Storage of Lactic Acid Bacteria Isolated form Honey Bees in Inverted Sugar Syrup

Dz. Rozitis

Faculty of Biology, University of Latvia, Kronvalda blvd. 4, Riga, Latvia, LV-1586

Abstract*— *Honey bee hives are influenced by many different biotic and abiotic factors. Several studies demonstrate that the presence of the lactic acid producing bacteria such as Lactobacillus, Bifidobacterium, and Leuconostoc in honey bee intestinal tracts has a positive effect on a bee hive's health. Bee hives which are lacking beneficial microflora could be supplied with it through a carrier media. The aim of this study was to evaluate several concentrations of inverted sugar syrup's suitability for carrying lactic acid bacteria for possible enhancement of honey bee microflora by using lactic bacteria strains previously isolated from honey bee gastrointestinal tract in Latvian apiaries. Since sugar syrup is commonly used for feeding honey bees during the wintering it was decided to use it as a carrier media. There were five isolates of lactic acid bacteria used to test three concentrations of sugar syrup and it was found out that the inverted sugar syrup concentration of 25% is most suitable for carrying tested isolates judged by their growth dynamics and attraction from the bees.

Keywords*— *Honey bees, Lactic acid bacteria, Sugar syrup, Carrying media

I. INTRODUCTION

Health state of honey bee hives (hive interior, bee bread, bee intestinal tracts, etc.) are determined by many different biotic and abiotic factors such as good beekeeping practice, geographic location of the apiary, honey bee genetics, seasonal conditions and others. Microbial composition of the bee hives is considered as once such factor [1]. Honey bee hive can harbor a great variety of microorganisms inside it and the bees themselves are a biota of countless populations of microbes. Microorganisms associated with honey bees can be classified as pathogenic and non-pathogenic [2], and potentially beneficial [3]. While pathogenic microflora gets the most attention due to its importance on revenues, non-pathogenic and beneficial microorganisms may turn out to be as much as important. Several studies are showing that presence of lactic acid bacteria from genera *Lactobacillus*, *Bifidobacterium* and *Leuconostoc* in honey bee intestinal tracts can increase immune response of honey bee larvae [3].

Inverted sugar syrup is well known feed stuff in beekeeping. It is added to bee hives during preparation for wintering in the cold climate areas. Usually inverted sugar is dissolved in water to prepare 73-75% (w/w) solution of inverted sugar syrup and fed to bees afterwards. Sugar syrup acts as an energy source and thus ensures successful wintering for the bee hives in cold climate areas [4]. It was decided to use exactly inverted sugar syrup in this study because of its well known application in beekeeping and ability of the lactic acid bacteria to ferment it and tolerate increased levels of sugar rich media [5].

This study was focused on lactic acid bacteria survival in inverted sugar syrup and will describe a potential use of it as a carrying media for lactic acid bacteria strains previously isolated from apiaries in Latvia.

II. MATERIAL AND METHOD

2.1 Preparation of The Sugar Syrup

There were three concentrations of inverted sugar syrup (Nordzucker, Germany) solution prepared for this study. Solutions of inverted sugar syrup of 20%, 25% and 30% (w/w) concentrations were prepared by diluting commercially available syrup of 72.9% (w/w) from beekeeper's store. Obtained solutions were then filtered through 0.2 µm Sartorius Minisart® high flow polyethersulfone membrane syringe filters for a cold sterilization. There were 5 x 18 ml of each concentration of solution filtered for further use and there were in total 15 polypropylene test tubes prepared.

2.2 Preparation of Bacterial Suspensions

There were 5 strains of lactic acid bacteria from genera *Lactobacillus and Leuconostoc* used in this study to test sugar syrup's impact on their survival during storage in refrigerator for specified period of time. The bacteria strains were previously isolated from honey bee intestinal tracts from several apiaries in Latvia. A pure culture of each strain was grown on MRS agar (Biolife Italiana, Italy) at 32 ^{o}C for 48 hours prior suspending it in 18 ml of all three prepared concentrations of

sterile inverted sugar syrup solution and well mixed on eccentric rotator Microspin FV-2400 (Biosan, Latvia). Homogenous suspensions with bacterial cells were then poured in micro test tubes, a volume of 1 ml in each micro test tube. In the end there were 90 micro test tubes for each prepared sugar syrup concentration and 270 micro test tubes in total. All micro test tubes were then stored in a refrigerator at 6±2 ^{o}C for the duration of the experiment which was 4 months.

2.3 Recultivation of suspended microorganisms

There were three parallel repetitions for each strain in each concentration made for better data reliability. There were decimal dilutions made for each tested strain and they were recultivated immediately after preparation of suspensions with the purpose to determine initial cell concentration, and the rest of the samples were put in a refrigerator to be recultivated after 1 week, 2 weeks, 1 month, 2 months and 4 months. During a scheduled recultivation a portion of samples was removed from the refrigerator and left to heat up to the room temperature (approx. 22 ^{o}C). There were decimal dilutions made same as at the initial recultivation on MRS agar (Biolife Italiana, Italy). Solutions were inoculated by using spread plate technique and incubated at 32 ^{o}C for 72 hours. Then the colony forming units (CFU) were counted on the plates and a concentration of CFU/ml was calculated for each repetition.

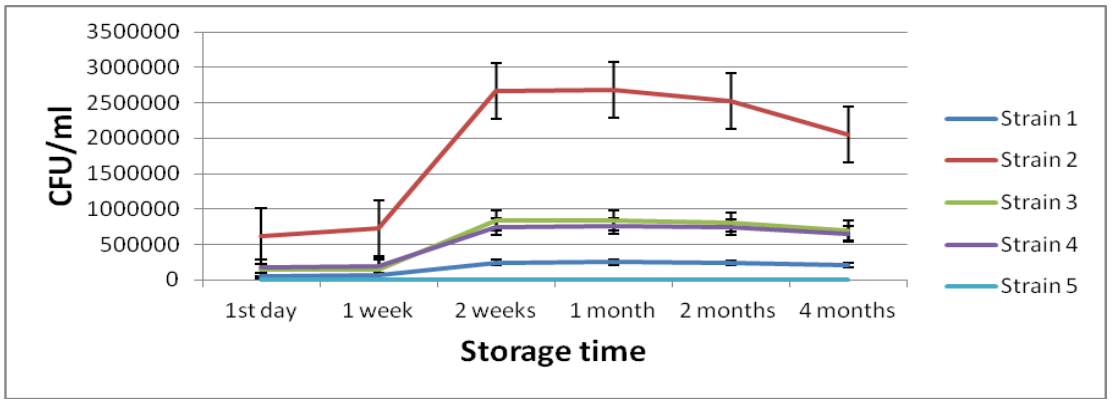
III. RESULTS AND DISCUSSION

As it can be seen in Figure 1, different concentrations of the inverted sugar syrup have a different impact on tested lactic acid bacteria strain growth and survival dynamics. An initial cell concentration measured as CFU in one milliliter of suspension differs from strain to strain since the cells were added on approximation. However, during the first two weeks of storage time in the refrigerator all strains showed an increase of CFU from initial counts and most of the growth was observed in 20% sugar syrup solution, by 6.9 times in average while in 25% solution it was 2.7 times and in 30% solution the growth was only 1.2 times in average. The “Strain 5” was the most active and increased by more than 15 times from initially prepared cell concentration in the 20% solution in 2 weeks. Meanwhile, the 30% inverted sugar syrup solution showed the least growth of the cells and during the first two weeks the cell concentration had increased by 1.2 times in average from initial concentration, while 25% was in the middle with 2.7 times of an increase.

It also can be observed in Table 1 that there is a visible decline in CFU count in all inverted sugar syrup concentrations after 2 months of storage and the concentration of 30% syrup shows CFU counts below initial state for all strains after 4 months of storage. Exception was the “strain 4” at 25% solution which still continued to increase by remarkable 47.1%.

By comparing dynamics of the lactic acid bacteria in all three concentrations it was found out that there is a statistical significance ($p < 0.05$) between their survival and growth in 25% and 30% inverted sugar syrup solutions while difference between 20% and 25% solutions can be assessed as insignificant ($p > 0.05$).

As the honey bees are attracted by as high as 75% concentrations of inverted sugar syrup it would be rational to feed them with as highly concentrated solution as possible. Therefore a significance of lactic acid bacteria survival and growth dynamics in different concentrations of the inverted sugar syrup is essential.



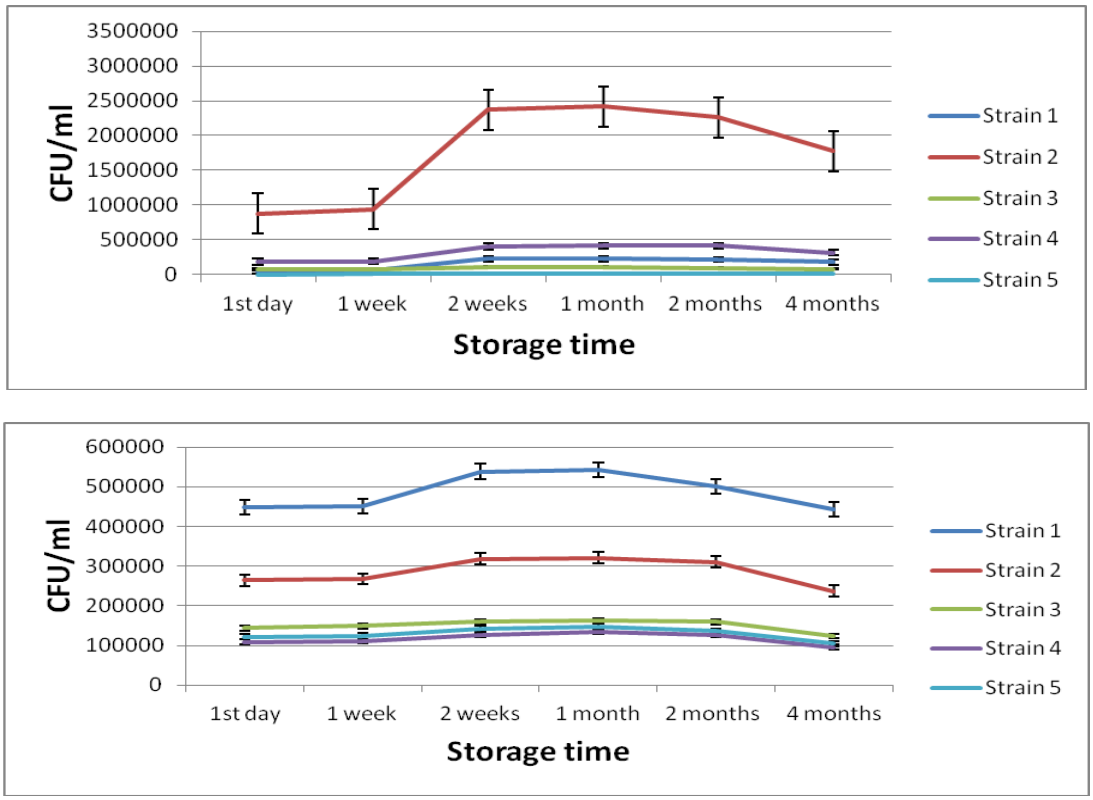


FIGURE 1: GROWTH AND SURVIVAL OF LACTIC ACID BACTERIA STRAINS IN A) 20% W/W SUGAR SYRUP SOLUTION B) 25% W/W SUGAR SYRUP SOLUTION C) 30% SUGAR SYRUP CONCENTRATION DURING STORAGE IN THE REFRIGERATOR AT 6±2 OC FOR THE PERIOD OF 4 MONTHS.

TABLE 1

AVERAGE CHANGES IN CFU/ML OF TESTED LACTIC ACID BACTERIA STRAINS IN 3 DIFFERENT CONCENTRATIONS OF INVERTED SUGAR SYRUP DURING STORAGE IN THE REFRIGERATOR AT 6±2 OC FOR 4 MONTHS.

Isolate	Concentration of inverted sugar syrup, %	Average changes in CFU/ml in percents against first day				
		1 week of storage, %	2 weeks of storage, %	1 month of storage, %	2 months of storage, %	4 months of storage,%
Strain 1	20	10.2	338.7	347.3	328.0	272.6
	25	10.3	371.4	376.4	351.7	276.9
	30	0.4	20.0	20.7	11.4	-1.2
Strain 2	20	18.9	324.8	338.0	312.0	235.8
	25	7.1	170.2	175.9	158.4	102.8
	30	1.1	20.1	21.2	17.2	-10.3
Strain 3	20	4.0	483.0	485.3	466.5	388.5
	25	4.5	53.5	56.1	35.1	8.8
	30	3.4	11.6	13.0	10.7	-14.0
Strain 4	20	17.0	335.9	342.5	333.6	277.9
	25	4.5	122.2	125.9	126.8	173.9
	30	0.9	16.3	23.0	16.3	-11.8
Strain 5	20	24.0	1442.6	1616.7	1468.6	1098.1
	25	9.7	121.1	157.6	142.6	117.2
	30	2.0	17.5	22.2	12.6	-14.1

IV. CONCLUSION

The inverted sugar syrup solution is a suitable carrier media for lactic acid bacteria previously isolated from honey bee intestinal tracts and can be used as a supplement for bee hives. A 25% concentration of inverted sugar syrup solution gives significantly better growth and survival dynamics than 30% solution while difference in 20% and 25% solutions is insignificant. Honey bees are attracted for higher sugar syrup concentrations therefore the 25% solution could be the best compromise between attraction for the bees and survival of the lactic acid bacteria over longer period of time.

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