# CHARACTERIZATION OF ACETIC ACID BACTERIA OF THE TRADITIONAL DATES VINEGAR IN THE OUARGLA BASIN (NORTHERN EAST ALGERIAN SAHARA)

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**Abstract.** Dates can be used as a raw material for the production of vinegar. This vinegar has significant interests in food, industry and health. Thus, acetic acid bacteria (AAB) introduced in the process of making traditional date vinegar (TDV), ensure its acidity. Six (6) pure strains of AAB *Acetobacter aceti* subsp. *aceti*, *Acetobacter aceti* subsp. *liquifaciens, Acetobacter aceti* subsp. *orleanensis, Acetobacter pasteurianus* subsp. *ascendens, Acetobacter pasteurianus* subsp. *lovaniensis* and *Gluconobacter oxydans* subsp. *industrius* isolated from traditional date vinegars (TDV) of the cultivars Deglet-Nour, Hamraya and Harchaya from the Ouargla basin, are biochemically tested to see their tendency to assimilate some hydrocarbon substrates in the form of miniaturized galleries (API 20 Strep and API 20 NE) . These strains are thus cultivated in a liquid medium based on yeast extract, glucose, ethanol and acetic acid (GYEA), modified with 2% to 10% ethanol and acetic acid, to test their resistance under industrial conditions. The tests are used to select the strains that perform best in acetic acid production (vinegar) and ensure their survival during the process. Two strains *Acetobacter aceti* subsp. *orleanensis* and *Acetobacter pasteurianus* subsp. *lovaniensis* can assimilate the majority of carbohydrate substrates which represents 66.67% (08 substrates). The strains grown in GYEA medium showed a total tolerance of 2% to 10% ethanol, but *Acetobacter aceti* subsp. *aceti* and *Acetobacter aceti* subsp. *liquifaciens* have very good growth at 10%. With acetic acid added to bacterial cultures, resistance to the condition is moderate between 2% and 10%, but the two strains *Acetobacter aceti* subsp. *aceti* and *Acetobacter aceti* subsp. *orleanensis,* are the most acidophilic at 10%. *Acetobacter aceti* subsp. *aceti* is the most suitable strain with ethanol and acetic acid stress. So it is the most profitable and likely to be able to withstand the incidents of the vinegar industry. This offers a choice for large industrial scale applications.

**Keywords:** Traditional date vinegar; acetic acid bacteria; *Acetobacter*; *Gluconobacter*; oxidation; ethanol tolerance; Acidophilia; Algerian Sahara.

## INTRODUCTION

Vinegar is a valuable additive, a complement and an effective preservative against spoilage [3]. The Saharan populations for a very long time, had made locally their own traditional date vinegar [17]. Vinegar fermentation is essentially a two-step process, the first being the anaerobic conversion of fermentable sugars to ethanol by yeasts, and the second being the aerobic oxidation of ethanol to acetic acid by acetic acid bacteria (AAB) [13, 14, 21].

AAB are strictly aerobic Gram-negative bacteria [5, 18], classified as an acetous group [16] common in nature. Currently, they belong taxonomically to the class *Alpha-proteobacteria* of the family *Acetobacteraceae* [5, 16, 18]. They are involved in different spontaneous food fermentations [29], which produce various organic acids from different sources. Most species have the ability to convert ethanol into acetic acid, and play a role during their fermentation processes, also have some beneficial effects [22], in the production of vinegar, traditional fermented milks, such as kefir and vitamin C production and as plant growth promoters (PGPR) [13]. The strains used for the production of vinegar belong to the genera *Acetobacter, Gluconacetobacter, Gluconobacter* [8] and *Komagataeibacter* because of their high capacity for the oxidation of ethanol and their resistance to the resulting acetic acid in the fermentation medium [12]. For this reason, the search for new strains that are likely to be tolerant and more profitable, remains the concern of the industry.

The work seeks to characterize some strains of AAB from traditional date vinegar (TDV) from the Ouargla basin (Algerian Northern Sahara) in relation to their culture medium. A study of the assimilation of carbon substrates and the tolerance of these bacteria to different concentrations of ethanol and acetic acid, in order to select the best indigenous (Saharan) strains of an industrial nature.

## MATERIAL AND METHODS

Six strains of AAB including; *Acetobacter aceti* subsp. *aceti*, *Acetobacter aceti* subsp. *liquifaciens, Acetobacter aceti* subsp. *orleanensis, Acetobacter pasteurianus* subsp. *ascendens, Acetobacter pasteurianus* subsp. *lovaniensis* and *Gluconobacter oxydans* subsp. *industrius,* isolated from traditional date vinegars (TDV) of different cultivars (Hchef Deglet-Nour, Deglet-Nour, Harchaya and Hamraya) from the Ouargla basin are used in the study. A set of fourteen (14) samples of TDV, prepared locally by some families from common dates or date waste by traditional means after the 2014-2019 date harvest season. The isolation of AAB required the use of selective culture agar media containing ethanol and $CaCO_3$. The process was carried out on Frateur medium, glucose, yeast extract and $CaCO_3$ (GYC) medium, modified nutrient agar medium and natural medium. The strains were then identified

phenotypically by standard catalase, oxidase and Gram tests. Physiological tests were performed on identification media, namely Carr, Hoyer, Haynes medium, gluconic acid formation agar, pigment production broth, cellulose production agar, glycerol agar, ketogluconic acid formation broth.

Biochemical analyses carried out on AAB to test their tendency assimilated hydrocarbon substrates such as esculin, α-galactose, ß-galactose, ribose, arabinose, lactose, trehalose, glucose, raffinose, mannitol, sorbitol and gelatine; as a source of carbon. An API 20 NE and API 20 Strep miniaturized gallery containing dehydrated substrates is used to demonstrate the enzymatic or fermentation activities of BioMérieux France brand sugars from a 0.5 Mac Farland bacterial suspension.

The study of AAB tolerance begin with the preparation of the pre-culture by making bacterial AAB of TDV suspects (10 mL) of GYEA medium and incubating at 30°C for one week, to obtain a culture of optical density at 600 nm of the order of 0.7 or 0.8 (the cells have reached the end of the exponential phase). The appropriate volume of inoculum is taken according to the formula; $C_1V_1=C_2V_2$ at $DO_{600nm}=0.5$ (the starting concentration). Ethanol and acetic acid tolerance were performed by inoculation the pre-cultures in a liquid medium based on 2% yeast extract, 2% glucose, 2% ethanol and 2% acetic acid (YGEA) modified with 2%, 4%, 6%, 8% and 10% ethanol, and 0%, 2%, 4%, 6%, 8% and 10% of acetic acid, equivalent in pH 6.49 ± 0.02, 3.47 ± 0.01, 3.29 ± 0.01, 3.18 ± 0.01, 3.06 ± 0.01, 2.94 ± 0.01. Incubated at 30°C for 168 hours. The amount of cells biomass produced is determined by measuring the absorbance of the culture at 600 nm using a UV-visible spectrophotometer every 24 h.

## RESULTS

The results of biochemical analyses of assimilation of 12 different substrates (esculin, α and ß galactose, ribose, arabinose, lactose, trehalose, raffinose, glucose, mannitol, sorbitol and gelatin) are presented in table 1.

An overview of the results in table 01 shows that all strains of the *genera Acetobacter* and *Gluconobacter* tested are capable of oxidizing esculin and α-galactose. The *Acetobacter aceti* subsp. *aceti* strain does not oxidize neither ß-galactose nor glucose, and does not ferment neither ribose, nor arabinose, nor mannitol, nor sorbitol, nor lactose, nor trehalose, nor raffinose, nor glucose but does degrade gelatin. On the other hand, *Acetobacter aceti* subsp. *liquifaciens* can assimilate and ferment glucose and able to degrade gelatine. For *Acetobacter aceti* subsp. *orleanensis* strain, has catabolyzed gelatin and ß-galactose. Arabinose, mannitol, lactose, raffinose are also fermented, unlike ribose, sorbitol, trehalose. For glucose, it is neither fermentable nor assimilable by the strain. *Acetobacter pasteurianus* subsp. *ascendens* is unable to oxidize ß-galactose and glucose, only ferments arabinose and does not degrade gelatin. Concerns *Acetobacter pasteurianus* subsp. *lovaniensis*, fermented arabinose, mannitol, lactose, raffinose and trehalose, with the exception of ribose, sorbitol and glucose, the oxidation of ß-galactose and glucose has not been proven, but the gelatin is degraded. The *Gluconobacter oxydans* subsp. *industrius* strain assimilated only glucose and degraded gelatin.

The comparison between strains according to their ability to assimilate carbohydrate substrates (Fig. 1) is organized in descending order; *Acetobacter aceti* subsp. *orleanensis* and *Acetobacter pasteurianus* subsp. *lovaniensis*, used 08 substrates (66.67%), then *Acetobacter aceti* subsp. *liquifaciens* and *Gluconobacter oxydans* subsp. *industrius,* assimilated 04 substrates (33.33%) and finally, *Acetobacter aceti* subsp. *aceti* and *Acetobacter pasteurianus* subsp. *ascendens* by 03 substrates (25.00%).

Thus, biochemical assimilation of some substrates as a source of carbon, shows the flexibility of the two strains *Acetobacter aceti* subsp. *orleanensis* and *Acetobacter pasteurianus* subsp. *lovaniensis* to assimilate several substrates via the enzymes they possess with 66.67% (08 substrates).

The growth results of the strains *Acetobacter aceti* subsp. *aceti*, *Acetobacter aceti* subsp. *liquifaciens, Acetobacter aceti* subsp. *orleanensis, Acetobacter pasteurianus* subsp. *ascendens*, *Acetobacter pasteurianus* subsp. *lovaniensis* and *Gluconobacter*

**Table 1.** Biochemical analysis of acetic bacteria

| Strains / Substrates | *Acetobacter aceti* subsp. *aceti* | *Acetobacter aceti* subsp. *liquifaciens* | *Acetobacter aceti* subsp. *orleanensis* | *Acetobacter pasteurianus* subsp. *ascendens* | *Acetobacter pasteurianus* subsp. *lovaniensis* | *Gluconobacter oxydans* subsp. *industrius* |
|---|---|---|---|---|---|---|
| ESC | + | + | + | + | + | + |
| α-GAL | + | + | + | + | + | + |
| ß-GAL | - | - | + | - | - | - |
| RIB | - | - | - | - | - | - |
| ARA | - | - | + | + | + | - |
| MAN | - | - | + | - | + | - |
| SOR | - | - | - | - | - | - |
| LAC | - | - | + | - | + | - |
| TRE | - | - | - | - | + | - |
| RAF | - | - | + | - | + | - |
| GLU(F) | - | + | - | - | - | - |
| GEL | + | + | + | - | + | + |
| GLU(A) | - | + | - | - | - | + |

+: Positive; -: Negative; ESC: esculin; α and ß GAL: galactose; RIB: ribose; ARA: arabinose; MAN: mannitol; SOR: sorbitol; LAC: lactose; TRE: trehalose; RAF: raffinose; GEL: gelatine; GLU(F): fermentation; GLU(A): oxydation.

*oxydans* subsp. *industrius*, for 168 hours, on a liquid medium YGEA modified by ethanol, are shown in Figures (2-7). Whose strains start their growth from the optical density 0.5 ± 0.005 at 600 nm.

Figure 2a shows the evolution of the biomass of *Acetobacter aceti* subsp. *aceti* strain as curves with relatively similar patterns of variation during the incubation period at different ethanol concentrations.

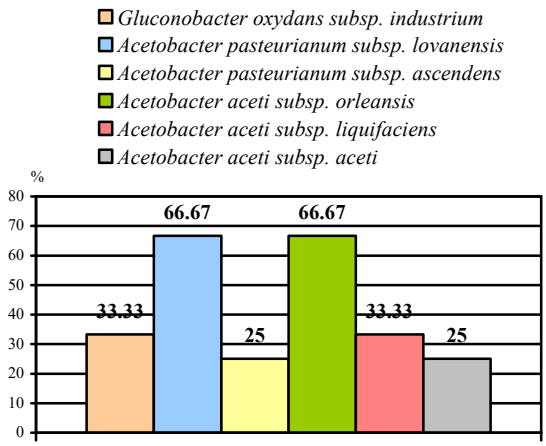


**Figure 1.** Assimilation of hydrocarbon substrates by acetic acid bacteria

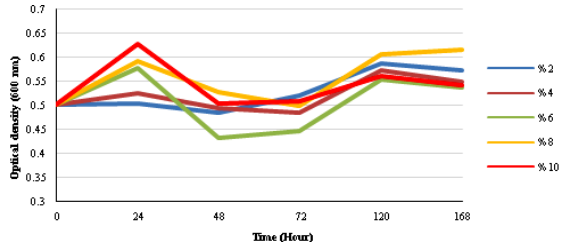


**Figure 2a.** Tolerance of *Acetobacter aceti* subsp. *aceti* strain from traditional date vinegar to ethanol concentrations

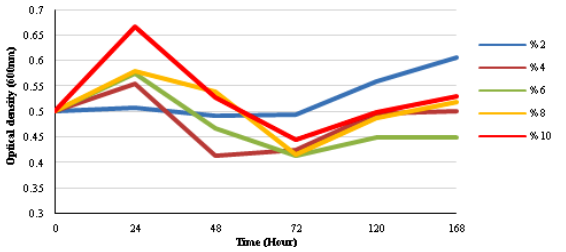


**Figure 2b.** Tolerance of *Acetobacter aceti* subsp. *liquifaciens* strain from traditional date vinegar with ethanol

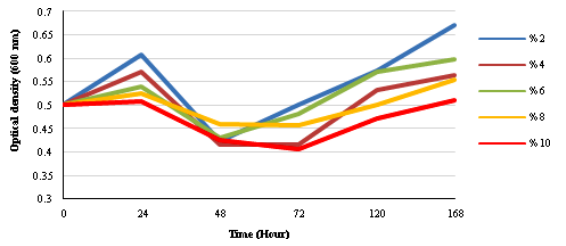


**Figure 2c.** Tolerance of *Acetobacter aceti* subsp. *orleanensis* strain from traditional date vinegar with ethanol

Variations showing an increase after 24 hours corresponding to optical densities 0.504 ± 013 (2%), 0.525 ± 0.004 (4%), 0.577 ± 0.003 (6%), 0.591 ± 0.003 (8%) and 0.627 ± 0.01 (10%), then a decrease after 48h, a resumption of growth from 72h to 120h with concentrations of 0.586 ± 0.004 (2%), 0.573 ± 0.006 (4%), 0.552 ± 0.008 (6%), 0.605 ± 0.005 (8%) and 0.560 ± 0.004 (10%), then a slight decrease. The good concentrations for growth are remarkable at 10% (0.627 ± 0.01), 8% (0.591 ± 0.003), 6% (0.577 ± 0.003), which translates into better acetic acid production in 24 hours, and good tolerance in 168 hours.

In Figure 2b the growth of *Acetobacter aceti* subsp. *liquifaciens* strain in different concentrations of ethanol appears similar during incubation. The evolution of biomass after 24 hours reaches 0.508 ± 0.001 (2%), 0.555 ± 0.002 (4%), 0.575 ± 0.010 (6%), 0.580 ± 0.003 (8%) and 0.666 ± 0.004 (10%), followed by a decrease until 72h. It was remarkable resumption of growth from 0.493 ± 0.008 (2%), 0.425 ± 0.002 (4%), 0.414 ± 0.007 (6%), 0.415 ± 0.004 (8%) and 0.444 ± 0.001 (10%), to 0.606 ± 0.004 (2%), 0.501 ± 0.008 (4%), 0.450 ± 0.006 (6%), 0.519 ± 0.002 (8%) and 0.530 ± 0.002 (10%) at 168 hours. Thus, bacteria tolerate concentrations ranging from 10% ethanol which is the most adequate where growth is maximum in reduced time (24h), which is expressed a good production of acetic acid in the medium after consumption of ethanol.

The growth of *Acetobacter aceti* subsp. *orleanensis over a* period of time at different concentrations of ethanol revealed tolerance forks ranging from 2% to 10% ethanol, shown on the curves (Fig. 2c). The optical density after 24 hours are 0.608 ± 0.003 (2%), 0.571 ± 0.008 (4%), 0.540 ± 0.005 (6%), 0.524 ± 0.011 (8%) and 0.509 ± 0.004 (10%). Then decrease from 48h to 72, the strain catches up with growth at 168h with 0.671 ± 0.058 (2%), 0.565 ± 0.011 (4%), 0.599 ± 0.001 (6%), 0.555 ± 0.006 (8%) and 0.511 ± 0.005 (10%). At almost 10% it is not a considerable growth, it is just an attempt to adapt to the environment during 168 hours. On the other hand, the strain prefers low concentrations of ethanol.

The evolution of growth *Acetobacter pasteurianus* subsp. *ascendens* represented in Figure 2d, showed decreasing optical densities from 2% to 10%, reaching 0.693 ± 0.004 (2%), 0.578 ± 0.004 (4%), 0.572 ± 0.003 (6%), 0.560 ± 0.004 (8%) and 0.521 ± 0.003 (10%) at 24h and decreasing at 48h. A significant increase appeared in 120h and 168h. With 0.797 ± 0.013 (2%), 0.596 ± 0.006 (4%), 0.568 ± 0.004 (6%), 0.598 ± 0.012 (8%) and 0.649 ± 0.002 (10%). The strain grew best with 2% ethanol after 168h with a tolerance to up of 4% and reached 10%.

The variation in growth of *Acetobacter pasteurianus* subsp. *lovaniensis* during 168 h incubation in different concentrations of ethanol are reported in Figure 2e.

The cells grew after 24 hours with optical densities of 0.522 ± 0.002 (10%), 0.542 ± 0.008 (8%), 0.608 ± 0.005 (6%), 0.618 ± 0.015 (4%) and 0.630 ± 0.010 (2%), then dropped between 48 and 72 hours. At 120h almost the maximum biomass is reached with 0.592 ± 0.002 (10%), 0.574 ± 0.002 (8%), 0.575 ± 0.001 (6%), 0.566 ± 0.006 (4%) and 0.606 ± 0.007 (2%). Therefore, low ethanol concentrations (2%, 4% and 6%) remain the most suitable for the cultivation of *Acetobacter pasteurianus* subsp. *lovaniensis* strain, reporting 10% resistance.

The concentration of *Gluconobacter oxydans* subsp. *industrius* strain obtained in Figure 2f, illustrates that all ethanol concentrations for the strain have almost similar patterns. Biomass increased during 24 h incubation with 0.542 ± 0.001 (10%), 0.544 ± 0.001 (8%), 0.591 ± 0.004 (6%), 0.606 ± 0.002 (4%)

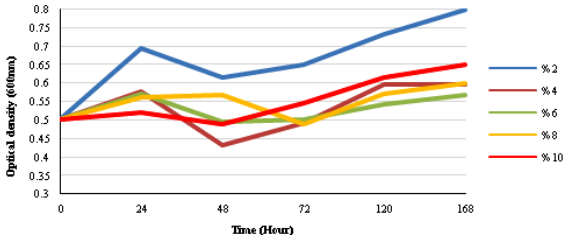


**Figure 2d.** Tolerance of *Acetobacter pasteurianus* subsp. *ascendens* strain from traditional date vinegar with ethanol

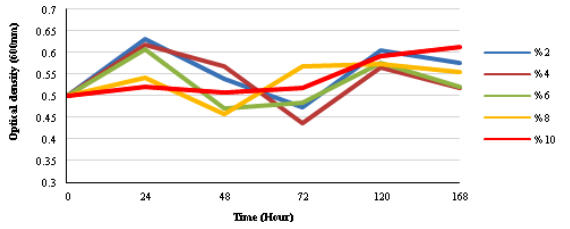


**Figure 2e.** Tolerance of *Acetobacter pasteurianus* subsp. *lovaniensis* strain from traditional date vinegar with ethanol

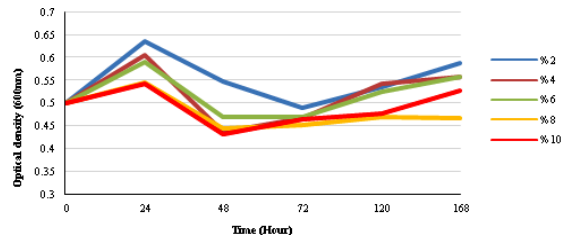


**Figure 2f.** Tolerance of the *Gluconobacter oxydans* subsp. *industrius* strain from traditional date vinegar with ethanol

and 0.636 ± 0.005 (2%). Then a decrease in growth, at 48 and 72 hours. The strain resumed its evolution at 168h in 2%, 4%, 6%, 8% and 10% with 0.586 ± 0.003, 0.557 ± 0.005, 0.558 ± 0.008, 0.467 ± 0.002 and 0.527 ± 0.015 respectively. The optimal growth range of the strain is between 2% and 6% ethanol but tolerates up to 10%.

The growth of the strains examined in Figures 2a-2f showed four characteristic phases of diauxic (two-phase) growth. A first phase of growth during 24 h incubation, followed by a slowing down phase between 48h and 72h, a maximum growth recovery between 120h and 168h, then slowing down ending at certain concentrations.

From the results of table 2, it seems that strains derived from traditional date vinegar of the cultivars Deglet Nour, Hchef Deglet Nour, Hamraya and Harchaya, can grow and be tolerated in media supplemented with ethanol concentrations between 2% and 10%. But *Acetobacter aceti* subsp. *aceti* and *Acetobacter aceti* subsp. *liquifaciens* are the most resistant with very good growth at 10%, followed by *Acetobacter aceti* subsp. *orleanensis*, *Acetobacter pasteurianus* subsp. *ascendens* with good growth at 8%. Finally, *Acetobacter pasteurianus* subsp. *lovaniensis* and *Gluconobacter oxydans* subsp. *industrius* which grow very good and good respectively in the presence of 6% ethanol.

The results of growth monitoring of the strains *Acetobacter aceti* subsp. *aceti*, *Acetobacter aceti* subsp. *liquifaciens*, *Acetobacter aceti* subsp. *orleanensis*, *Acetobacter pasteurianus* subsp. *ascendens,* *Acetobacter pasteurianus* subsp. *lovaniensis* and *Gluconobacter oxydans* subsp. *industrius* for 168 hours, on a liquid medium YGEA modified with acetic acid, are shown as curves in Figures 3a – 3f. Whose strains start their growth from 0.5 ± 0.006 at 600 nm.

The variation of biomass of *Acetobacter aceti* subsp. *aceti* from an optical density of 0.5 at different concentrations of acetic acid is shown in the form of curves with correlatively identical rates of change during incubation (Fig. 3a). An increase in growth after 24 hours with increasing optical densities; 0.553 ± 0.008 (0%), 0.555 ± 0.003 (2%), 0.582 ± 0.002 (10%), 0.568 ± 0.004 (4%), 0.617 ± 0.005 (8%) and 0.780 ± 0.006 (6%). So 6% acetic acid represents the growth peak, then a decrease towards the two side (4% and 8%) and further towards 2% and 10%. A decrease until 120h, where the strain resumes growth with 0.377 ± 0.003 (2%), 0.451 ± 0.004 (4%), 0.733 ± 0.003 (6%),

**Table 2.** Comparison of growth of AAB at different ethanol concentration

| Strain \ Concentration | 2% | 4% | 6% | 8% | 10% |
|---|---|---|---|---|---|
| *Acetobacter aceti* subsp. *aceti* | + | + | +++ | +++ | +++ |
| *Acetobacter aceti* subsp. *liquifaciens* | + | ++ | ++ | ++ | +++ |
| *Acetobacter aceti* subsp. *orleanensis* | +++ | +++ | ++ | ++ | + |
| *Acetobacter pasteurianus* subsp. *ascendens* | +++ | ++ | ++ | ++ | + |
| *Acetobacter pasteurianus* subsp. *lovaniensis* | +++ | +++ | +++ | + | + |
| *Gluconobacter oxydans* subsp. *industrius* | +++ | ++ | ++ | + | + |

Very good: +++, good: ++, moderate: +.

0.541 ± 0.002 (8%) and 0.363 ± 0.004 (10%) at 168h. With the exception of 0% acetic acid the curve is almost stable.

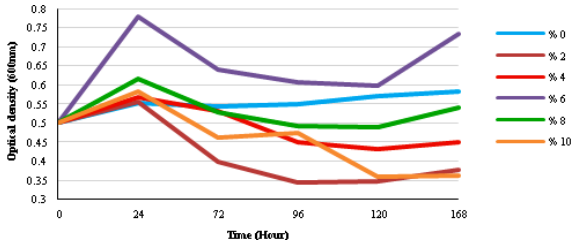


**Figure 3a.** Tolerance of *Acetobacter aceti* subsp. *aceti* strain from traditional date vinegar with acetic acid

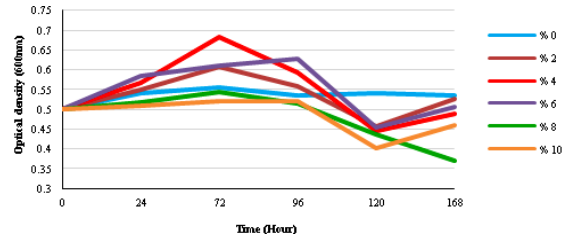


**Figure 3b.** Tolerance of *Acetobacter aceti* subsp. *liquifaciens* from traditional date vinegar with acetic acid

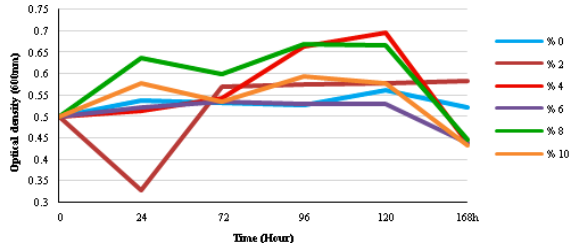


**Figure 3c.** Tolerance of *Acetobacter aceti* subsp. *orleanensis* strain from traditional date vinegar with acetic acid

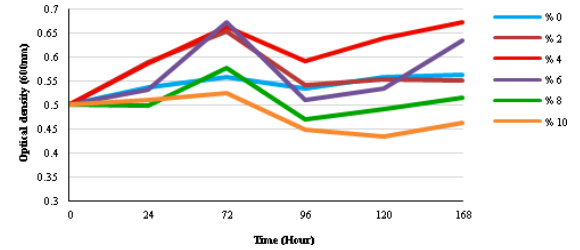


**Figure 3d.** Tolerance of *Acetobacter pasteurianus* subsp. *ascendens* strain from traditional date vinegar with acetic acid

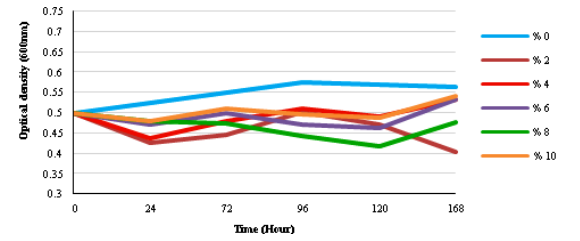


**Figure 3e.** Tolerance of *Acetobacter pasteurianus* subsp. *lovaniensis* strain from traditional date vinegar with acetic acid

Figure 3b shows a slower majority growth of *Acetobacter aceti* subsp. *liquifaciens* up to 72 hours from bottom to top by 0.608 ± 0.005 (2%), 0.611 ± 0.002 (6%), 0.544 ± 0.004 (8%) and 0.522 ± 0.003 (10%). A regression at 120h, then a return to growth at 168h, of which, 0.527 ± 0.002 (2%), 0.489 ± 0.005 (4%), 0.505 ± 0.004 (6%), 0.369 ± 0.002 (8%) and 0.461 ± 0.005 (10%). In the absence of acetic acid, there is no significant growth. Therefore the optimum growth is around 0.682 ± 0.002 (4%) acetic acid.

*Acetobacter aceti* subsp. *orleanensis* (Fig. 3c), evolved in the direction of 0.323 ± 0.002 (2%), 0.512 ± 0.023 (4%), 0.522 ± 0.002 (6%), 0.576 ± 0.004 (10%) and 0.637 ± 0.012 (8%) at 24 hours, but majority decay at 72 hour. Increase in 120 hours [0.696 ± 0.007 (4%), 0.528 ± 0.005 (6%), 0.665 ± 0.002 (8%) and 0.577 ± 0.001 (10%)], and regressed afterwards. The bacterium in the acetic acid-free medium hardly exceeds 0.560 during the 168 hours. The drop in biomass for 0.323 ± 0.002 (2%) of acetic acid after 24 hours may be due to a lack of oxygen.

Figure 3d express slow growth of *Acetobacter pasteurianus* subsp. *ascendens*. After 72h, where the optical densities at 0.673 ± 0.007 (6%), 0.663 ± 0.002 (4%) and 0.653 ± 0.005 (2%) are almost juxtaposed, and far from 0.577 ± 0.001 (8%) and 0.525 ± 0.004 (10%). Then a decrease at 96 h, and growth resumes at 168 h with 0.551 ± 0.005 (2%), 0.673 ± 0.002 (4%), 0.633 ± 0.006 (6%), 0.514 ± 0.009 (8%) and 0.463 ± 0.009(10%) hence the tolerance of the bacteria. On the other hand, the evolution grows 0% acetic acid, always maintains about 0.5 ± 0.004 and 0.562 ± 0.003.

The addition of acetic acid to the culture of *Acetobacter pasteurianus* subsp. *lovaniensis* causes stress to the bacteria, which is expressed by curves showing close and decreasing paces after 24 h, where the optical density is 0.480 ± 0.001 (10%), 0.472 ± 0.002 (8%), 0.470 ± 0.002 (6%), 0.438 ± 0.003 (4%) and 0.425 ± 0.005 (2%) with acetic acid concentrations increasing from 2% to 10% (Fig. 3e). In the absence of acetic acid, the strain grows slowly to 0.575 ± 0.006 and 0.562 ± 0.006.

The optical density of *Gluconobacter oxydans* subsp. *industrius* obtained after 72 hours in decreasing order is the 0.671 ± 0.002 (4%), 0.634 ± 0.002 (6%), 0.630 ± 0.019 (2%), 0.615 ± 0.020 (8%) and 0.561 ± 0.033 (10%). It decrease from 72 h to 168 h by 0.434 ± 0.007 (4%), 0.402 ± 0.006 (6%), 0.427 ± 0.013 (2%), 0.398 ± 0.003 (8%) and 0.468 ± 0.004 (10%) (Fig. 3f).

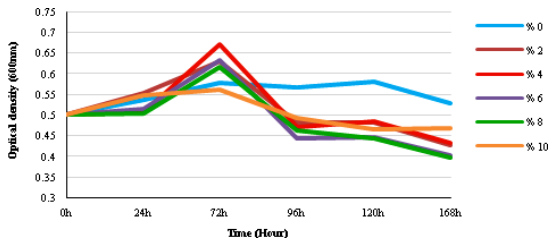


**Figure 3f.** Tolerance of *Gluconobacter oxydans* subsp. *industrius* strain from traditional date vinegar to acetic acid

**Table 3.** Comparison of growth of AAB at different acetic acid concentration

| Strain \ Concentration | 0% | 2% | 4% | 6% | 8% | 10% |
|---|---|---|---|---|---|---|
| *Acetobacter aceti* subsp. *aceti* | + | + | ++ | +++ | ++ | ++ |
| *Acetobacter aceti* subsp. *liquifaciens* | + | ++ | +++ | ++ | + | + |
| *Acetobacter aceti* subsp. *orleanensis* | + | + | + | + | +++ | ++ |
| *Acetobacter pasteurianus* subsp. *ascendens* | + | +++ | +++ | +++ | ++ | + |
| *Acetobacter pasteurianus* subsp. *lovaniensis* | + | --- | --- | - | - | - |
| *Gluconobacter oxydans* subsp. *industrius* | + | ++ | +++ | ++ | ++ | + |

Very good: +++, good: ++, moderate: +, bad: -, very bad: ---

Cultivation of the *Gluconobacter oxydans* subsp. *industrius* strain in the absence of acetic acid (0%) remains reduced between 0.580 ± 0.005 (120h) and 0.530 ± 0.001 (168h). The exponential phase (before 72 hours) can be explained by an exceptional adaptation and assimilation of the excess acid produced and added to the medium by the glyoxylate shunt. But the second phase of decline (from 72h to 168h), may be due to the inability of *Gluconobacter oxydans* subsp. *industrius* to respond to a very high stress of acetic acid accumulated in the stressed environment. This causes cell lysis.

The strains tested at different concentrations of acetic acid and compared in Table 3, showed a total tolerance up to 10% (pH= 2.94). The exception was *Acetobacter pasteurianus* subsp. *lovaniensis*. Including the order of growth at high rate of acitic acid is, *Acetobacter aceti* subsp. *aceti*, *Acetobacter aceti* subsp. *orleanensis*, *Acetobacter aceti* subsp. *liquifaciens, Acetobacter pasteurianus* subsp. *ascendens,* and *Gluconobacter oxydans* subsp. *industrius*. In the absence of acetic acid, the growth of the strains remains practically constant. *Acetobacter aceti* subsp. *aceti* and *Acetobacter aceti* subsp. *orleanensis* resisted better in an acid shock of about 10% (pH= 2.94 ± 0.01).

Thus, the strain *Acetobacter aceti* subsp. *aceti,* which adapts faster to the stresses of ethanol and 10% acetic acid. For this, it may be a choice for future use on an industrial scale, which requires an optimization of the production parameters before inoculation into the fermenter.

## DISCUSSION

In light of our results, AAB strains isolated from traditional date vinegar produced locally in the basin of Ouaragla (Algerian Northern Sahara) were found to show a major industrial effect. Strains that have provided the enzyme ß-glucosidase, hydrolyzed esculin to glucose monomer. The ɑ-galactose and ß-galactose are hydrolyzed using the enzymes ɑ-galactosidase and ß-galactosidase. To degrade gelatin, a gelatinase enzyme must be produced by the strains of *Acetobacter* and *Gluconobacter* tested, to provide the amino acid Alanine, Hydroxyproline, Isoleucine, Leucine, Methionine, Phenylalanine, Valine, Aspartic Acid, Glutamic Acid …etc. The fermentation of glucose, ribose, arabinose, lactose, trehalose, raffinose, mannitol and sorbitol reveals an acidification of the media after production of organic acids by AAB. AAB have the ability to oxidize in fermentation many types of substrates to high-value end products, including acetic acid, the main constituent of vinegar, ascorbic acid (vitamin C), gluconic acid, ketogluconic acid, cellulose and dextrans production [23]. The best carbon sources for *Acetobacter* strains are, in descending order, ethanol, glycerol and Na-DL-lactate, while for *Gluconobacter,* D-mannitol, sorbitol, glycerol, D-fructose and D-glucose [26]. For the productivity of cellulose, it is preferable to use dual carbon sources e.g. glucose and ethanol, modified sugars and polyols [7]. According to Kadere et al. (2008) [9], strains of the genus *Acetobacter* isolated from coconut are capable of fermenting arabinose, xylose, ribose, glucose, galactose, mannose, melibiose and trehalose. They do not ferment amylagdine, cellibiose, esculin, fructose, lactose, maltose, mannitol, melezitose, na-gluconate, raffinose, rhamnose, salicin, sorbitol, sucrose. They can grow on gelatin, without liquefaction. *Acetobacter pasteurianus* according to Konate et al. (2014) [10] of Ivorian wine, were capable of oxidizing xylose, glycerol, glucose, ethanol, acetic acid, lactic acid and incapable of oxidizing galactose, mannose, sucrose, mannitol, sorbitol and fructose. Romero-Cortes et al. (2012) [19], emerged AAB from cocoa, oxidizing ethanol but does not hydrolyze cellulose.

Chinnawirotpisan et al. (2003) [2], report that strains of the genus *Acetobacter* always show three characteristic growth phases in alcoholic environments. The first is the oxidation of alcohol (ethanol) to acetic acid by the enzyme alcohol dehydrogenase (ADH), the second is not a growth stage and the third is the over-oxidation of the accumulated acetic acid by aldehyde dehydrogenase (ALDH) to $CO_2$ and $H_2O$. For the *Gluconobacter oxydans* subsp. *industrius* strain, the third phase consists of an adaptation and tolerance to the acetic acid produced without over-oxidation. Ethanol damages the cell membrane, denatures proteins, interferes with metabolism and causes cell lysis in bacteria [18] and thus for biomolecules such as DNA, RNA, ribosomes and enzymes [27]. Higher concentrations have not been tested because the composition of traditional balsamic and date vinegar is different from industrial vinegar and rarely exceeds 10% alcohol. The concentration of ethanol is not a limiting factor for the growth of AAB [6]. An optimal concentration of 13.45 ± 0.99% ethanol is optimized for good acetic fermentation [8]. After alcoholic fermentation, even when ethanol concentrations of 5-10% are toxic to AAB, some strains are able to survive in very high ethanol concentrations of up to 15% [11].

Beheshti-Maal (2014) [1], showed that strains of *Acetobacter* isolated from date palm in Iran, were resistant in ethanol concentrations between 5 and 9% and 2.5% and 6% respectively. For a good vinegar process, an alcohol concentration of 10-13% is essential [4]. Our results are in agreement with previous studies reporting growth in ethanol and acetic acid at stressed conditions.

The acid suppresses bacterial growth, witch was an activator up to the value of 2% [15]. The acetic acid strength of a good vinegar should be about 6% (at least 4% acetic acid) [4], the minimum acetic acid content in vinegar should be 4% [8]. This explains the choice of the strain introduced in vinegar. The most attractive species of the genus *Komagataeibacter, Komagataeibacter europaeus, Komagataeibacter intermedius, Komagataeibacter oboediens, Gluconacetobacter entanii* and *Komagataeibacter maltaceti,* were isolated from industrial vinegar bioreactors where the acetic acid concentration was over 6%. These species are capable of resisting acetic acid up to 15-20%, while species of the genus *Acetobacter* are harmed when acidity rises to 7-8%. This property makes the *Komagataeibacter* species well adapted for application in the fermentation of immersed vinegar [27]. The activity of DHA is much more stable under acidic conditions in bacteria of the genus *Acetobacter* than in bacteria of the genus *Gluconobacter,* which explains why *Acetobacter* produce a higher amount of acetic acid [25]. Adaptive responses of microbial cells to the toxic environment include changes in membrane composition to facilitate the penetration of acetic acid and their dissociation in the cytoplasm (high pH). The cells are exposed to the acetic acid present in the culture medium composition, and that produced from ethanol, during vinegar production. The oxidation process is performed by enzymes bound to the periplasmic surface of the internal cytoplasmic membrane. The acetic acid produced diffuses passively into the cytoplasm and is probably transported out of the cell by an efflux pump. In addition to this efflux system, the cell must also adapt to high concentrations of acetic acid in its membrane and also prevent the diffusion of acetic acid into the cytoplasm [28]. *Acetobacter* acidophilic strains isolated by Saeki et al. (1997) [20], tolerated 15-21% acetic acid but their optimal growth was between 4 and 8%. The optimum pH for growth and acetic acid production had a wider range from 3 to 5. AAB are also able to grow at lower pH values where bacterial activity has been detected at pH values below 3 [24]. The strain *Acetobacter aceti* subsp. *aceti,* isolated in Ouargla basin (Algerian Northern Sahara), has good mechanisms of adaptation to its environment, which is likely to be a better current industrial offer.

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