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DEGRADATION OF ORGANOPHOSPHORUS COMPOUNDS BY LACTIC ACID BACTERIA ISOLATED FROM TOMATO FRUITS.

KanyDIARRA[1,2], AïsséBAH[1], Mohamed Ag BARAIKA[2], OusmaneDIARRA[1,3], Brahima TRAORE[1,3], BoubacarMadioditAladiogo MAIGA[4],Mah FANÉ[1], Atia TRAORE[1], Fanta KabaCAMARA[1], Adounigna KASSOGUE[1], Fassé SAMAKE[1,3,] AmadouHamadounBABANA[1].

[1]Research Laboratory in Microbiology and Microbial Biotechnology (LaboREM-Biotech), Faculty of Sciences and Techniques, University of Sciences, Techniques and Technologies of Bamako, Mali BPE: 3206

[2]USTTB Faculty of Pharmacy, National Institute of Public Health (INSP).

[3]Institute of Applied Sciences (ISA) / University campus of KABALA phase II Tel: 20 70 77 43/ 20 70 77 46 / Website: www.isamali.org

[4]Central Veterinary Laboratory (LCV) 8 Km Road of Koulikoro BP 2295 Bamako, Mali Phone : (223) 20-24-33-44/20-24-23-04/20-24-23-05 Fax : (223) 20- 24 -98-09 Email : mali@abovetmali.org

*Corresponding author: bahaisse2014@gmail.com.

ABSTRACT:

The tomato is a fruit rich in nutrients and very popular in Mali, despite its many benefits, producers were faced with many problems. Samples of tomato fruits were taken in the circle of Kati at October 10, 2022. The main objective of this work was isolated and characterizes lactic acid bacteria in the tomato fruits ability of degrading organophosphorus pesticides (OPP) chlorpyrifos.Besides, the antagonistic activity was determined against the phytopathogenic and spoilage bacteria on tomato fruit. Twenty-three lactic acid bacteria and twenty-one pathogenic bacteria were isolated from tomato fruits of the two varieties Mongal and Cobra. However, the Cobra variety had high bacterial count compared to the Mongal variety. Identification by Gallérie API 20E shows the presence of pathogenic and spoilage bacteria such as *Enterobacter cloacae* (2) 100% pathogenic bacteria generally isolated in CSF, *Pasteurellapneumotropica* (T5) 95% *Pantaeo*ssp (T13), and (T14). Five lactic acid bacteria isolates (BL16, BL4, BL10, BL6, BL5) among the twenty-three lactic acid isolates showed degradation organophosphate pesticide (OPP) Chlorpyrifos. The results showed that certain lactic acid bacteria which have inhibitory activities against phytopathogens; can survive in the presence of OPPs and degrade them significantly in a short time. Among lactic acid bacteria, BL16 and BL5 showed the highest degradation capacity. Moreover, these lactic acid bacteria strains showed excellent growth ability at different NaCl concentration (4% 6% 8% 10%) and at the temperature of 44°C.

Keywords: Degradation, organophosphorus, lactic acid bacteria, tomatoes.

I.INTRODUCTION:

Tomatoes constitute an important agricultural industry and have become a staple food for humans in many countries. The cultivated tomato (*Solanumlycopersicum*) is native to the New World, probably Mexico, from wild species native to the Andes region of South America (Shankara, 2005). The tomato was brought to Europe in the 16th century and later spread to many parts of the world, including Africa.Tomato fruits are eaten both raw and cooked and play a role in preventing several diseases such as cancer or cardiovascular diseases (Sharoni and Levi, 2006). This protective effect has been mainly attributed to its valuable bioactive components such as antioxidants (Borguini and Torres, 2009), such as carotenes (lycopene which gives the red color to tomatoes). Besides, Organophosphates (Parathion, Malathion, Dichlorvos, Chlorfenvinphos, Paravit®, Phosdrin, Diazinon or anti scabies, Vapona®, Fatek®…) are pesticides commonly used in agriculture. Certain substances are no longer sold in Western countries but are probably used in Asian or African countries. They are ester, amide or sulphur derivatives of phosphoric acids. They are poorly soluble in water, not very volatile, but very lipid soluble.Currently, methods of degrading pesticide residues in food, such as conventional processing technologies (washing with various oxidants, peeling, cooking and chemical oxidants) and emerging advanced oxidation technologies (ozone, ultrasound, ultraviolet light and non-thermal plasma), have been reported by many researchers (Azam et al., 2020; Pandiselvam et al., 2020; Yigit and Velioglu, 2019). Yet none of them is completely satisfactory. Such methods cannot effectively remove harmful substances or cause secondary pollution. Additionally, some extreme processing conditions may affect the sensory quality of foods or result in the loss of nutritional components (Cengiz et al., 2018). Additionally, no specific treatment for pesticide poisoning has been clinically confirmed and the traditional method of gastric lavage is tedious and ineffective (Shieh et al., 2019). Thus, new strategies against pesticide toxicity must be developed. In recent years, the biodegradation method has been widely favored by domestic and foreign scholars, with the advantages of low cost, safety and efficiency, non-toxic and harmless, no secondary pollution and other significant advantages and irreplaceable (Liu et al., 2019). Many studies have reported that certain lactic acid bacteria (LAB), including *Lactococcuslactis, Leuconostocmesenteroides, Lactobacillus rhamnosus*and *Lactobacillus brevis*; can degrade OPPs in vitro (Pinto et al., 2019; Wochner et al., 2018). It was found that *Lactobacillus plantarum* 1.0315, *Lactobacillus plantarum* 1.0624, and *Lactobacillus plantarum* 1.0622 could degrade chlorpyrifos and phorate in whole corn silage (Zhang et al.,

2016). Additionally, there is considerable evidence that probiotic lactic acid bacteria have antioxidant properties.

These properties, whose can be effective against oxidative stress in vivo (Luti et al., 2020. Based on these special functions, daily consumption of lactic acid bacteria could prove to be a protective dietary strategy for populations exposed to pesticides. Therefore, the aim of this study is to test OPP-degrading lactic acid bacteria, and select potential strains.

II.MATERIAL AND METHODS:

STUDY SITE:

The tomato fruits were collected in M'pébougou of Kati circle, 19 km from Kati town. Two variety of tomato fruits were Collected (Mongal and Cobra variety). The tomatoes samples were packaged aseptically, carried in sterile plastic bags, and then transported to the laboratory for various analyses.

METHOD:

SAMPLING:

The two-tomato fruit variety sampleswere taken randomly due to 500g per batch (Mongal and Cobra).

Fig 1.The two varieties of Tomatoes fruits.

N. B= (a): Cobra variety, (b) Mongale variety.

ISOLATION OF BACTERIA FROM TOMATO FRUITS:

To asses, bacteria strains were carried out on 25g samples of tomatoes mechanically homogenized in 225 ml containing a solution of (NaCl at 0.85%, and bacteriological peptone at 0.1%). A dilution series was carried out and samples of 0.1 mL spread and cultured on different selective media: (i) the obtained suspensions were diluted in tenfold series and plated onto different culture media: tryptic soy agar (TSA) (30 °C for 48–72 h), Man-Rogosa-Sharpe (MRS) agar medium for LAB growth (30 °C for 48–72 h) (Alegre et al., 2011;

Wouters et al., 2012). After incubation, microorganisms presenting different phenotypes were enumerated, selected, and purified on the same medium. Pure bacterial cultures were stored at − 20°C in the different broth media containing 25% glycerol.

BIOCHEMICAL AND PHYSIOLOGICAL CHARACTERIZATION:

MORPHOLOGICAL CHARACTERIZATION AND CATALASE TEST:

The test consists of direct observation (with the naked eye) from colonies obtained on agar media MRS agar, TSA agar, PCA Agar. It provides information on the shape, size, appearance, consistency, smell, outline and color of the colonies studied.The presence of catalase results in the appearance of effervescence due to the release of oxygengrowth at different temperatures. The lactic acid isolates were inoculated in MRS broth and incubated at different temperatures of 30°C, 37°C, and 44°C for 72 hours.

GROWTH IN THE PRESENCE OF NaCl:

LAB were tested for their abilities to support different concentrations of NaCl (4%, 6%, 8%, and 10%),) incubated at 30°C for 72 hours on MRS agar medium. Assess by the appearance of a disorder and the measurement of the optical density; by Double beam UV spectrophotometry (Beam PC 8 Scanning Auto Cell UVD-3000), according to Khedida et al., (2009) their growth.

ANTIBACTERIAL ACTIVITY:

The antibacterial activity test was performed in vitro using the agar-well-diffusion method reported by (bah et al., 2019).The antagonistic activity of the lactic acid bacteria strains such as (L10, L23, L5, L9 L17, L15, L18). Antibacterial activity determined by distinguishing translucent halo zones around lactic acid bacteria strains and measuring the diameters of the inhibition zones. According to the diameter of inhibition (mm), LAB were grouped as strains with weak inhibitory activity ($d \leq 12$), medium activity ($12 \leq d \leq 15$), and strong inhibitory activity ($d > 15$). All antibacterial tests performed in triplicate.

DEGRADATION OF OPPS BY LACTIC ACID BACTERIA:

The MRS medium was placed in conical tubes under sterile conditions was added to the MRS medium for a final concentration of 50 mg/L and then homogenized, vortex for 30 s (Yuan et al., 2021). Bacterial suspensions (10^5 CFU/mL) were inoculated into Petri dishes containing MRS medium contaminated with OPPs. At the same time, the control sample would be carried out without a suspension of bacteria, containing only MRS agar and OPPs. All samples will be incubated at 37 ◦C for 72 h.

III.RESULTS:

The bacteria strains collection counted (n=44) associated with tomato fruits from the locality of the commune of Kati were obtained. The bacteria strains counted , was carried out on three different media (TSA, MRS, PCA) in order to select various bacterial genera on tomato fruits. So, 23 lactic acid bacteria was evaluated for physiological characteristics on Man-Rogosa-Sharpe (MRS) agar and 21 pathogenic bacteria on plate count agar (PCA), Tryptic Soy Agar (TSA).

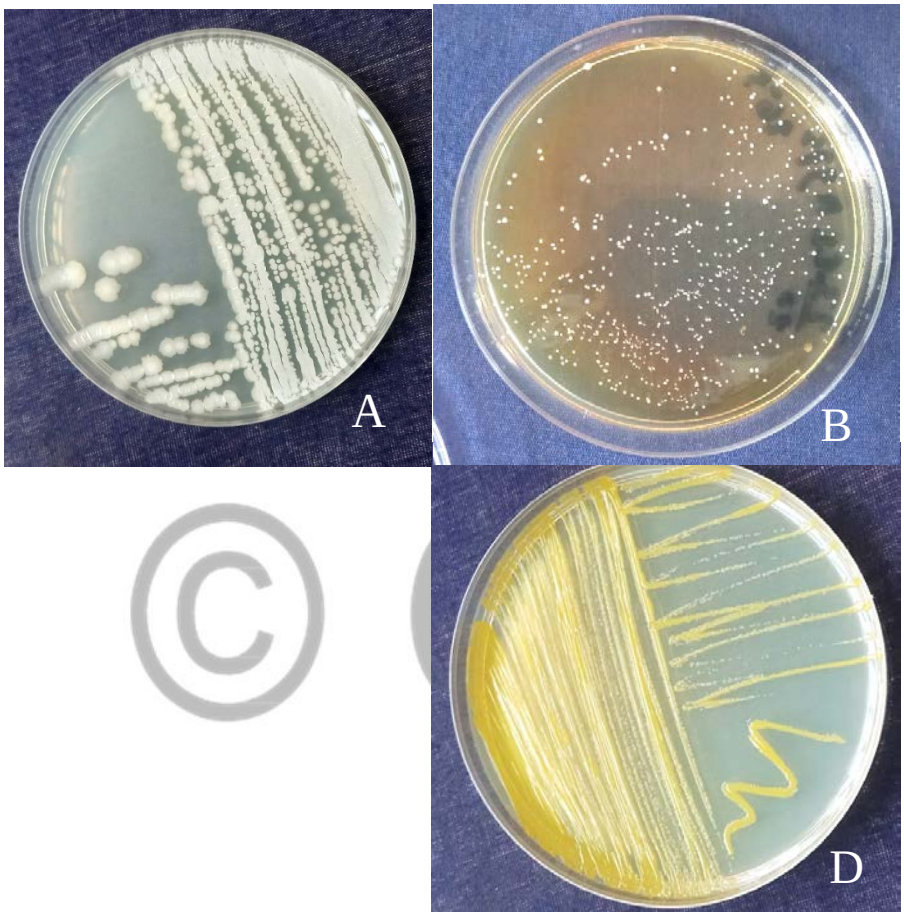

Fig 2. Bacteria strains profiles on the mediums cultures different.

(A, D): Pathogenic bacteria on PCA agar and (B): lactic acid bacteria on MRS agar and (C): Pathogenic bacteria on TSA agar.

MORPHOLOGICAL CHARACTERIZATION:

The pure cultures underwent macroscopic examinations (studies of cultural characteristics), on medium culture different.

TABLE I. The bacteria isolated depending on the variety of tomato fruits.

Tomatoes variety	**Culture media**	**Profiles**	**Incubation conditions**	**CFU/mL**	**CFU/mL**
Mongal	MRS Agar	Midsized white	37°C	$1,6\ 10^6$	
Mongal	MRS Agar	Fine white	30°C	10^6	
Total					$2.6\ 10^6$
Mongal T crushed	TSA Agar	Small orange	30°C	$72.5\ 10^3$	
	TSA Agar	Big yellow	30°C	$72.5\ 10^3$	
	TSA Agar	White	30°C	$62.5\ 10^3$	
Total					$212\ 10^3$
	PCA Agar	Dark yellow	30°C	$17.5\ 10^3$	
	PCA Agar	Limpid	30°C	$22.5\ 10^5$	
	PCA Agar	White	30°C	$7.5\ 10^5$	
	PCA Agar	Small yellow	30°C	$27.7\ 10^5$	
Total					$57.875\ 10^5$
Cobra	MRS Agar	Midsized white	37°C	$10\ 10^5$	
Cobra	MRS Agar	Fine white	30°C	$3.4\ 10^6$	
Total					3.410106
Cobra T crushed	TSA Agar	Small yellow	30°C	$20\ 10^3$	
	TSA Agar	Light yellow large	30°C	$30\ 10^3$	
	TSA Agar	Transparent	30°C	$17.5\ 10^3$	
Total					67.510^3
	PCA Agar	Dark yellow	30°C	$20\ 10^4$	
	PCA Agar	Orange	30°C	$13\ 10^4$	
	PCA Agar	White	30°C	$20\ 10^4$	
	PCA Agar	Fine transparent	30°C	$110\ 10^4$	
Total					$163\ 10^4$

TABLE II. Confirmation test for Gram staining with KOH (3%) and catalase for the lactic bacteria.

Forms	**Catalase**	**KOH**	**GRAM**

	Negative	Positive	Negative	Positive	Negative	Positive
Bacilli	13	0	13	0	0	13
Cocci	8	2	10	0	0	10

The Gram staining result divided the isolates into two groups: Gram-negative bacteria, Gram positive, and confirmed by the 3% KOH test as illustrated in the figure below. The isolates ranged from whitish to cream color on the medium culture after microscopic examination 23 Gram-positive rods, bacilli, such as 21 Gram-negative were founded

Fig 3. Bacteria strain mixed in 3% potassium hydroxide solution showing positive effect.

BIOCHEMICAL AND PHYSIOLOGICAL CHARACTERIZATION OF LACTIC ACID BACTERIA:

The catalase test allowed the distinction between certain negative Gram bacteria that were catalase-positive compared to others. The presence of gas bubbles was observed for some genera see table 3.Negative Catalase, and Gram-positive bacilli was high the number of in lactic acid bacteria strains, see table above.

GROWTH AT DIFFERENT TEMPERATURES AND GROWTH IN THE PRESENCE OF NaCl:

The lactic acid bacteria strains (23) were characterized according to their physiological state. The results reveal that all lactic acid bacteria strains could grow under stress at different concentrations of NaCl. In particular at 4%, 6%, 8% NaCl the growth was favorable and weak growth at 10% NaCl. Temperature function 90% of LAB grow at 44°C suggesting that thermotolerant lactic acid bacteria strains are strongly represented in tomato fruit.

TABLE III.The physiological characteristics of lactic acid bacteria.

Strain coded	**4%**	**6%**	**8%**	**10%**	**44°C**
BL2	++++	++++	+++	++	++

BL3	++++	++++	++	+	+
BL4	++++	++++	+++	++	++
BL8	++++	++++	+++	+	+++
BL9	++++	++++	+++	+	++
BL10	+++	++	+	+	++
BL11	++++	++++	+++	++	+++
BL16	++++	+++	++	+	+++
BL13	++++	++++	+++	++	++
BL12	++++	++++	+++	++	++++
BL6	++++	++++	+++	++	++++
BL5	++++	++++	++	+	+++
BL14	++++	+++	+	++	++
BL1	+++	+++	++	++	
BL7	+++	+++	++	+	+
BL17	++++	+++	+++	++	+
BL18	++++	++++	+++	++	++
BL19	++++	++++	++	++	+
BL20	+++	+++	++	+	+
BL21	++++	++++	+++	+++	++
BL22	++++	+++	++	++	+
BL23	++++	+++	++	++	++
BL15	+++	++	++	+	+

BIOCHEMICAL CHARACTERIZATIONS OF PATHOGEN ISOLATES:

After purification, the pure cultures showed different macroscopic aspects (through their studies of cultural characteristics). Thus, the Result of the biochemical characters, show*Enterobacteriaceae*presented in tomato fruits (Fig 4).

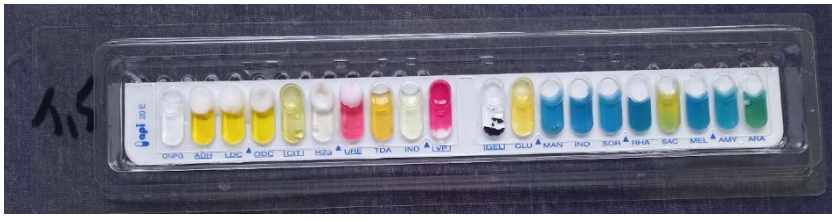

Fig 4.*Pasteurellapneumotropica*profiles.

Enterobacter cloacae (2) 100% pathogenic generally isolated in CSF, *Pasteurellapneumotropica*(5) 95% *Pantaeo*ssp (13), and (14) this species is generally common in plants. The most dominant species were*pantaeo*.

ANTIBACTERIAL ACTIVITY:

LAB strains were screened for antibacterial activity against of phytopathogenic bacteria. According to their inhibitory activity on pathogens, lactic acid bacteria strain a grouped into three classes: weak inhibitor activity with d≤ 12mm, medium activity with diameter 12 mm≤ d ≤ 15 mm, and strong inhibitor activity, diameter d> 15 mm. The results showed that 80% of the LAB collection presented inhibitory activity against, at least, four pathogenic bacteria out of the 21 investigated. Among those LAB species, BL 10, BL 6, BL 7, BL 8, BL 9 presented strong ; inhibitory activity against of the tested pathogenic bacteria T19.

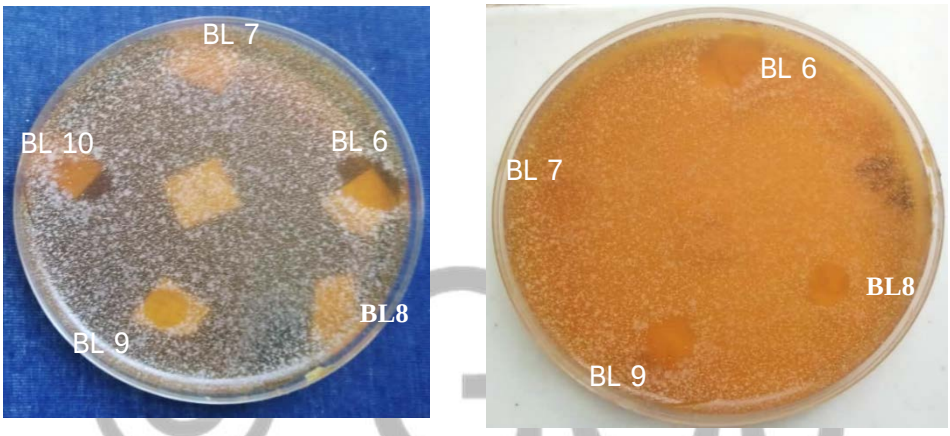

Fig 5. Antagonistic activity of lactic acid bacteria.

BL 10, BL 6, BL 7, BL 8, BL 9 = lactic acid bacteria;
T19 = Pathogenic bacteria.

DEGRADATION OF OPP BY LACTIC ACID BACTERIA:

Screened of Five lactic acid bacteria strains, OPP degrading was used to explore with OPP (chlorpyrifos). When the initial concentration of OPP in the MRS agar medium 50 mg/L, five lactic acid bacteria strains were showed the degradation capacity of OPPs with a wide range of degradation rates (26.08%). The highest degradation rate was observed of strains BL16, BL5, BL4. The number of colonies counted was greater than 300 on the MRS agar culture medium containing 50 mg/L chlorpyrifos.

TABLE IV. Growth of lactic acid bacteria strains in the presence of OPP.

Stains	Control	50 mg/L
L4	++++	++++
L5	++++	++++
L10	++++	+++

L16	++++	++++
L6	++++	++
L23	++++	-
L9	++++	-
L3	++++	-
L19	++++	-
L17	++++	-
L1	++++	-
L2	++++	-
L13	++++	-
L15	++++	-
L18	++++	-
L20	++++	-
L14	++++	-
L21	++++	-
L22	++++	-
L8	++++	-
L11	++++	-
L12	++++	-

IV.DISCUSSION:

The diversity of tomato fruit ecosystems presents a challenge for lactic acid bacteria strains to adapt to environments, and these abilities vary considerably among species and strains.In this study, we describethe bacteria community morphological on tomato fruits from two variety (figure1). The result was revealed that 52.27% (n = 23) of lactic acid bacteria and 47.72% (n = 21) of clinical pathogenic bacteria and typical phytopathogenic bacteria. Besides, the Mongal variety lactic acid bacteria was 2.6 10^6 CFU/mL, TSA agar medium 212 10^3 CFU/mL and on PCA agar 57.875 10^5 CFU/mL. In the variety Cobra, lactic acid bacteria was counted 3.410 10^6 CFU/mL on MRS agar medium, TSA agar medium 67.5 10^3 and PCA agar 163 10^4 CFU/mL (Table 1). The work of the present study are consistent with the results obtained by Kizheva et al., (2022) on Cross-pathogenic bacteria detected in infected tomatoes (*Solanumlycopersicum L.*) and peppers (Capsicum annuum L.) in Bulgaria. Indeed, 23 lactic acid bacteria strains obtained in the two variety of tomatoes agree with the work of Fessarda and Remize (2019) who characterized 77 lactic acid bacteria of different genera in the tropical fruits of papaya, tomato and sliced cabbage. According to those of (Di Cagno et al., 2016,

2011b, 2009a), who detected *Lc. mesenteroides* from fresh fruits and vegetables, such as raw prickly pear, sweet cherry and raw peppers *Lc. pseudomesenteroides*. Indeed, considerable diversity of LAB we found in tow variety of tomato fruits, in Katy circle. In the present study, two forms of bacteria was found, according to Gram staining, microscopic examination and other biochemical reactions they carried out. The lactic acid bacteria (LAB), strains, the colonies of LAB werenegative catalase, Gram positive and negative KOH. The results, according with studies by Saif and Abu (2016) on Efficacy of lactic acid bacteria isolated from some fruits and vegetables. The growth at different concentrations of NaCl and different temperatures shows that lactic acid bacteria could grow under stress conditions. A 90% of the strains grow at 44°C, these are in agreement with the result of Benavides et al., (2016); in which all selected isolates growth at temperatures of 15°C and 45°C and showed greater tolerance to sodium chloride at 15°C and treatment with 2%, 4%, and 6% NaCl (table 3). These results show that the majority of our lactic acid bacteria strains are thermotolerant; that they can grow in vivo under the climatic conditions of the environment.

The antagonistic activity of the twenty-three lactic acid bacteria strains against the pathogenic bacteria *Pantoae* sp., *Enterobacter cloacae, Pasteurellapneumotropica*was evaluated. The LAB strains were capable of inhibiting the growth of pathogenic bacteria isolated on tomatoes fruits. The results show that 80% among the twenty-three LAB strains presented inhibitory activity against the indicator strains, at least four pathogenic bacteria out of the twenty-one ; the results of the present study are similar to the work of Bah et al., (2019), when determining the inhibitory activity of lactic acid bacteria isolated in the spontaneous fermentation of tomato fruits against *Enterobacter cloacae*, *Citrobacterfarmeri*, *Escherichia coli*, *Bacillus cereus*, *Salmonella enteritidis*, *Staphylococcus aureus* MRSA, *Listeria monocytogenes*, *Enterococcus faecalis*, *Enterococcus faecium*. Liu et al., (2013), found that 26.6% of lactic acid bacteria strain capable of inhibiting the growth of *Burkholderiacepacia*, these strains belong to the *Pseudomonas genera*. Twenty-one pathogenic bacteria they obtained in the two variety of tomatoes and the identification by API 20E gallery, revealed the presence of spoilage and pathogenic bacteria of fruits and vegetables in tomato fruits, such as *Enterobacter cloacae* clinical strain and *Pantoea* sp., *Pasteurellapneumotropica*, phytopathogens. However, genera, *Pantoea*and *Enterobacter*, theyknown opportunistic that pathogens in humans, but infections generally require an immunocompromised host.Kizheva et al., (2022); who crossed *Pantoea* sp., *Enterobacter cloacae*, into the endophytic population of infected tomato and pepper plants, found consistent results. We founded that the five isolates can degrade OPPs to varying degrees, but the degradation rates were considerably wide. It indicated that a pesticide environment with a high n concentration would not affect the growth of lactic acid bacteria (Li et al., 2018). However, BL16 and BL5 continued to

show the best degradation capacity among the five isolates. Lactic acid bacteria isolated from tomato fruits in the Kati circle degraded OPP from 72 hours of incubation at 37°C. In a short time compared to that of Anwar et al., (2009). Which the lactic acid bacteria strain degrades from 8 days of incubation.

V.CONCLUSION:

This study tested the ability of twenty-three strains of lactic acid bacteria to degrade OPP. Significant OPP degradation was observed in all five lactic acid bacteria strains tested. Among them, BL16, BL4 and BL5 showed the strongest degradation capacity. The five lactic acid bacteria strains showed inhibitory activity against pathogenic bacteria in tomato fruits. Overall, these results indicated that the use of lactic acid bacteria was a safe and highly effective method for the removal of OPPs from complex media such as tomato plants of all variety. In addition, these LAB isolates exhibited strong growth at very high temperature conditions and relatively good salinity under field application conditions. These findings showed that it was possible to develop LAB-based probiotics capable of attenuating oxidative damage caused by pesticides *in vivo.*

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