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Tayseer Shamran Al-Deresawi

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Keywords

Acetobacter xylinum, bacterial fermentation, food industry, Gluconacetobacter xylinus, Vinegar waste

RESEARCH PAPER

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Abstract

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Keywords: *Acetobacter xylinum*, Bacterial fermentation, Food industry, *Gluconacetobacter xylinus*, Vinegar waste

1. Introduction

Vinegar forms an essential pillar of the global food chain, ranging from fermentation processes used to produce vinegar from widely different starting raw materials such as fruits, grains, or alcoholic beverages [1]. An example is the vinegar industry in Iraq, an important practice of the food industry that accounts for a major contribution to local culinary and economic productions, primarily using local fruits and grains. Unfortunately, there is struggling with the management of the industry's waste products, which consist of excess biomass, sediments, and fermentation by-products, which remain particularly problematic and poorly managed [2]. Waste generated in vinegar production is either disposed of in municipal landfills or dumped into aquatic systems, severely harming the environment. Similar waste management challenges can be observed in global practices. However, local manufacturing is lacking regulatory practices and infrastructure, making the management of waste products even more challenging. Since the vinegar industry generates large quantities of waste organic chemicals that cause environmental problems, such as water pollution with acetic acid industrial chemical leaching, soil pollution with organic waste, and air pollution with volatile organic compounds. The past few years have seen a growing interest in the valorization of industrial side streams and waste by microbial fermentation, which can offer sustainable solutions to waste management while potentially producing valuable products [3,4].

The isolation and use of microbial fermentation, especially bacterial isolates, has drawn attention here. Bacteria have a broad range of metabolic characteristics that allow them to grow under a wide range of conditions and convert complex organic carbon substrates into various products through a series of fermentation pathways. The ability of vinegar industry waste bacterial isolates to tolerate a

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wide range of conditions and have a variety of enzymes, such as cellulases, amylases, proteases, lipases, pectinases, and xylanases, makes them well-suited as a novel tool in various biotechnological applications [5,6].

Research on fermenting bacterial isolates from acetic acid (vinegar) industry wastes has increased and become intensified by the need of society for implementing sustainable waste-management procedures and the economic incentives of generating a value-added product. Different studies have tackled different parts of the process, showing the isolation and characterization of bacterial strains, optimization of fermentation parameters, understanding of metabolic pathways and yields of the metabolites, and production of product quality [7–10].

Many milestone studies from recent years have isolated and identified bacterial isolates from vinegar industry waste, describing their biological diversity and their metabolic capabilities. It was pioneer-isolated and identified *Acetobacter* and *Lactobacillus* species as the main fermentative bacteria from vinegar industry waste, demonstrating that they possess superior cellulolytic and pectinolytic activities. Bacterial microorganisms were isolated as novel strains of bacteria for xylose fermentation from vinegar industry waste, offering new opportunities for xylose-rich substrates in biorefinery applications [11,12].

Moreover, fermentation conditions were optimized to enhance both the product yield and process efficiency. The pH and temperature were optimized in the fermentation process of vinegar industry waste to maximize ethanol production by a selected bacterial isolate. They reported significant enhancement of ethanol production due to optimization, further improving the economic feasibility of vinegar waste valorization efforts [13].

Besides ethanol, bacterial fermentation of the byproducts of the vinegar industry also offers the opportunity for the bioproduction of other industrially important biochemicals by converting the raw material to various useful products such as organic acids, enzymes, and bioactive compounds with industrial prospects in the food, pharmaceutical, and biofuel sectors. This opens the way to isolate and identify new bacterial cultures from vinegar industry waste that can be used for fermentation [14–19].

In conclusion, the fermentation of bacterial isolates recovered from vinegar industry wastes is an attractive and environmentally friendly option for the valorization of waste and the production of bioproducts with added value. Recent work exploring the microbial populations involved, their metabolic capacities, and the different operational strategies to optimize the fermentation process paves the way for future progress in biotechnological applications of these organisms. According to this, the current study was conducted to isolate and characterize fermenting bacterial isolates from these wastes in Wasit Province, Iraq.

2. Materials and methods

2.1. Samples and regular cultivation methods

The use of vinegar industry waste is an important sector of the food industry worldwide; however, this critical sector is neglected in many parts of Iraq. According to this, the current study was conducted to isolate and characterize fermenting bacterial organisms from these wastes in Wasit Province, Iraq. Samples of vinegar waste were collected from local businesses, such as by-products from food processing. These samples were prepared first by placing 10 ml of the waste in 90 ml Hestrin-Schramm (HS) medium [19,20] (Table 1) and incubated at 30 °C for 70 h. After incubation, one loopful of the white-pellicle growth on the top of the HS medium was taken and subjected to further direct and indirect cultivation. The direct cultivation included placing this growth on HS solid medium plates and incubating at 30 °C for 70 h. The indirect method contained the utilization of the loopful growth in serial dilution (10^{-1} to 10^{-4}) using NaCl solution, and then 0.1 ml of each dilution was placed onto HS solid medium plates and incubated for 30 °C for 70 h. The growth (single colonies only) from each method was further sub-cultivated at 30 °C for 70 h for purification purposes. The purified growth was examined using morphological and biochemical tests.

2.2. DNA extraction and PCR

The colonies were subjected to a DNA extraction method and followed the manufacturer's protocol. The DNA was evaluated using a NanoDrop system. The PCR method depended on the primers shown in Table 2.

Table 1. Components of Hestrin-Schramm (HS) medium.

Components	(w/v)
D-glucose	2.0%
Peptone water	0.5%
Yeast extract	0.5%
Na_2HPO_4	0.27%
Citric acid	0.12%
Acetic acid	0.2%
Ethanol	0.5%
Cycloheximide	0.01%
PH	6.0

Table 2. Primers of the study.

Primer	Sequence
Al 6	TTGCATGGATCCTGCGGCTGGAT CACCTCC
B23	GAATCAGGATCCGAATGCCCTTAT CGCGCTC
bcsAI F	TCCATATCGGGCAGCGCGTG
bcsAI R	CCCAGGAACAAGAACGCCAGC
bcsAII F	GTGCCTCAGCTATTTCCAGA
bcsAII R	AAATGGTGCGGCGTCTGC
16S A. zylum F	GAGGAACCTGCGTTCGATTAG
16S A. zylum R	TACACTGGGAATTCCACAACC
GLoco 16S R	AGAAAGGAGGTGATCCAGCC
GLoco 16S F	GAGTTTGATCCTGGCTCAG

The PCR method was uniplex for the *Zylum*, *GLoco*, and bcsAII. This included a total volume of 25 μl, which contained 12.5 μl mastermix, 5 μl DNA, 1 μl (10 pmol) for each primer direction, and 5.5 μl H_2O. The thermal cycler was set for the following conditions: 95 °C-3 mins, 94 °C-45 s, 57 °C-1 min, 72 °C-1 min of one-cycle primary denaturation, 35 cycles (denaturation, annealing, and extension), and one-cycle final extension. The products were run on 1.6% agarose electrophoresis.

The uniplex PCR for bcsAI and A6 included a total volume of 25 μl, which contained 12.5 μl mastermix, 5 μl DNA, 1 μl (10 pmol) for each primer direction, and 5.5 μl H_2O. The thermal cycler was set for the following conditions: 95 °C-3 mins, 94 °C-45 s, 59 °C-1 min, 72 °C-1 min of one-cycle primary denaturation, 30 cycles (denaturation, annealing, and extension), and one-cycle final extension. The products were run on 1.6% agarose electrophoresis. The products were visualized utilizing a UV-imager.

3. Results and discussion

The findings of the morphological features revealed the presence of Coccobacillus microorganisms with smooth and mucoid colonies (Table 3).

The biochemical tests of the bacterial isolates revealed various reactivity. Results from the biochemical tests on bacteria isolates retrieved from vinegar industry waste agree with existing microbiological literature. Catalase positivity for most isolates indicates consistency with the reports that catalase is widely found in environmental bacteria. Oxidase positivity for a minority (not exceeding one-third of the isolates) conforms to studies of oxidase as typical for those bacteria that are aerobic or facultatively anaerobic. Citrate utilization for a few isolates conforms with studies of organic acid utilization, which suggests an emergence from preferential adaptations to environments with limited oxygen and high in organic acids. Regarding the varying urease activity, indole production, and mannitol fermentation, they conform with studies of bacterial populations with varied metabolic potentials, which are thought to confer a fitness advantage under various environmental conditions. These results agree with those by Ref. [11]. These are mentioned in Table 4.

The vinegar industry waste revealed favors *Acetobacter xylinum* over *Gluconacetobacter xylinus* due to various components in the ecosystem. It is thought that *A. xylinum* metabolic versatility provides it with the flexibility and adaptation to flourish in the acidic and ethanol-rich conditions of the vinegar waste streams. Its ability to utilize ethanol efficiently may

Table 3. Morphological characteristics of bacterial isolates from vinegar industry wastes.

No.	Gram stain	Shape of Colony	Color of Colony	Texture	Surface	Shape of Cell	Elevation of Colony	Margin of Colony
1	- ve	Circular	white	mucous	Smooth	Coccobacillus	convex	Entire
2	- ve	Circular	off-white	Creamy	Smooth	Coccobacillus	convex	Entire
3	- ve	Circular	Transparent golden	Creamy	Smooth	Coccobacillus	convex	Entire
4	- ve	Circular	Transparent golden	Creamy	Smooth	Coccobacillus	convex	Entire
5	- ve	Circular	off -white	Creamy	Smooth	Coccobacillus	convex	Entire
6	- ve	Circular	Transparent golden	Creamy	Smooth	Coccobacillus	convex	Entire
7	- ve	Circular	white	Creamy	Smooth	Coccobacillus	convex	Entire
8	- ve	Circular	golden	mucous	Smooth	Coccobacillus	convex	Entire
9	- ve	Circular	golden	Creamy	Smooth	Coccobacillus	convex	Entire
10	- ve	Circular	golden	Creamy	Smooth	Coccobacillus	convex	Entire
11	- ve	Circular	white	Creamy	Smooth	Coccobacillus	convex	Entire
12	- ve	Circular	light brown (Peggy)	Creamy	Smooth	Coccobacillus	convex	Entire
13	- ve	Circular	golden	Creamy	Smooth	Coccobacillus	convex	Entire
14	- ve	Circular	off -white	Creamy	Smooth	Coccobacillus	convex	Entire
15	- ve	Circular	off-white	Creamy	Smooth	Coccobacillus	convex	Entire
16	- ve	Circular	light brown (Peggy)	Creamy	Smooth	Coccobacillus	convex	Entire
17	- ve	Circular	white	mucous	Smooth	Coccobacillus	convex	Entire
18	- ve	Circular	white	mucous	Smooth	Coccobacillus	convex	Entire
19	- ve	Circular	Transparent golden	Creamy	Smooth	Coccobacillus	convex	Entire
20	- ve	Circular	Transparent golden	Creamy	Smooth	Coccobacillus	convex	Entire

Table 4. Biochemical tests of bacterial isolates from vinegar industry wastes.

No.	Oxidase	Catalase	Citrate	Urease	Indole	Mannitol	Tryptophan
1	–	+	–	–	–	–	–
2	–	+	+	–	+	+	–
3	–	+	–	–	–	+	–
4	–	+	–	+	–	–	–
5	+	–	–	–	–	–	–
6	–	+	–	–	–	–	–
7	–	+	+	–	+	+	–
8	–	+	–	–	–	+	–
9	–	+	–	+	–	–	–
10	+	–	–	–	–	–	–
11	–	+	–	–	–	–	–
12	–	+	+	–	+	+	–
13	–	+	–	–	–	+	–
14	–	+	–	+	–	–	–
15	+	–	–	–	–	–	–
16	–	+	–	–	–	–	–
17	–	+	+	–	+	+	–
18	–	+	–	–	–	+	–
19	–	+	–	+	–	–	–
20	+	–	–	–	–	–	–

also give it an advantage in competition with other microbes living in the waste. This agrees with the findings as in Ref. [21]. Also, the finding is in match with those by Ref. [22], who found that ethanol can deactivate bacterial contamination in the waste.

The findings of the PCR demonstrated the presence of *G. xylinus* and *A. xylinum* in 3/20 (15%) and 17/20 (85%) of the isolates, respectively. The PCR confirmed these isolates. The isolates were recognized by their composition of various tested genes with variable presence (Table 5).

Table 5. PCR results of bacterial isolates from vinegar industry waste.

NO	*GLoco*	*zylum*	*bcsAI*	*bcsAII*	A6
1	−	+	−	−	+
2	−	+	−	−	+
3	−	+	−	+	+
4	−	+	+	−	+
5	+	−	−	+	+
6	−	+	−	−	+
7	−	+	+	−	+
8	−	+	−	+	+
9	+	−	−	−	+
10	−	+	+	−	+
11	−	+	−	+	+
12	−	+	−	−	+
13	−	+	+	−	+
14	+	−	−	+	+
15	−	+	−	−	+
16	−	+	−	−	+
17	−	+	+	−	+
18	−	+	−	+	+
19	−	+	−	−	+
20	−	+	−	−	+

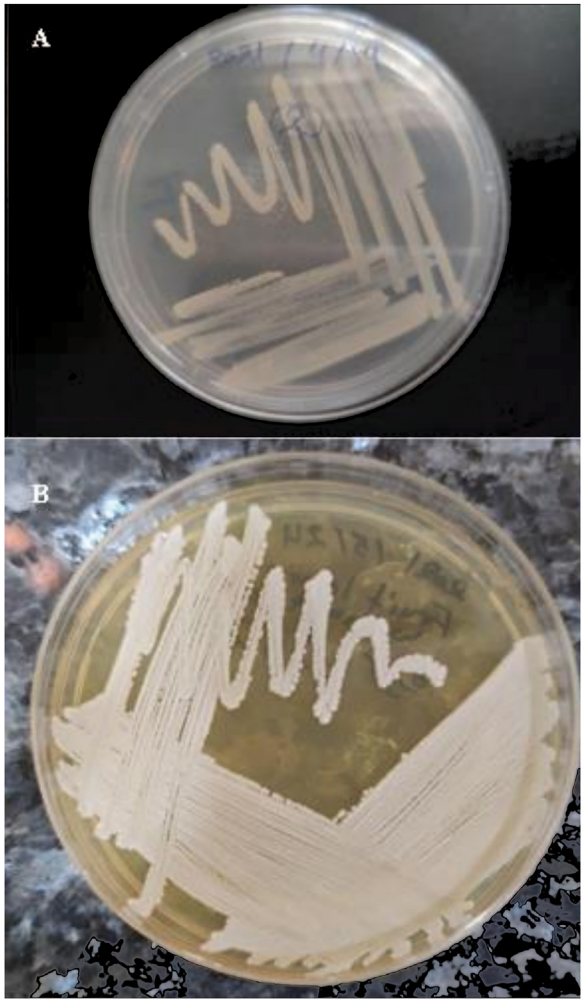

Fig. 1. Image of bacterial colonies from vinegar industry waste. A. Gluconacetobacter xylinus. B. Acetobacter xylinum.

Furthermore, the machinery of *A. xylinum* to produce cellulose might have been shaped to be more suited to work at the conditions under which it is growing in the fluctuating conditions found in the vinegar waste and be able to grow rapidly enough to produce the large cellulose needed for covering the whole surface as a continuous pellicle, globule, or bead (Fig. 1) (see Fig. 2).

Or perhaps because *A. xylinum*; is the only microorganism growing on cellulose. This growth would be protected against competition from other microorganisms that might be present in the vinegar waste, as it agrees with the results as in Ref. [23]. *G. xylinus* is also present in vinegar waste and can form chain-like structures or rods rather than the huge globules produced by *A. xylinum*, to aggregate into a strongly adhering mass or pellicle rather than growing as a circle or a multicellular mass, and that covers the bottom of the jar as in the case of *A. xylinum*. *G. xylinus* produces cellulose too, and its cellulose might be purer because it produces cellulose in a controlled environment, a point that is important for its current use in the production of bacterial cellulose for several industrial uses, such as in the food, cosmetic, and pharmaceutical industries [24]. Given its presence in vinegar waste, we also tried to cultivate *G. xylinus*. However, the percentage of this bacterium in the mix was much lower. Why would this be the case? Why have we recovered two *Acetobacter* species and not only one? *G. xylinus* has more specific niche requirements and might be more susceptible to physiochemical stress, competition or predation. *G. xylinus* can also form a pellicle, but its metabolic versatility is much narrower than that of *A. xylinum* [25] (see Fig. 2).

This knowledge of the ecological factors, impacting their distribution and prevalence across these vinegar wastes, would be critical to optimizing the use of these bacteria and their applications in waste management strategies in biotechnologies [26]. The genetic and biochemical bases of the bio-cellulose synthesis pathways in these bacteria as well as their interactions with other microorganisms in the waste would represent another avenue of future research to elucidate their ecological roles in the microbial communities inhabiting the waste and exploit their potential for bioremediation or bioconversion purposes.

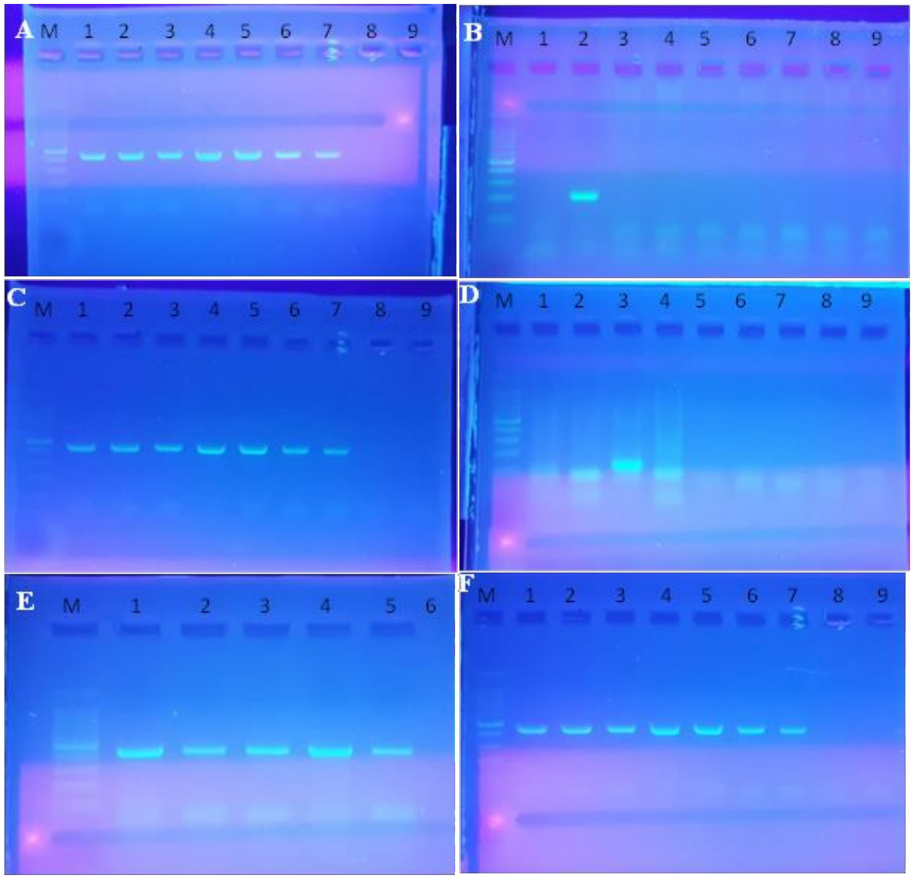

Fig. 2. Image of 1.6% agarose electrophoresis of PCR that targeted A. GLoco, B. zylum, C. bcsAI, D. bcsAII, and E. A6 in bacterial isolates from vinegar industry waste. M: Ladder: 1500-100 bp.

Vinegar-waste microbiomes are shaped by ecological factors that drive *A. xylinum* to outcompete *G. xylinus*. The abundance of *A. xylinum* over *G. xylinus* in vinegar waste suggests that ecological factors – such as adapting to highly acidic and extremely high-ethanol conditions – drive the dominance of *A. xylinum* in vinegar-waste microbiomes. First, *A. xylinum* is a Gram-negative bacterium, which gives it a metabolic advantage over similarly efficient Gram-positive vinegar bacteria [27]. Ethanol conversion to acetic acid is a central player in vinegar production and probably provides *A. xylinum* with an evolutionary competitive advantage over other microorganisms in the waste.

Furthermore, *A. xylinum* cellulose production machinery is finely tuned to function excellently under the fluctuating environmental proprieties encountered in vinegar wastes. The bacterium grows cellulose microfibrils to produce extracellular thick pellicles or mat-like structures at the air-liquid interface, protecting it from environmental stressors such as fluctuations in temperature and pH [28]. This resistance to an ephemeral environment promotes its survival in dynamic circumstances.

On the other hand, although *G. xylinus* is also able to produce cellulose, it could be limited by the specific challenges of vinegar waste. This Gram-negative bacterium possesses one of the most powerful cellulose-manufacturing systems known so far, and this feature has already been applied in biomedical engineering or food packaging (e.g., film production, food product coating). Nevertheless, its more restricted metabolic repertoire and apparent sensitivity to environmental stress could make it less abundant in vinegar waste in comparison with *A. xylinum* [29,30].

4. Conclusion

A. xylinum shows common in waste from vinegar-producing factories, while *G. xylinus* is less abundant there. Such differences in the abundance of similar abiotic-adapted bacteria reflect the complexity of microbial ecology shaped by environmental factors.

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