

Production of Apricot Vinegar Using an Isolated *Acetobacter* Strain from Iranian Apricot

Keivan Beheshti Maal, Rasoul Shafiei and Noushin Kabiri

Abstract—Vinegar or sour wine is a product of alcoholic and subsequent acetous fermentation of sugary precursors derived from several fruits or starchy substrates. This delicious food additive and supplement contains not less than 4 grams of acetic acid in 100 cubic centimeters at 20°C. Among the large number of bacteria that are able to produce acetic acid, only few genera are used in vinegar industry most significant of which are *Acetobacter* and *Gluconobacter*. In this research we isolated and identified an *Acetobacter* strain from Iranian apricot, a very delicious and sensitive summer fruit to decay, we gathered from fruit's stores in Isfahan, Iran. The main culture media we used were Carr, GYC, Frateur and an industrial medium for vinegar production. We isolated this strain using a novel miniature fermentor we made at Pars Yeema Biotechnologists Co., Isfahan Science and Technology Town (ISTT), Isfahan, Iran. The microscopic examinations of isolated strain from Iranian apricot showed gram negative rods to cocobacilli. Their catalase reaction was positive and oxidase reaction was negative and could ferment ethanol to acetic acid. Also it showed an acceptable growth in 5%, 7% and 9% ethanol concentrations at 30°C using modified Carr media after 24, 48 and 96 hours incubation respectively. According to its tolerance against high concentrations of ethanol after four days incubation and its high acetic acid production, 8.53%, after 144 hours, this strain could be considered as a suitable industrial strain for a production of a new type of vinegar, apricot vinegar, with a new and delicious taste. In conclusion this is the first report of isolation and identification of an *Acetobacter* strain from Iranian apricot with a very good tolerance against high ethanol concentrations as well as high acetic acid productivity in an acceptable incubation period of time industrially. This strain could be used in vinegar industry to convert apricot spoilage to a beneficiary product and mentioned characteristics have made it as an amenable strain in food and agricultural biotechnology.

Keywords—Acetic Acid Bacteria, *Acetobacter*, Fermentation, Food and Agricultural Biotechnology, Iranian Apricot, Vinegar.

I. INTRODUCTION

ACETIC Acid Bacteria (AAB), the main producers of Acetic acid, are related to class Alphaproteobacteria, family *Acetobacteraceae* that currently includes nine genera namely *Acetobacter*, *Acidomonas*, *Asaia*, *Gluconoacetobacter*, *Gluconobacter*, *Kozakia*, *Neoasaia*, *Saccharibacter* and *Swaminathania* [1]. AAB are prevalence on the several fruits, flowers and other natural resources and responsible for their decay and spoilage [2], [3].

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Among different genera of AAB, *Acetobacter* is the most significant genus for production of vinegar industrially. According to FDA (Food and Drug Administration, USA), vinegar as a sour solution, contains not less than 4 grams of acetic acid in 100 cubic centimeters at 20°C that is produced through alcoholic and successively acetic fermentation of sugary and starchy substrates [2], [4]-[6]. These gram negative rod shaped strains consume ethanol as their carbon source and convert it to acetic acid. So far the native *Acetobacter* strains have been isolated using several natural resources e.g. grape, coconut, date and palm [5], [7]-[8], Iranian white-red cherry [2], Iranian peach [4], [9], palm tree and palm wine [10], apple, Jamaican cherry, longan, mango, pineapple and rambutan [11]-[12]. Currently vinegar industries are seeking for new origins for vinegar production with attractive tastes as several researches have focused in production of new vinegar types using different AAB and through various precursors such as sugarcane [6] (Kocher et al., 2006), rice [13], bee honey [14] and balsam [3], [15]. In this research we have focused in isolation and identification of new AAB from native Iranian fruits. Also we were looking for strains with high vinegar production as well as suitable tolerance against high ethanol concentrations and production temperature.

II. MATERIALS AND METHODS

Iranian apricot extract preparation: The heterogeneous samples of both intact and spoiled summer fruit, Iranian apricot, were gathered from various fruit stores in Isfahan, Iran using sterile containers and incubated in a good ventilated cabinet at room temperature, 25-30° C, for 2 weeks. After rising sour smelling from incubated container that suggested the activity of AAB, the Iranian apricot spoilages were pressed, squeezed, homogenized and passed through a sterile basket with recognized pore size. The cores of apricots were isolated and extracts were transferred to sterile 2 liter bottles, capped and incubated at 30° C for 7 day anaerobically. For inhibiting bottle's explosion due to alcoholic fermentation, a few tiny punctures were made on top of the bottles for extra gas drainage.

Microbial culture media and instruments: The laboratorial culture media that we used were included GYC standard medium [yeast extract, 10 g/l; D-glucose, 50 g/l; CaCO₃, 30 g/l; agar, 25 g/l; distilled water, 1000 ml], Frateur medium [yeast extract, 10 g/l; CaCO₃, 20 g/l; ethanol, 20 g/l; agar, 20 g/l; distilled water, 1000 ml], Carr medium [yeast extract, 3%; agar, 2%; bromocresol green, 0.002%; ethanol, 2% (v/v); distilled water, 1000 ml] and modified Carr media with 3%, 4%, 5%, 6%, 7%, 8%, 9% and 10% ethanol concentrations. The industrial culture medium [ethanol, 2%; acetic acid, 2%;

yeast extract, 1%] was applied for initial enrichment and isolation of AAB from Iranian peach. All the chemicals were from the Merck. The main instruments that we used were incubator (Heraeus, Germany), shaker incubator (Jahl, JSH 20L, Iran), autoclave (Iran Tolid, Iran), miniature glass fermentor (Pars Yeema Biotechnologists Co., ISTT, Isfahan, Iran), Spectrophotometer (Milton Roy, USA), high speed refrigerated centrifuge (Hitachi, 20PR, Japan), microscope (Nikon, Japan) and stereomicroscope (Wild, Germany).

AAB enrichment and isolation from Iranian apricot: Fifty milliliters of homogenized Iranian peach extract after 7 days incubation at 30° C were collected and centrifuged at 10000 rpm for 20 minutes. The supernatants were discarded and the pellets were added to miniature glass fermentors containing of 400 ml industrial broth medium. The fermentors then were placed in a 30° C incubator while aeration was carrying out through an appropriate sparging system for 7 days. Each 24 hours the acetic acid elevations were examined through titration assay. The containing of fermentors with acetic acid percentage more than 4% were cultured on AAB selective culture media using streak plate method and incubated at 30° C for 4 days.

Macroscopic, microscopic and biochemical properties of isolated strains: The macroscopic traits of isolated colonies from Iranian apricot, morphological characteristics of colonies were investigated using stereomicroscopy. Microscopic and biochemical examinations of pure individual colonies were carried out using gram staining, catalase and oxidase reactions, ability of overoxidizing in Carr medium and ability of consuming calcium carbonate (transparenting around of colonies) in Frateur medium.

Investigation of strain's tolerance to various ethanol concentrations: The isolated strains of AAB from Iranian apricot were cultured on modified Carr media with 5%, 7%, and 9% ethanol concentrations using streak plate method and incubated at 30° C for 96 hours.

Acetic acid titration assay: In all industrial broth media, both before and after inoculation with isolated strains from Iranian peach, the titration assay of acetic acid percentage were took placed as follow: five milliliters of the broth medium were added to 20 ml of distilled water in a 250 ml flask and mixed thoroughly with 5 drops of phenol phetalein [phenol phetalein, 0.1 g; ethanol, 60 g; distilled water, 40 g] and then 0.5 normal sodium hydroxide [NaOH, 20 g/l; distilled water, 1000 ml] were added using 200 ml burette to mentioned mixture until appearance of pale pink color in the flask. The volume of consumed NaOH was measured and the acetic acid percentage in each medium was computed.

III. RESULTS

The addition of Iranian apricot extract to industrial culture media in laboratorial fermentors resulted in an increase of acetic acid percentage that in turn led to enrichment of AAB in

fermentors after 7 days incubation at 30° C. Cultivation of subsequent suspension to selective Carr medium resulted in growth of blue convex and circular colonies with diameter less than 3 mm after 24 hours incubation at 30°C. Also cultivation of enriched industrial culture medium to standard

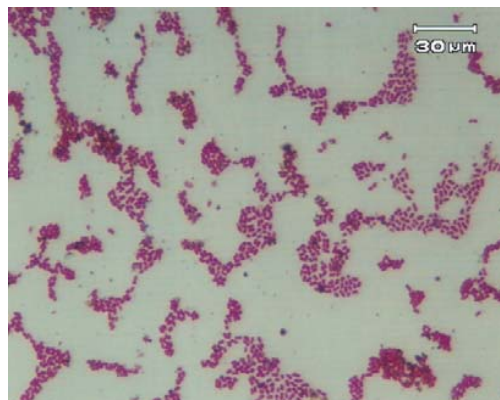


Fig. 1 The gram staining of isolated *Acetobacter* strain from Iranian apricot after 24 hours incubation at 30° C in Carr and Frateur culture media

Frateur medium resulted in the same colonies with gray-greenish color around which transparent zones have been appeared. Gram staining of pure individual colonies showed gram negative rods to cocobacilli as it has been shown in figure 1. Other biochemical and confirmatory tests of isolated strain were shown in Table 1.

TABLE I
THE BIOCHEMICAL TESTS OF ISOLATED ACETOBACTER STRAIN FROM IRANIAN APRICOT IN CARR AND FRATEUR CULTURE MEDIA

Biochemical Examinations	Tests Results from	
	Carr Medium	Frateur Medium
Oxidase	-	-
Catalase	+	+
Overoxidation	+	-
CaCO ₃ Utilization	-	+
Aerobic Growth	+	+
Anaerobic Growth	-	-

The macroscopic, Microscopic and biochemical examinations of isolated strain from Iranian apricot showed that this strain related to AAB group. Overoxidation in Carr medium resulted in conversion of blue color of medium to yellow after 24-48 hours and then reconversion of yellow color to blue after 72-96 hours. Also utilization of CaCO₃ and creation of transparent zones around the colonies in Frateur medium confirmed that the isolated strain was *Acetobacter*. The inoculation of pure *Acetobacter* strain to industrial culture

media showed high acetic acid productivity after 24-144 hours incubation at 30° C and 100 RPM aeration speed. The acetic acid percentage after 24, 48, 72, 96, 120 and 144 hours incubation were 2.35%, 3.42%, 4.51%, 6.43%, 7.25% and 8.53% respectively.

The figure 2 shows the acetic acid production by this strain after 6 days incubation at 30° C. The growth of isolated *Acetobacter* strain on modified Carr media with 5%, 7% and 9% ethanol concentrations confirmed its potential tolerance against high ethanol concentration's shock. Its growth rate in 5% ethanol concentration after 96 hours incubation has been shown in Figure 3.

Acetic Acid Production in Incubation Time

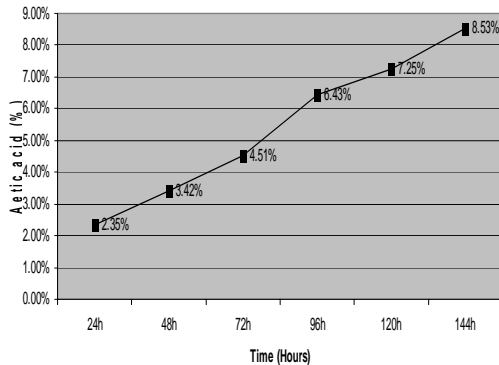


Fig. 2 The production of acetic acid by isolated *Acetobacter* strain from Iranian apricot in industrial culture medium after 144 hours incubation at 30° C using ISTT miniature fermentors with 100 RPM aeration speed

Growth rates in 5% Ethanol

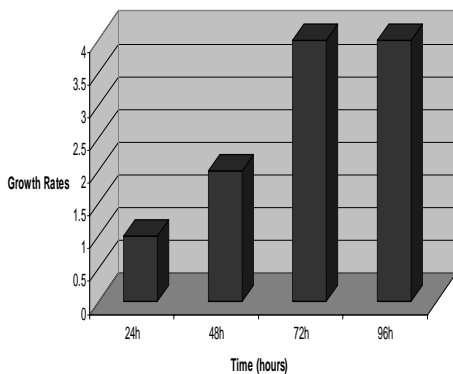


Fig. 3 The growth rate of isolated *Acetobacter* strain from Iranian apricot in modified Carr medium with 5% ethanol after 96 hours at 30° C

The growth rate of isolated *Acetobacter* strain from Iranian apricot in 7% ethanol concentration after 96 hours incubation

has been indicated in figure 4. The growth rate of this strain in 9% ethanol concentration after 96 hours incubation has been shown in Figure 5.

Growth Rates in 7% Ethanol

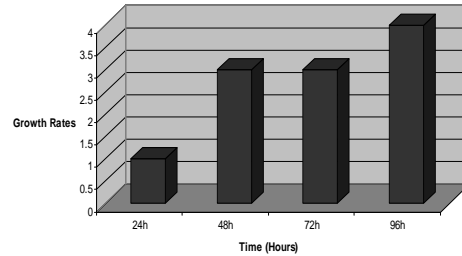


Fig. 4 The growth rate of isolated *Acetobacter* strain from Iranian apricot in modified Carr medium with 7% ethanol after 96 hours at 30° C

Growth Rates in 9% Ethanol

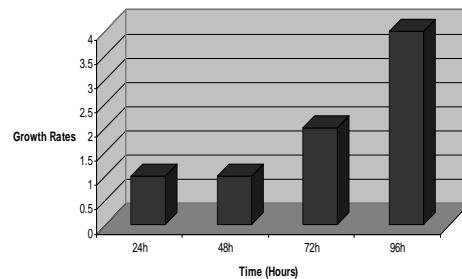


Fig. 5 The growth rate of isolated *Acetobacter* strain from Iranian apricot in modified Carr medium with 9% ethanol after 96 hours at 30° C

Comparison of its growth rates in Carr medium (2% ethanol concentration) and modified Carr media with 5%, 7% and 9% ethanol concentrations after 4 days incubation at 30° C has been illustrated in figure 6.

Growth rates in Different Ethanol Concentrations after 4 Days

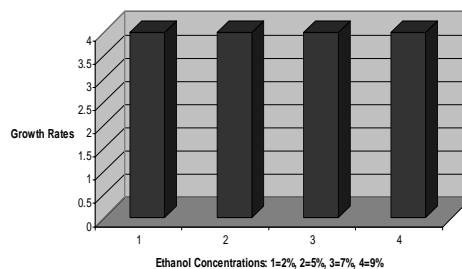


Fig. 6 The comparison of growth rates of isolated *Acetobacter* strain from Iranian apricot in Carr medium and modified Carr media after 4 days incubation at 30° C

IV. DISCUSSION

Kadere et al. isolated an *Acetobacter* strain from coconut and indicated its growth at 25, 30 and 40° C and then produced coconut toddy (mnazi) vinegar using this strain [5]. A few thermotolerant *Acetobacter* strains isolated from apple, Jamaican cherry, longan, mango, pineapple and rambutan using a culture medium containing of 4% ethanol amongst which the one isolated from rambutan have produced the highest acetic acid amount at 37° C after 7 day incubation period [12] (Moryadee et al., 2008). Ilha et al. (2000) reported production of a new vinegar type by several AAB from bee honey but didn't explain any tolerance of their responsible strains against high temperature or ethanol concentrations [14]. Kocher et al. (2006) have studied the production of vinegar by sugarcane using corn cobs, bagasse and wood shavings as immobilizers and *Acetobacter aceti* NRRL746. They showed that the fermentation time was reduced to 13 days by proposed recycling method [6]. An *Acetobacter* strain namely ASVO3 has been isolated from pineapple by Sossou et al. (2009). While they applied this strain for producing vinegar at 30° C and for 23-25 incubation periods they clarified that the concentration of ethanol had no effect on growth of *Acetobacter* sp. ASVO3 [11]. Beheshti Maal and shafiei (2010a) have reported the isolation and characterization of an *Acetobacter* strain from Iranian white red cherry. They have specified that the mentioned isolated strain had growth at 34-36° C in high ethanol, 5-9%, concentrations and after 4 day incubation duration [2]. Beheshti Maal and Shafiei (2010b) isolated a thermotolerant *Acetobacter* strain from Iranian peach and showed that strain could grow at high ethanol concentrations, 7-10%, at 30° C after 96 hours on modified Carr media. They have indicated that the isolated *Acetobacter* has grew on 2.5% and 5% ethanol concentrations at 34° C after 24 hours but had no growth on the same ethanol concentrations at 36° C after 24 hours, instead had growth after 48 hours at 36° C. Also this strain had an acceptable growth on 2.5% and 5% ethanol concentrations at 38° C and 40° C [4]. Ndoye et al. (2007) have reported a thermotolerant *Acetobacter*, *A. senegalensis*, that they isolated from mango (*Mangifera indica* L.). They showed that their strain had growth at 35° C as an optimum temperature [1]. In this research we isolated and identified a new *Acetobacter* strain from Iranian apricot that we obtained from Isfahan, Iran. We suggested this strain could produce high percentages of acetic acid, 8.53%, after 144 hours incubation as a short period of time industrially. Also it could be used in fermentation of a new type of vinegar, apricot vinegar. While the apricot is a very delicious summer fruit with nutritional advantages, but it is a very susceptible fruit against microbial spoilage. We showed that this strain could use mentioned spoilages as precursor for producing of apricot vinegar as a superior food supplement. These properties could be considered as very dramatic characteristics for industrial strains that are qualified for large-scale commercial fermentations in vinegar industry and industrial microbiology. In conclusion, this is the first report of isolation and identification of an *Acetobacter* strain

from a native fruit of Iran, Iranian apricot. The isolated *Acetobacter* strain could grow at 7% - 9% ethanol concentrations that suggest a very suitable strain for large-scale vinegar fermentation in microbial biotechnology as well as a new amenable strain for bioremediation uses in environmental microbiology.

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