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Utilization of Agricultural waste as one of the environmental issues: employing the bio-conversion potential of lactic acid bacteria

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ABSTRACT : Reprocessing food and agriculture waste (FAW) is of great interest, these residues can be converted into fruitful products, and lactic acid bacteria (LAB) are at the forefront of this approach. Therefore, the present study is aimed at exploring LAB bioconversion potential. Herein, Lactiplantibacillus plantarum MC39 (MZ769311) was used for the fermentation assay. The inhibitory effect against multi-drug resistant Klebsiella pneumoniae W8(MZ769363) and Enterobacter hormaechei U25 (MZ769310) was used as indicator for the productivity. Rice bran, wheat bran, alfalfa hay, reed straw, potato peels, banana peels, orange peels, and fava bean peels were used as fermentation substrates with different carbohydrate contents. The results showed that the maximum inhibitory effect was attained when orange peels were used after 96h of fermentation, followed by fava bean peels after 72h of fermentation. These findings suggest that the carbohydrate content of fermentation residues has a significant effect on organism's viability, and orange peels could be a suitable feedstock substrate in solid state fermentation, producing an antimicrobial agent that can inhibit MDR Gram-negative pathogens.

***KEYWORDS:** Orange peels waste, Lactiplantibacillus plantarum MC39, biotechnological application*

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I. INTRODUCTION

The problem of food and agriculture waste (FAW) becomes very obvious all over the world. Each year, about one- third of food products are lost throughout the food chain. Approximately 1.3 billion tons of waste are generated, beginning with agricultural production and continuing through post-harvest, storage, processing, distribution, and consumption [1][2]. According to the Food and Agriculture Organization (FAO), about 14% of worldwide food production is wasted before reaching consumers [3]. According to another report, only 1300 million tons of food waste out of 1600 million tons were edible [3].

In Egypt, more than 30 million tons of agriculture residues are produced after harvesting each year. What aggravates the problem is the absence of environmental awareness and low level of knowledge of the farmers which result in wrong behavior in handling agro-waste. The farmers get rid of the agro-waste by burning. This method results in different harmful effects on the environment. Burning agro-wastes results in poisonous oxides and hydro carbonate (the black cloud), as well as reducing the microbial activity on the soil. Also, storing these wastes in the field provides a suitable environment for the pests that attack the new crops [4]. In addition, the FAO's most recent report stated that households waste more than 15 kg of food each year. These wastes still being dumped in the landfills causing environmental problems as methane emissions [2]. As a result, the handling and utilization of agro-waste must be done in a safe and environmentally friendly manner.

Food waste utilization not only has a significant effect on the environment but also has economic importance. Food wastes are considered rich sources of carbohydrates, oils, proteins, and fats that can be used for producing different useful by- products such as biogas, ethanol, molasses, dietary fibers, pigments, essential oils and flavors besides, different organic acids [2][5]. Organic acids, and lactic acid in particular, considered one of the most important by-products can be synthesized from food wastes specially, fruits and vegetables. Lactic acid can be employed in different sectors. In the food industries as a microbial control, inactivate food borne pathogens. Also, it can be used to improve the flavor, provide aroma and regulate pH [1]. In addition to pharmaceutical and cosmetic sectors as skin lightening, moisturizing, and antimicrobial agents [6] [7]**.**

A high amount of lactic acid can be synthesized by fermentation using different strains of LAB such as *L. plantarum*, *L. manihotivorans*, and *L. paracasei* [8][9][10]**.** Instead of expensive raw materials needed for LAB cultivation, fruit and vegetable wastes can be used as natural carbon sources enriched with cellulose, hemicellulose and starch [11]**.** Several studies have found that by incorporating these enriched carbohydrates residues into the formulated optimum medium for LAB cultivation, LAB can be used in the bioconversion of these residues in a faster, simpler pathway than chemical technology [10] [12].

Although many publications have focused on the various uses of food waste, there is still a lack of research on food waste fermentation for antimicrobial substances production and application in pathogen inactivation. As a result, the current study concentrated on lactic acid bacteria and their bioconversion potential of agro-wastes, producing an antimicrobial agent controlling MDR Gram-negative pathogens.

II. MATERIAL AND METHODS

2.1. Strains

Klebsiella pneumoniae W8 (Accession no.: MZ769311) and *Enterobacter hormaechei* U25 (Accession no.: MZ769363) were employed as MDR targets in the present study. *Lactiplantibacillus plantarum* MC39 (Accession no.: MZ769310) was employed in the fermentation assay. Sources and identification procedures of these strains were described previously [20].

2.2. Substrates

Different food and agriculture wastes, including rice bran, wheat bran, alfalfa hay, reed straw, potato peels, banana peels, orange peels, and fava bean peels, were screened separately to determine the most favorable natural carbon source for the growth and production of antimicrobial substance by *Lb.plantarum* MC39

2.3. Fermentaation assay

Agriculture residues were dried and milled with a mechanical blender into uniform particles. Five grams of each source were added, separately, as an alternative carbon source to 50 ml of MRS broth that was used as a basal medium; the other content of the growth medium was kept unchanged. The medium was adjusted to pH 7 and then sterilized. After autoclaving, the medium was inoculated with (10^6 CFU/ml) fresh overnight culture of *Lb.plantarum* MC39 then incubated aerobically at 30^{o}C for different incubation periods (48h, 72h, and 96h) at130 rpm on a rotatory shaker[13] [14].

At the end of each treatment, cell-free supernatant (CFS) was obtained by filtration using Whatman filter paper followed by centrifuging (4000g, 20 min). The antimicrobial activity was determined by the agar-well diffusion method [15]**.** A volume of 100 µl of CFS was inoculated separately into wells (7mm) perforated in Muller- Hinton agar plates that had lawn cultures (0.5 McFarland standard) of MDR indicators. The plates were refrigerated for 24h before being incubated at 37◦C for another 24h. The inhibition zone diameter (mm) was measured. The control set was designed by cultivating *Lb.plantarum* MC39 without any changes in quantities or content of MRS broth as a growth medium.

Statistical analysis

The results were expressed as the mean of three replicates ± standard error (SE) and the means were compared for statistical significance by one-way ANOVA (significance level (P<0.05), the confidence interval was 95% using SPSS software version 15 (SPSS, Richmond VA, USA) followed by applying post hoc procedure (Duncan’s multiple range test). Figures were drawn with the Origin 2017 program for graphing and data analysis.

III. RESULTS

Various food and agriculture wastes, including wheat bran, rice bran, reed straw, alfalfa hay, potato peels, orange peels, banana peels and fava bean peel, were used as alternative, natural carbon sources and examined to determine the most favorable one for productivity. The carbohydrate content of these residues was outlined in Table (1). Results in Table (2) illustrated by Figures (1-4) showed that the highest viability of *Lb.*

plantarum MC39 was achieved in the presence of orange peels after 96h of fermentation, resulting in inhibitory effects against *K. pneumoniae* W8 and *E. hormaechei* U25 with inhibition zones of 22mm, and 12mm respectively, followed by fava bean peels with inhibitory effects against W8 and U25 strains with inhibition zones of 4mm and 6mm, respectively, after 72h of fermentation.

Table (1): Carbohydrate content of experimental food and agro-waste

Food and agro-waste	Carbohydrate content %	References
Orange peels	57.06	[21]
Banana peels	55.34	[19]
Potato peels	52	[22]
Straw reed	46	[23]
Wheat bran	22	[24]
Rice bran	14	[25]
Fava bean peels	18.93	[26]
Alfalfa hay	2.1	[27]

Table (2): Antibacterial activity of *Lb.plantarum* in MRS broth supplemented with different agro-wastes and cultivated for different fermentation times

Fermentation time / Agro-wastes	Inhibition zone diameter (mm)					
	K.pneumonia W8			*E.hormaechei* U25		
	48h	72h	96h	48h	72h	96h
Wheat bran	0±0.0d	0±0.0c	0±0.0b	0±0.0c	0±0.0c	0±0.0b
Rice bran	0±0.0d	0±0.0c	0±0.0b	0±0.0c	0±0.0c	0±0.0b
Reed straw	0±0.0d	0±0.0c	0±0.0b	0±0.0c	0±0.0c	0±0.0b
Alfalfa hay	0±0.0d	0±0.0c	0±0.0b	0±0.0c	0±0.0c	0±0.0b
Potato peels	0±0.0d	0±0.0c	0±0.0b	0±0.0c	0±0.0c	0±0.0b
Orange peels	0±0.0d	0±0.0c	22mm±0.582a	0±0.0c	0±0.0c	12mm±0.317a
Banana peels	0±0.0d	0±0.0c	0±0.0b	0±0.0c	0±0.0c	0±0.0b
Fava bean peels	4mm±0.106c	4mm±0.105b	0±0.0b	0±0.0c	6mm±0.158b	0±0.0b
Control	40mm±1.06a	26mm±0.635a	24mm±0.582a	24mm±0.635a	22mm±0.529a	12mm±0.317a

Data are the mean ± standard error. Different letters in the column indicate significant differences among treatments using a one-way ANOVA followed by the Duncan's multiple range test ($P < 0.05$).

Control: *Lb.plantarum* MC39 cultivated in MRS broth without any changes in quantities or content of the medium.

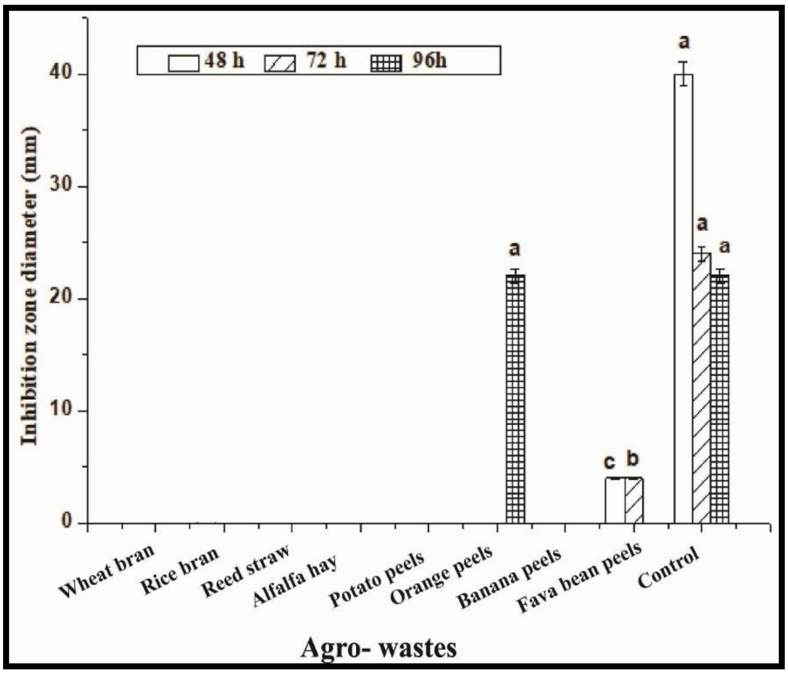

Fig.1. Antimicrobial activity of *Lb. plantarum* MC39, inoculated in MRS broth supplemented with different agro-wastes using *Klebsiella pneumoniae* W8 as indicator

Data are the mean of three replicates ± standard error. Error bars represent the standard error of the mean. Different letters indicate significant differences among treatments using one-way ANOVA followed by the Duncan's multiple range test ($P < 0.05$).

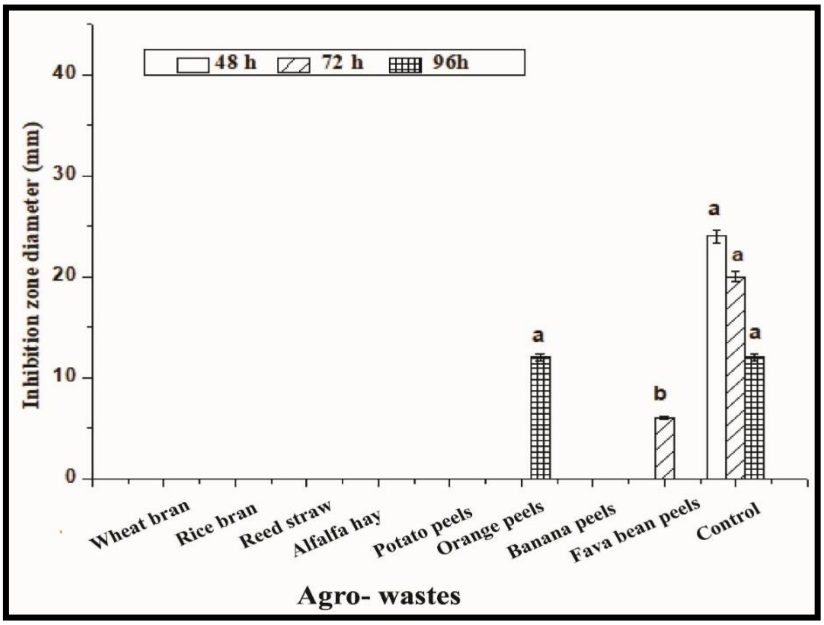

Fig.2. Antimicrobial activity of *Lb. plantarum* MC39, inoculated in MRS broth supplemented with different agro-wastes using *Enterobacte hormaechei* U25 as indicator

Data are the mean of three replicates ± standard error. Error bars represent the standard error of the mean. Different letters indicate significant differences among treatments using one-way ANOVA followed by the Duncan's multiple range test ($P < 0.05$).

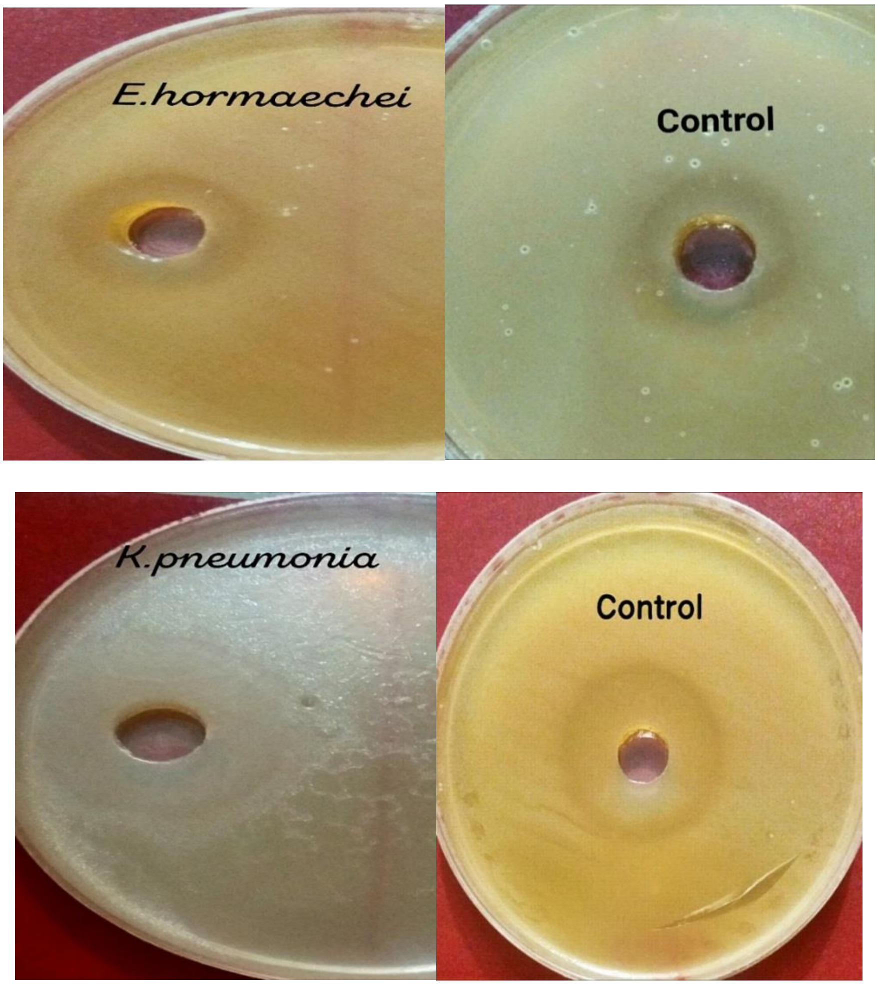

Fig.3. Antimicrobial activity of *Lb. plantarum* MC39, inoculated in MRS broth supplemented with orange peels after 96h of fermentation using *Klebsiella pneumonia* W8 and *Enterobacte hormaechei* U25 as indicators

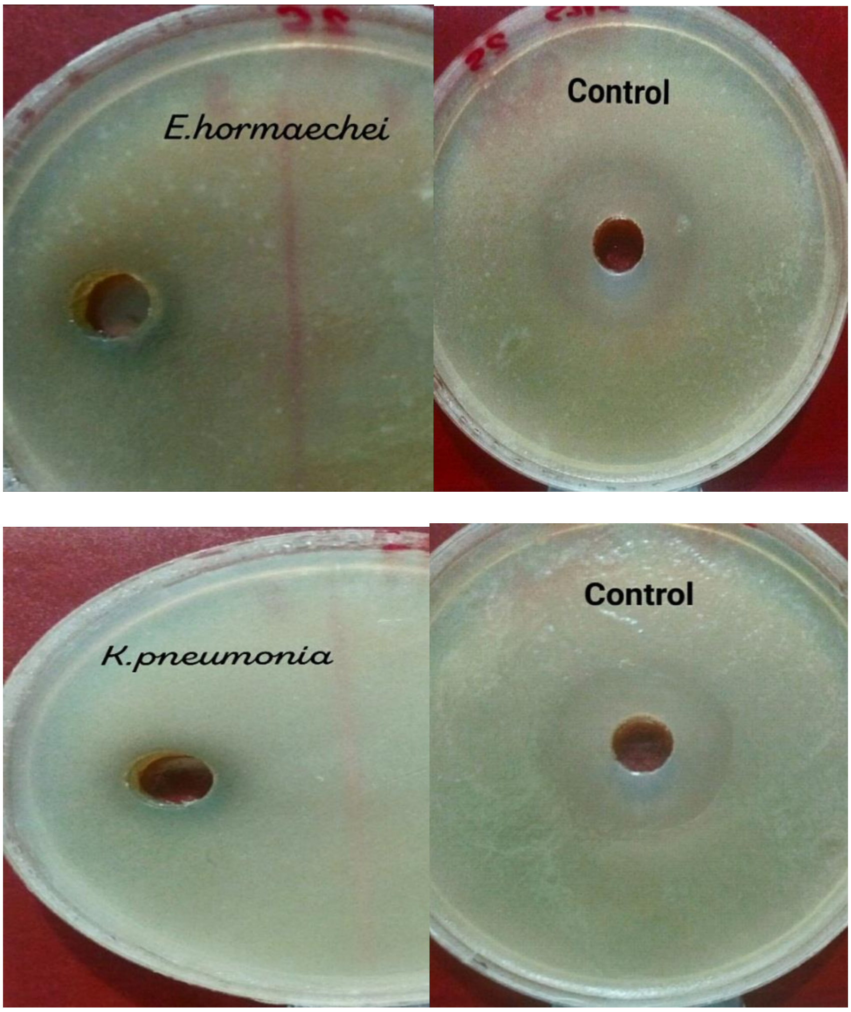

Fig.4. Antimicrobial activity of *Lb.plantarum* MC39, inoculated in MRS broth supplemented with fava bean peels after 72h of fermentation using *Klebsiella pneumonia* W8 and *Enterobacte hormaechei* U25 as indicators

IV. DISCUSSION

The global economic loss caused by food and agricultural wastes (FAWs) is estimated to be around 750 billion dollars per year. As a result, "food wastage" occupies a prominent position in the United Nations' 2030 Agenda for Sustainable Development [16]. One of the most important method to overcome this problem is to use the potential bioconversion of LAB for degradation and utilization these residues which produce fruitful by-products such as lactic acid that can be used as an antimicrobial agent against MDR Gram-negative pathogens.

In the present study, different food and agriculture wastes including wheat bran, rice bran, reed straw, alfalfa hay, potato peels, orange peels, banana peels and fava bean peels were used as renewable carbohydrate sources for *Lb. plantarum* MC39 fermentation. The results showed a significant effect on *Lb. plantarum* MC39 viability depending on the carbohydrate content of fermented substrates. The maximum viability of MC39 strain was attained in the presence of orange peels that contain the highest carbohydrates (57.06%) compared to other feedstock residues. This viability was illustrated in the inhibitory action against MDR *K. pneumoniae* W8 and *E. hormaechei* U25 with inhibition zones of 22mm and 12mm respectively after 96h of fermentation. Our results were in line with those obtained by **[17],** they reported an 84% (w/w) yield of D-lactic acid from orange peel hydrolysate using *Lactobacillus delbrueckii*.

In the present study, productivity of the MC39 strain decreased with the decrease in carbohydrate content, as illustrated by a weak inhibitory effect of 4mm and 6mm against MDR *K. pneumoniae* W8 and *E. hormaechei* U25 respectively after 72h using fava bean peels as a fermentable substance and not detected in the case of alfalfa hay that contains a very low content of carbohydrates.

Although banana peels, potato peels and straw reed contain a sufficient content of carbohydrates to produce lactic acid (55.34%, 52%, and 46% respectively), no inhibitory effect has been detected. That is due to the limited content of fermentable reducing sugars. Therefore, fermentation is not practically possible, and enzymatic hydrolysis is needed to increase the content of fermentable reducing sugar. The works [18] [19] backed up our finding.

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