such as the strain, the nutrients concentration, the availability space for the mycelial growth and the physico-chemical conditions such as temperature and pH [41].Moreover, some microbial strains were reported to be able to utlize the OTA as a source of carbon in the case of lack of organic nutrients [42]. A total reduction of OTA production of tested *A. carbonarius* strains by the cells of all tested *L. plantarum* strains varied from round 92 to about 99.9 %. Our study hihglighted that many *L. plantarum* species exhibited OTA removal ability as previously obtained by Luz et al. [43]. The individual inoculation of two previously greater inhibitors of mold growth as *L.plantarum* D12 and D13 to coffee cherries showed high OTA reduction in dry green coffee beans of 82.2 and 63.2% respectively. This OTA reduction could be due to the ability of *L. plantarum* for adherence to the surface of coffee cherries [44]. The high OTA levels detected in inoculated coffee cherries with OTA producing *A. carbonarius* strains showed that production of OTA could be due to the previous contamination of coffee cherries by mycotoxigenic fungi [45].However, the OTA-producing fungal contaminants of *Arabica* and *Robusta* coffee in Phillipines are *A. niger* and *A. ochraceus* [46]. Other study reported that A. *niger* and *A. carbonarius* are the main OTA producing molds found in coffee cherries [47]. OTA was reported to be produced during both preharvest [22] and post-harvest processing of coffee cherries [48]. The inoculation of antifungal LAB strains to coffee cherries reduced strongly the OTA contents. These results could be explained by the

production of various compounds with antifungal effects which could damage fungal hyphae and conidia by LAB [49]. Furthermore, the low OTA levels found in inoculated coffee beans confirm that the tested LAB have strong OTA removal ability as showed by Del Prete et al. [50]. The results could be due the ability of some bacterial metabolites produced at high concentrations to lyse of OTA-producing molds cells[44] and to disrupt their fonctionality [51]. The results showed also that *L. plantarum* D12 reduced OTA at 39.6% in coffee beans. This could be explained by the higher ability to adsorb and/or sequester OTA [52]or use it as a carbon by cells of LAB source for the their growth [43].

In our study, there was no significant difference between beverages derived from detoxified coffee cherries and those made from non-inoculated coffee in terms of attributes including "acidity", "bitterness", "astringency", "body in the cup", "sourness" and "overall quality". The similarity about sensory attributes could be ascribed to the fact that the field tests were not conducted in controlled atmosphere, the microbial interactions that favored the dominance of endoegnous microorganisms and to the influence of uncontrolled microorganisms [34]. The low intensity scored by beverages from inoculated coffee cherries could be due to the lower amount of VOCs produced by LAB in inoculated green as compared to the naturally fermented green coffee [53]. Furthermore, the results showed also that the beverage prepared from the farmer control sample had a more intense coffee aroma. Main aroma compounds such as esters, higher alcohols, aldehydes and ketones, formed during roasting from organic compounds precursors would therefore be responsible for the intensity of the coffee odour of the beverage from the farmer control samples [54]. Finally, the inoculation of *L. plantarum* LAB D12 and LAB D13 certainly had an impact on the production of VOCs involved in beverrages sensory qualities. According to Pereira et al. [54], the volatile organic compounds (VOCs) produced by LAB included ester, alcohol, alkane, acid, hydrocarbon, ether and nitrogen-containing. Bertrand et al. [28] concluded that these VOCs seemed to be associated

with a decrease in many aromatic quality attributes. Consequently, we can hypothesise that some VOCs play the inhibitory effetcs against the mycelial growth as like the *Bacillus* strains [55].So, inoculated LABs did not negatively influence the sensory qualities of the coffee beverages.

CONCLUSION

LAB cells were efficient against mycelial growth of *A. carbonarius*. All antifungal *L. plantarum* strains exhibited OTA removal ability by adsorption.The findings of this study are very relevant, especially considering the critical toxic effect of OTA as well as the increasing OTA occurrence worldwide. The purpose of screening for LAB with the ability to reduce OTA production in green coffee was clearly demonstrated. The addition of antifungal and anti-mycotoxigenic LAB strains to coffee cherry did not generally influence the sensory attributes of beverage produced thereof except the intensity of odour. This study highlighted that tested *L. plantarum* strains are very promising biological candidates for various fermented foods safety such as coffee cherries cocoa beans and wine.

REFERENCES

1Toledo VM, Moguel P. Coffee and Sustainability: The multiple values of traditional shaded coffee. J. Sustain. Agric, 2012;36:353 - 377.

2Wang Y, Peng X, Yang Z, Zhao W, Xu W, Hao J, Wu W, Shen XL, Luo Y, Huang K. iTRAQ Mitoproteome analysis reveals mechanisms of programmed cell death in Arabidopsis thaliana induced by Ochratoxin A. Toxins (Basel), 2017;9(5):167.

3Levi CP, Trenk HL, Mohr HK. Study of the occurrence of ochratoxin A in green coffee beans. J. Assoc. Off. Anal. Chem., 1974;57(5):866- 870.

4Taniwaki MH, Pitt JI, Teixeira AA, Iamanaka BT. The source of ochratoxin A in Brazilian coffee and its formation in relation to processing methods. International journal of food microbiology, 2003;82(2):173-179.

5Gerez CL, Torino IM, Rollan G, Fond de Valdez G. Prevention of bread mold spoilage by using lactic acid bacteria with antifungal properties. Food Control. 2009;20(2):144-148.

6Kedjebo KBD, Guehi TG, Kouakou B, Durand N, Aguilar P, Fontana A, Montet D. Effect of post-harvest treatments on the occurrence of ochratoxin A in raw cocoa beans. Food Addit Contam Part A. 2016; 33(1):157-166.

7El Khoury R, Mathieu F, Atoui A, Kawtharani H, Khoury AE, Afif C, Maroun RG, El Khoury A.. Ability of soil isolated actinobacterial strains to prevent, bind and biodegrade Ochratoxin A. Toxins, 2018;9(7):222.

8 Leitão AL. Occurrence of Ochratoxin A in Coffee: Threads and Solutions-A Mini-Review. Beverages.2019;5(2):36.

9Mounjouenpou P, Durand N, Guiraud JP, Tetmoun M, Abeline S, Gueule D, Guyot B. Assessment of exposure to ochratoxin-A (OTA) through ground roasted coffee in two cameroonian cities: Yaounde and Douala. International Journal of Food Science and Nutrition Engineering, 2013;3 :35-39.

10 Pfohl-Leszkowicz A, Manderville RA. Ochratoxin A: an overview on toxicity and carcinogenicity in animals and humans. Mol. Nutr. Food Res. 2007;51(1);61-69.

11 Abrunhosa L, Paterson RR, Venâncio A. Biodegradation of ochratoxin A for food and feed decontamination. Toxins, 2010;2(5):1078-1099.

12Chulze SN, Palazzini JM, Torres AM, Barros G, Ponsone ML, Geisen R, Schmidt-Heydt M, Köhl J. Biological control as a strategy to reduce the impact of mycotoxins in peanuts, grapes and cereals in Argentina. Food Addit. Contam. Part A, 2014;**32(4):**471-479.

13Schillinger U, Geisen R, Holzapfel WH. Potential of antagonistic microorganism and bacteriocins for the biological preservation of foods. Trends food sci. Tech. 1996;71(5):158-64.

14Trias R, Baneras L, Montesinos E, Badosa E. Lactic acid bacteria from fresh fruit and vegetables as biocontrol agensts of phytopathogenic bacteria and fungi. Int. J. microbiol. 2008;11:231-236.

15 Ren X, Zhang Q, Zhang W, Mao J, Li P. Control of aflatoxigenic molds by antagonistic microorganisms: Inhibitory behaviors, bioactive compounds, related mechanisms, and influencing factors. Toxins, 2020;12(1):24.

16Dalié DKD, Deschamps AM, Richard-Forget F. Lactic acid bacteria–Potential for control of mould growth and mycotoxins: A review. Food control, 2010;21(4):370-380.

17 Oliveira PM, Zannini E, Arendt EK. Cereal fungal infection, mycotoxins, and lactic acid bacteria mediated bioprotection: from crop farming to cereal products. Food Microbiol. 2014;37:78-95.

18 Ostry V, Malir F, Toman J, Grosse Y. Mycotoxins as human carcinogens - the IARC Monographs classification. Mycotoxin research, 2017;33:65-73.

19 Djossou O, Perraud-Gaime I, Mirleau FL, Rodriguez-Serrano G, Karou G, Niamke S, Ouzari I, Boudabous A, Roussos S. Robusta coffee beans post-harvest microflora: Lactobacillus plantarum sp. as potential antagonist of Aspergillus carbonarius. Anaerobe, 2011;17(6):267-272.

20Djossou O, Roussos S, Isabelle PG, Macarie H, Germain K, Yoan L. Fungal population, including Ochratoxin A producing Aspergillus section Nigri strains from Ivory Coast coffee bean. African Journal of Agricultural Research, 2015;10(26):2576-2589.

353 21Sebastian P, Herr P, Fischer U, König H. Molecular identification of lactic acid bacteria
354 occurring in must and wine. South African Journal of Enology and
355 Viticulture, 2011,32(2) :300-309.
356 22Atoui A, Mathieu F, Lebrihi A. Targeting a polyketide synthase gene for Aspergillus
357 carbonarius quantification and ochratoxin A assessment in grapes using real-time PCR. Int.
358 J. Food Microbiol. 2007;115(3):313-318.
359 23 Hubka V, Kolařík M. β-tubulin paralogue tubC is frequently misidentified as the benA
360 gene in Aspergillus section Nigri taxonomy: primer specificity testing and taxonomic
361 consequences. Persoonia, 2012;29(1):1-10.
362 24 Axel C, Brosnan B, Zannini E, Peyer L, Furey A, Coffey A, Arendt E. Antifungal
363 activities of three different Lactobacillus species and their production of antifungal
364 carboxylic acids in wheat sourdough. Appl. Microbiol. Biot. 2016;100:1701-1711.
365 25 Sanchez-Hervas M, Gil JV, Bisal F, Ramon D, Martinez-Culebras PV. Mycobiota and
366 mycotoxin producing fungi from cocoa beans. Int. J. Food Microbiol. 2008;125(3):336-
367 340.
368 26 Bragulat MR, Abarca ML, Cabanes FJ. An easy screening method for fungi producing
369 Ochratoxin A in pure culture. Int. J. Food Microbiol. 2001;71(2-3):139-144.
370 27Sanchez K, Chambers IV, E. How does product preparation affect sensory properties? An
371 example with coffee. Journal of Sensory Studies, 2015. 30(6), 499-511.
372 28Bertrand B, Boulanger R, Dussert S, Ribeyre F, Berthiot L, Descroix F, Joët T. Climatic
373 factors directly impact the volatile organic compound fingerprint in green Arabica coffee
374 bean as well as coffee beverage quality. Food chemistry, 2012;135(4):2575-2583.
375 29Neto DPC, Pereira GVM, Finco AMO, Letti LAJ, Silva BJG, Vandenberghe LPS, Soccol
376 CR. Efficient coffee beans mucilage layer removal using lactic acid fermentation in a

stirred-tank bioreactor: Kinetic, metabolic and sensorial studies. Food Bioscience, 2018;26:80-87.

30 Leong KH, Chen YS, Pan SF, Chen JJ, Wu HC, Chang YC, Yanagida F. Diversity of lactic acid bacteria associated with fresh coffee cherries in Taiwan. Current microbiology, 2014;**68** :440-447.

31 Vale AS, Pereira GVM, Neto DPC, Sorto RD, Goés-Neto A, Kato R. Soccol C R. Facility-specific 'house' microbiome ensures the maintenance of functional microbial communities into coffee beans fermentation: implications for source tracking. Environmental Microbiology Reports, 2021;13(4):470-481.

32 Nazhand A, Durazzo A, Lucarini M, Souto EB, Santini A. Review Characteristics, Occurrence, Detection and Detoxification of Aflatoxins in Foods and Feeds, Foods. 2020;9(5):644.

33 Lu, L, Tibpromma S, Karunarathna SC, Jayawardena RS, Lumyong S, Xu J, Hyde KD. Comprehensive Review of Fungi on Coffee. Pathogens, 2022;11(4):411.

34 Kouadio IA, Koffi LB, Nemlin JG, Doss MB, Effect of Robusta (Coffea canephora P.) coffee cherries quantity put out for sun drying on contamination by fungi and Ochratoxin A (OTA) under tropical humid zone (Côte d'Ivoire). Food Chem. Toxicol. 2012;50(6):1969-1979.

35 Martinez SJ, Simão JBP, Pylro VS, Schwan RF. The altitude of coffee cultivation causes shifts in the microbial community assembly and biochemical compounds in natural induced anaerobic fermentations. Frontiers in Microbiology, 2021;12:671395.

36 Møller CODA, Freire L, Rosim RE, Margalho LP, Balthazar CF, Franco LT, de Souza Sant'Ana A, Corassin CH, Rattray FP, Oliveira CAFD. Effect of lactic acid bacteria strains on the growth and aflatoxin production potential of Aspergillus parasiticus,

and their ability to bind aflatoxin B1, ochratoxin A, and zearalenone in vitro. Frontiers in Microbiology, 2021;12:655386.

37 Shehata MG, Badr AN, El Sohaimy SA, Asker D. Awad TS. Characterization of antifungal metabolites produced by novel lactic acid bacterium and their potential application as food biopreservatives. Annals of Agricultural Sciences, 2019 ;64(1) :71-78.

38 Dong Q-Q, Hu H-J, Luo X-G, Wang Q-T, Gu X-C, Zhou H, Zhou W-J, Ni X-M, Zhang T-C. Complete genome sequence of Lactobacillus plantarum CGMCC 8198. Genome Announc., 2017;5(6),1559-16.

39 Fitri F, Tawali AB, Laga A, Dwyana Z. Enzyme activity assay of lactic acid bacteria from civet (paradoxurus hermaphroditus) digestive tract. Advances in Animal and Veterinary Sciences, 2021;9(10):1649-1654.

40 Martins ML, Martins HM, Gimeno A. Incidence of microflora and of ochratoxin A in green coffee beans (Coffea arabica). Food Additives and Contaminants, 2003;20(12):1127-1131.

41 Le Lay C, Mounier J, Vasseur V, Weill A, Le Blay G, Barbier G, Coton E. In vitro and in situ screening of lactic acid bacteria and propionibacteria antifungal activities against bakery product spoilage molds. Food Control, 2016;60:247-255.

42 Zhao M, Wang XY, Xu SH, Yuan GQ, Shi XJ. 2019. Degradation of ochratoxin A by supernatant and ochratoxinase of Aspergillus niger W-35 isolated from cereals. World Mycotoxin Journal, 2019;13(2):287-298.

43 Luz C, Ferrer J, Mañes J, Meca G. Toxicity reduction of ochratoxin A by lactic acid bacteria. Food and chemical toxicology, 2018;112 :60-66.

44 Lappa IK, Mparampouti S, Lanza B, Panagou, EZ. Control of Aspergillus carbonarius in grape berries by Lactobacillus plantarum: A phenotypic and gene transcription study. International journal of food microbiology, 2018;275 :56-65.

45 Wang X, Wang Y, Hu G, Hong D, Guo T, Li J, Li Z, Qiu M. Review on factors affecting coffee volatiles: from seed to cup. Journal of the Science of Food and Agriculture, 2021. 102(4):1341-1352.

46 Barcelo JM, Barcelo RC, Post-harvest practices linked with ochratoxin A contamination of coffee in three provinces of Cordillera Administrative Region, Philippines. Food Additives & Contaminants: Part A, 2018;35(2):328-340.

47 Joosten HMLJ, Goetz J, Pittet A, Schellenberg M, Bucheli P. Production of ochratoxin A by Aspergillus carbonarius on coffee cherries. International journal of food microbiology, 2001;65(1-2):39-44.

48 Batista LR, Chalfoun SM, Silva CF, Cirillo M, Varga EA, Schwan RF Ochratoxin A in coffee beans (Coffea arabica L.) processed by dry and wet methods. Food control, 2009;20(9):784-790.

49 Hirozawa MT, Ono MA, Suguiura IMDS, Bordini JG, Ono EYS. Lactic acid bacteria and Bacillus spp. as fungal biological control agents. Journal of Applied Microbiology, 2023;134(2):lxac083.

50 Del Prete V, Rodriguez H, Carrascosa AV, De Las Rivas B, Garcia-Moruno E, Munoz R. In vitro removal of ochratoxin A by wine lactic acid bacteria. Journal of Food Protection, 2007;70(9):2155-2160.

51 Salas ML, Mounier J, Valence F, Coton M, Thieery A, Coton E. Antifungal microbial agents for food biopreservation – A review. Microorganisms, 2017;5(3):37.

52 Piotrowska M. The Adsorption of Ochratoxin A by Lactobacillus Species. Toxins, 2014;6(9):2826–2839.

53 Nor SM, Yusof NM, Ding P. Volatile organic compound modification by lactic acid bacteria in fermented chilli mash using GC-MS headspace extraction. In IOP Conference Series: Earth and Environmental Science, 2021;765(1):012043.

54Pereira GVD, Neto DPC, Magalhães AIJ, Vásquez ZS, Medeiros ABP, Vandenberghe LPS, Soccol CR. Exploring the impacts of postharvest processing on the aroma formation of coffee beans – A review. Food Chemistry, 2018;272:441-452.

55Ling L, Zhao Y, Tu Y, Yang C, Ma W, Feng S, Lu L, Zhang J. The inhibitory effect of volatile organic compounds produced by Bacillus subtilis CL2 on pathogenic fungi of wolfberry. Journal of Basic Microbiology, 2021;61(2):110-121.