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Isolation of *Alicyclobacillus acidoterrestris* from Commercial Spoiled apple Juice and Study on some Influence Parameters on its Growth in Apple Juice

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ABSTRACT: The acid-tolerant and heat-resistant bacterium *Alicyclobacillus acidoterrestris* is a spoilage problem in pasteurized and heat-treated fruit juices. This study was undertaken to identify the bacterium and metabolic products contributing to a disinfectant taint in shelf-stable fruit juice and to determine some of the growth conditions for the organism. Generally the aim of this work was to study the influence of temperature (25-65 °C), total soluble solids (different °Brix) and various types of fruit juice on growth of the *A. acidoterrestris*. A strain of *Alocyclobacillus acidoterrestris* was isolated from spoiled commercial apple juice. Isolated strain growth was studied in different temperatures, different type of juices and brix, was on its growth was investigated. According to the statistical results, all parameters had significant effect on the isolated strain. Also results showed that the isolated strain of A. acidoterrestris had maximum growth in 45°C **and brix 12. The resistance of *A. acidoterrestris* at the assayed temperatures was confirmed. Spore resistance increases with higher SS and pH values. In the other hand the growth of isolated strain wasn't observed in Red Grape juice. This occurrence proved inhibition of phenolic compound of Red Grape. Also temperature was the parameter that had the greatest influence on the bacterial growth.**

Keywords: Brix, IFU, Incubation, Alocyclobacillus acidoterrestris

Abreviation: ss: solid soluble

INTRODUCTION

Alicyclobacillus acidoterrestris is a thermoacidophilic spore forming bacteria, which has the ability to survive traditional thermal process. Acidophilic spore formers were first isolated in 1967 from hot springs in Japan and later from soil. Those from hot springs had a higher optimum growth temperature and were termed Bacillus acidocaldarius. Strains isolated from soil were termed *B. acidoterrestris*. In 1992, the creation of a new genus, *Alicyclobacillus*, was proposed, to comprise the species *B. acidocaldarius, B. acidoterrestris, and B. cycloheptanicus*. Comparative rDNA sequence analyses showed that the 3 strains were sufficiently different from other *Bacillus* spp. to warrant reclassification in a new genus. Also, *Alicyclobacillus* spp. are unique in their fatty acid profiles, containing ώ -alicyclic fatty acid as the major natural membranous lipid component. *Alicyclobacillus acidoterrestris* has been reported to cause juice spoilage, manifested as an off flavor and light cloudiness (Walls, *et al*, 2000).

Several studies have found that *A. acidoterrestris* can grow at temperatures from 20 to 55°C and pH values form 2.5 to 6.0.

This bacterium has been isolated from various types of habitats, but studies showed it is commonly found in soil and water. Since its discovery, this bacterium has been associated in the spoilage of many foods, especially acidic juices, such as orange juice, apple juice and fruits blend (Forestier *et al*, 2009).

Uchino and Doi reported the isolation of spore-forming bacteria from a hot spring site that grew under acidic and geothermal conditions, and has very similar characteristics to *Bacillus coagulans* (Uchino, 1967). In the 1980s, an acidophilic species was isolated from apple juice and identified as a new type of spoilage bacterium. Originally named bacillus acidoterretris this organism was later classified in a new genus, *Alicyclobacillus* (Wisse, 1998). Alicyclobacillus strains survive and grow in acidic beverages, and are responsible for the flat sour type spoilage and the production of guaiacol (Evangelina, 1999, Goldberg 2002, Mitsuda, 2003, Walls, 2000, Yamazaki, 1996). The source of *Alicyclobacillus* contamination is soil, and *Alicyclobacillus* contamination of fruit juices is typically introduced via soil residue remaining on the fruit surface (Matsubara, 2002, Michelle, 2005, and Walls, 2000).

The presence of *Alicyclobacillus acidoterrestris* has been reported in the United States and Europe causing economic loss due to the recall of the final product (Forestier, *et al*, 2009).But no studies have been made in Iran regarding the presence of *A. acidoterrestris* in fruit juices.

The main objective of this investigation was isolation of *A. acidoterrestris* strain from spoiled commercial apple juice and then affected parameters on its growth was investigated.

MATERIALS AND METHODS

A. Method of isolation of A. acidoterrestris in fruit juice

The preparation of samples and detection of *Alicyclobacillus* was developed according to IFU protocol. According IFU method, YGC-agar, K-agar and BAT-agar was used as culture media for detection and Confirmation Tests for *Alicyclobacillus* isolation. K agar is a novel isolation medium developed for the Food industry (Uchino, 1967, Walls, 2000). Differentiation of species was done by Differential Temperature Method. This method is based on the fact that *A. acidoterrestris* has a relatively low optimum temperature for growth compared to the other *Alicyclobacillus* species (Yokota, 2007). Study about effective parameters on *Alicyclobacillus acidoterrestris* growth Parameters such as type of fruit juice, temperature of incubation, pH and the Brix of the fruit juice play an important role in *Alicyclobacillus* growth (Yokota, 2007).

Effect of fruit juice type: Not all species of Alicyclobacillus grow in fruit juices under normal environmental conditions. Some species, such as A. *acidoterrestris*, *A. hesperidum* and *A. acidiphilus*, can grow in juices or soft drink products containing fruit juice. The type of juice greatly affects the growth of *Alicyclobacillus* (Orr 2000). In this study, growth of isolated strain in apple juice, orange juice, red grape juice, white grape juice, peach juice was studied. *A. acidoterrestris* spores (20-50 CFU/ml) were inoculated into 100 ml of different fruit juices with brix 12 and then incubated at 45°C for 2 weeks (Yokota, 2007).

Effect of Temperature: The temperature for growth of Alicyclobacillus species is within a range from 20°C to 70°C. For *A. acidoterrestris*, a species particularly known for its ability to cause undesirable odor (guaiacol) in soft drinks, the temperature range for growth in culture media is 20-55°C (Jensen. N, 1999), similar behavior is also observed when fruit juices are the growth medium (Michelle, 2005). To study about isolated strain growth in different temperatures, *A. acidoterrestris* spores (20-50 CFU/ml) were inoculated into 100 ml of apple juice and then incubated at 25-65°C for 8 days (Yokota, 2007).

Effect of Brix: The optimum pH for growth of isolated strain in culture media was studied. *A. acidoterrestris* spores (20-50 CFU/ml) were inoculated into 100 ml of apple juices with different brix and then incubated at 45°C for 2 days. Different range of brix obtained by adding water or sugar in apple juice (6-24%).

Log N/N0 (N = Count of *A. acidoterrestris* in initial time of incubation & N0= Count of *A. acidoterrestris* after 2 days of incubation) was estimated (Yokota. R, 2007).

B. Statistical analysis

All of the data were analyzed statistically with data processing softwares and figured with Microsoft excel 2007.

RESULTS

Effect of fruit juice type and Brix

The growth rate of isolated strain is much related to type of juice (Table 1).

Table 1: Effect of type of juice on isolated *A. acidoterrestris.*

Fruit juice/ content percentage of juice	Brix	*Alicyclobacillus acidoterretris* growth
Apple juice / 100%	12	+
Orange juice 100%	12	+
White grape juice/ 100%	12	+
Red grape juice/ 100%	12	-
Peach juice/ 100%	12	+
So cherry juice/ 100%	12	+
Mixed juice / 100% (90% Apple juice+ 10% Red grape juice)	12	+
Mixed juice / 100% (80% Apple juice+ 20% Red grape juice)	12	+
Mixed juice / 100% (70% Apple juice+ 30% Red grape juice)	12	-

The inoculated strains grew very well in apple juice, orange juice, white grape juice, peach juice and had weak growth in sour cherry juice and mixed juice (90% Apple juice + 10% Red grape juice - 80% Apple juice+ 20% Red grape juice) (Table 1).

Effect of Brix of Fruit Juice

Statistical analysis showed that effect of brix on the growth of isolated of strain was significant. According to the results (Fig. 1), optimum brix for isolated *A. acidoterrestris* growth is 12%. As the brix of juice increased from 6 to 12%, bacterial growth increased too.

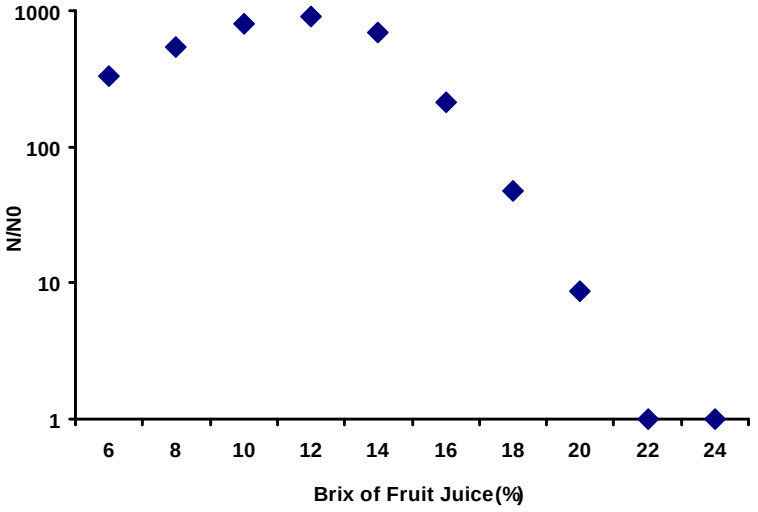

Fig.1. Effect of Brix on *A. acidoterrestris* growth in apple juice.

Effect of Temperature and time

Temperature and time had significant effects on growth of isolated strain, Also there were significant effects among these factors on growth of isolated strain (Table 2).

According to the results and comparison of data average (Fig. 2), isolated strain had maximum growth in 45°C and followed 40°C and 35°C respectively. In temperatures of 25°C, 55°C and 60°C had seen decline in initial amount of isolated strain in incubation period (Fig. 2).

Table 2: Results of analysis of variance of studied variable.

Source of difference	df	LogN/N0
R	2	0.072ns
Temperature(t)	4	2.756**
Time(t)	7	33.786**
time×temperature	28	4.802**
Error	78	0.052
Cv%	-	10.47%

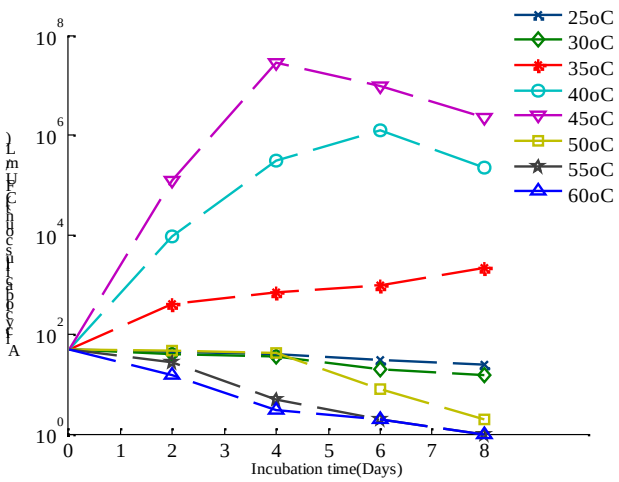

Fig. 2. Effect of Temperature and time on *A. acidoterrestris* growth in apple juice.

This suggests that if the products are kept at room temperature, the possibility of deterioration is very low, in the presence of this *Alicyclobacillus* strain.

DISCUSSION

According to results, type of fruit juice is an effective parameter on growth of *A. acidoterrestris*. The disability to growth of *A. cidoterrestris* in red grape juice is attributed to the presence of natural phenolic compounds, which have antimicrobial activity (Splittstoesser, 1998). A variety of polyphenols extracted from grapes were also investigated for their inhibitory effect on *A. acidoterrestris*, and the results indicated cumaric acid and ferulic acid had a strong inhibitory affect with a MIC (minimal inhibitory concentration) of 150-200 mg/L 10 (Eiroa, 1999). Goto and co-workers tested the growth behavior of several strains of *A. acidoterrestris* in a variety of fruit juices, and have concluded that the behavior of the strains depends on the type of juice and also on the source of isolation of the strains (Goto, 2003).

Brix was an effective factor on bacterial growth too. As increasing of brix from 6% to 12%, caused enhancement of bacterial growth. This result confirmed by Maldonado *et al*. They in their research showed that brix influence spore viability, because spore resistance increases with higher SS (Maldonado, 2005). But influence bacterial growth decrease with higher than 12% brix (Fig. 1). Reason of this result is related to decreasing of optimum content of water activity that is necessary for bacterial growth.

In regard to results, bacterial growth was observed from 35°C to 45°C. Rivera Forestier *et al* in their research showed that *A. acidoterrestris* can grow at temperatures from 20 to 55°C too (Forestier, 2009). The ability to survive high temperature is due to their unique membrane composition that possesses ώ -cyclohexane fatty acids (Forestier, 2009). Maidonado *et al*., confirmed the resistance of *A. acidoterrestris* at the assayed temperatures too. Also they showed that temperature was the parameter that had the greatest influence on the Decimal reduction time (Maldonado, 2005).

With the regard to studied environmental parameters on isolated *A. acidoterrestris* growth, this strain can be a major danger for fruit juice processors. Thus more study on using of natural compounds to prevention of this strain growth is the future project.

All obtained results in this article is validate for mentioned isolated starin. Thus, the results may be applied to the acidic food industry, especially the manufacture of fruit juice and fruit juice-containing drinks that have problem with similar microorganisms.

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