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RESEARCH

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Beneficial effect of honeybee-specific lactic acid bacteria on health and activity of *Apis mellifera* L. colonies

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Abstract

Background Objective of this work was to investigate the impact of isolated honeybee-specific-lactic acid bacteria (Hbs-LAB) that isolated from worker's bee intestinal tracts on bee health as well as bee colony activity parameters. Independent assays were conducted from February to July, 2021 in apiary yard of Faculty of Agriculture, Cairo University, Giza, Egypt. Colonies were allocated to 2 experimental groups each of 10 colonies, Control colonies were fed with sugar syrup, while the treated colonies were fed with sugar syrup supplemented with mixture of Hbs-LAB (*Lactobacillus brevis*-HBE2, *Lactobacillus casei*-HBE5, and *Enterococcus Faecalis*-HBE1, 3 gm bacterial belts (1:1:1) mixed with 1.5 L of sugar syrup.

Results The treated colonies recorded high significant differences in all activity parameters (worker's and drone's brood areas, amount of honey and pollen stored, and no. of combs covered with bees) than controlled colonies. In addition, total protein in workers hemolymph samples of treated colonies showed more number of protein bands and the hemolymph molecular low weight protein bands was 99.24 ± 0.3, whereas the high weight protein bands reached 183.87 ± 0.3 KDa, while for untreated colonies the hemolymph molecular low weight protein bands (KDa) were 70.99 ± 0.3 and the high weight protein bands reached 171.57 ± 0.2 KDa.

Conclusions The Hbs-LAB proved to be a natural protocol that will positively impact the beekeepers' economy by providing a higher yield of bee products as well as improve the protein values in worker's hemolymph which is a superior tool for colonies resistance against several diseases that attack their hives.

Keywords Honeybee workers, Specific lactic acid bacteria, Probiotics bacteria, Bee activity parameters and protein bands

Background

Recently, managed honeybee (*Apis mellifera* L.) colonies had been sharply declining all across the world. Declines of honeybee colonies are due to colony collapse disorder (CCD), the phenomenon that may occur because of many reasons; infections, pesticides, contaminated water, antibiotic use, inadequate diet and inappropriate breeding management have all been suggested as possible reasons of these large-scale losses (Borges and Goodwin 2021). Because honeybees are not only economically important for pollination, while they are also important producers of bee products. As pressures on honey bee colonies increase, probiotics bacteria-supplemented food can shield honey bees against American and European foulbrood and aid in pathogen defense, nutrition, and environmental protection (Mishukovskaya et al. 2020; Budge et al. 2016).

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Probiotics are live microorganisms, when supplemented with food and ingested in adequate amounts, provide health benefits to the host by improving the intestinal microbial balance (Bonilla-Rosso and Engel 2018; Chernitskiy et al. 2019). Probiotics isolated from gut bacteria are frequently used in many dietary supplements and functional foods (Daisley et al. 2020; Kešnerová et al. 2020). Since antibiotics are forbidden in animal feed in many countries, natural substances are being investigated to improve animal health (Audisio 2017; Elzeini et al. 2021).

Probiotics are now widely used in a variety of animal husbandry fields. They are used to preventing and treat intestinal infections, as well as to restore the microflora of the digestive tract when antibiotics or antibacterial chemotherapeutic drugs have been used (Alberoni et al. 2016). Probiotics help to improve digestion and boost non-specific immunity (Elzeini et al. 2021; Safonov 2020; Schmidt and Engel 2016). Along with its direct pH-lowering impact, acidificants also have an antibacterial effect that stops the growth of potentially harmful bacteria and fosters the emergence of helpful species. Long-lasting benefits to honeybee longevity have been observed following relatively short probiotic supplementation periods and without the need for host colonization, demonstrating an intermittent dosing schedule that also reduced hive disturbance. The isolated lactic acid bacteria are among the probiotic microorganisms that are well known (LAB, such as Lactococcus, Lactobacillus, Streptococcus, and Enterococcus), and Bifidobacterium have a long history of safe use (Raymann and Moran 2018; Al-Ghamdi et al. 2018). Supplements with probiotics and prebiotics can raise colony productivity, and hence profits, when added to the spring stimulation feeding of bee colonies (Alberoni et al. 2016; Andreeva et al. 2018; Endo and Salminen 2013).

It is worth mentioning that research on the efficacy of probiotic bacteria extracted from the gastrointestinal tracts on honeybee colony activities is limited as a consequence. Thus, the purpose of this study was to evaluate the use of Hbs-LAB strains previously isolated from foragers honeybee workers' gut as additives to the supplementary feed for honeybee activities, honey production, collecting pollen, covered combs with bees, sealed areas of worker as well as drone brood, and the effect of Hbs-LAB on protein level in the worker's hemolymph samples.

Methods

Preparation of Hbs-LAB strains

Tested strains were previously isolated from the honeybee workers gut and identified molecularly based on the 16S rRNA gene sequences as *Lactobacillus brevis*MH191230, *Lactobacillus casei*KT273339, and *Enterococcus Faecalis* MG890204 (Elzeini et al. 2021; Gomaa and Rushdy 2014). Bacterial isolates were cultured in MRS medium at 37 °C in a 5% carbon dioxide prior to incubation for overnight (18 h). The inoculated count was adjusted at OD600 = 0.1 (10^7 CFU/mL) using spectrophotometer.

Experimental bee's colonies

Twenty honeybee colonies of local first hybrid Carniolan bees (*Apis mellifera carnica*), divided into two experimental groups each of 10 colonies, first group were used as controls and only feed with sugar syrup, and the second group was feed with Hbs-LAB1 $\times 10^6$ added to syrup. Each group approximately was at equal strength and headed by newly mated queens of the same age (Schmidt and Engel 2016).

The studies were carried out during the honeybees' active season, at the Faculty of Agriculture's apiary yard in Giza, Egypt, from February to July of 2021. Control colonies were fed with sugar syrup only, two times every week on the other hand, the treated colonies were fed with 500 ml/colony sugar syrup supplemented with mixture of Hbs-LAB (*Lactobacillus brevis*-HBE2, *Lactobacillus casei*-HBE5, and *Enterococcus Faecalis*-HBE1), two times every week and the sugar syrup concentration (125 g/L) was chosen based on previous laboratory results (Pătruică et al. 2012).

Honeybee activity parameters

During the assays, the general state of the colonies was described using the activity parameters as a guiding principle. According to Fathy et al. (2017), the following variables were noted for each examined colony both treated and untreated with (Hbs-LAB):-

- The average area of sealed worker's brood (cm^2) every 14 days intervals/colony.
- The average area of sealed drone's brood (cm^2) every 14 days intervals/colony.
- The average of stored honey (cm^2) every 14 days intervals/colony.
- The average of stored pollen and bee bread (cm^2) every 14 days intervals/colony.
- The average number of combs covered with bees every 14 days intervals/colony.

SDS polyacrylamide gel electrophoresis

The electrophoretic analysis was used to identify the changes that occurred in the protein structure in the hemolymph of honeybee workers which fed on Hbs-LAB. Hemolymph samples were collected from the treated and untreated honeybee workers by puncture of dorsal

cuticle between the 2nd and 3rd tergites using fine capillary glass tubes with EDTA (ethylenediaminetetraacetic acid) according to Yowell and Flurkey (2006). Using the Laemmli method (Laemmli 1970), the total protein concentration (mg/dl) in honeybee workers hemolymph was determined. The computerized Gel was used to analyze the protein fraction, rate of flow, and molecular weight of electrophoretically separated serum protein, reporting using Gel- Pro- Analyzer V.3.0 (Mass. Comp., Cairo-Egypt).

Statistical analysis

Data are expressed as mean ± standard deviation (SD) values using the MSTAT-C program (MSTAT 1991) version 2.10; two-way analysis of variance (ANOVA) and Duncan's multiple range tests were used to examine changes in biological parameter activity.

Results

Determine the activity parameters of honeybee colonies

The average area of sealed worker's brood (cm²/col.)

As shown in Fig. 1, there were clearly significant differences in the average area of sealed worker brood reared in the treated colonies fed with sugar syrup supplemented with Hbs-LAB than untreated colonies fed with sugar syrup only, and the control colonies were recorded 211.15 ± 5.12, 206.07 ± 6.10, 239.25 ± 3.33, 278.43 ± 3.45, 290.21 ± 2.44 and ,302.33 ± 4.10 cm^2, respectively. Whereas the treated colonies reached higher worker brood areas in February, March, April, May, June, and July months, the mean values were scored 289.30 ± 6.22, 309.70 ± 5.36, 350.30 ± 6.21, 369.30 ± 5.34, 455.01 ± 4.34, and 520.70 ± 7.32 cm^2, respectively.

The average area of sealed drone's brood (cm²/col.)

Data illustrated in Fig. 2 showed the average area of sealed drone brood reared in the treated colonies during the experimental period, the treated bee colonies obtained high value of drone brood area in April and May, 88.30 ± 3.45 and 82.30 ± 4.21 cm^2, respectively. Whereas the control colonies reached high drone brood area in June and July months, 58.70 ± 3.10 cm^2 and 62.30 ± 3.80 cm^2, respectively.

The average area of stored bee's honey (cm²/col.)

The immature and capped honey kept in the apiary was extracted and collected at the end of the flowering season (February to July). The recorded honey yields were significantly higher in the colonies that fed on Hbs-LAB. In comparison with the control hives, the amounts of stored honey were reached in control colonies, 451.10 ± 4.21 and 457.40 ± 3.32 cm^2 in June and July, respectively, whereas the stored honey area in the treated colonies was scored 533.70 ± 5.56 and 540.70 ± 6.33 cm^2 in the same months (Fig. 3).

The average area of stored pollen and bee bread (cm²/col.)

Results illustrated in Fig. 4 showed clearly significant differences in treated bee colonies with untreated colonies; untreated colonies scored lower values of pollen stored compared with treated colonies. The control colonies recorded 90.10 ± 3.21, 106.65 ± 4.33, 112.10 ± 3.56, 89.95 ± 4.22, 90.65 ± 4.01 and 78.00 ± 3.67 cm^2 in

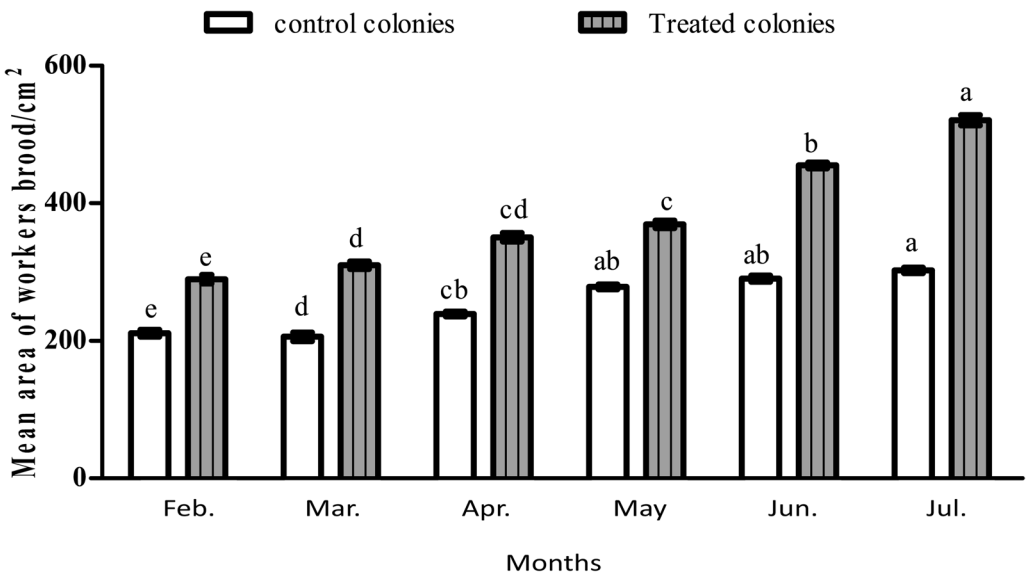

Fig. 1 The average (± SE) area of sealed workers brood/cm^2 in control and treated colonies

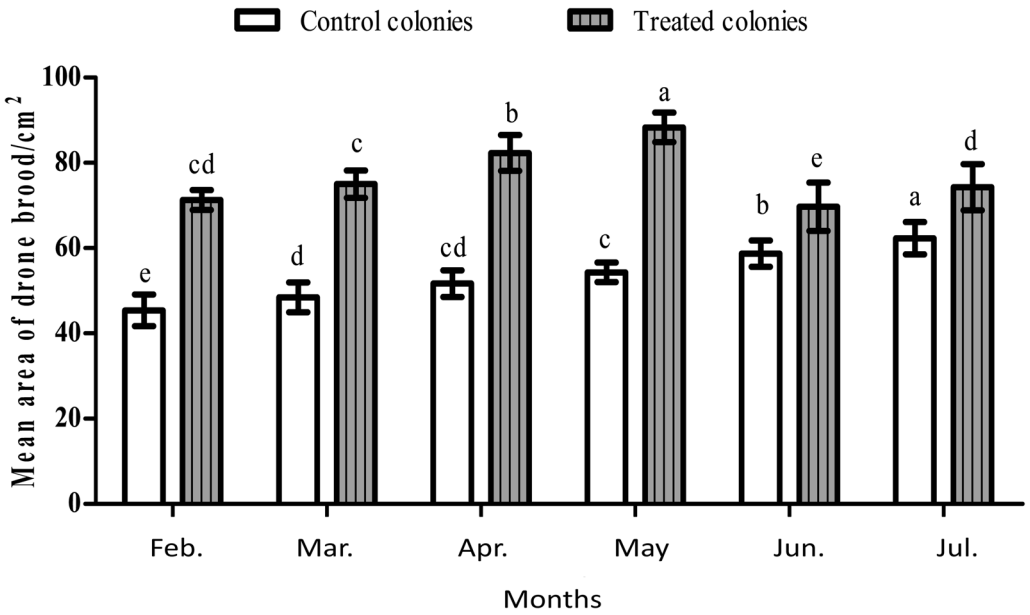

Fig. 2 The average (± SE) area of sealed drone's brood/cm^2 in control and treated colonies

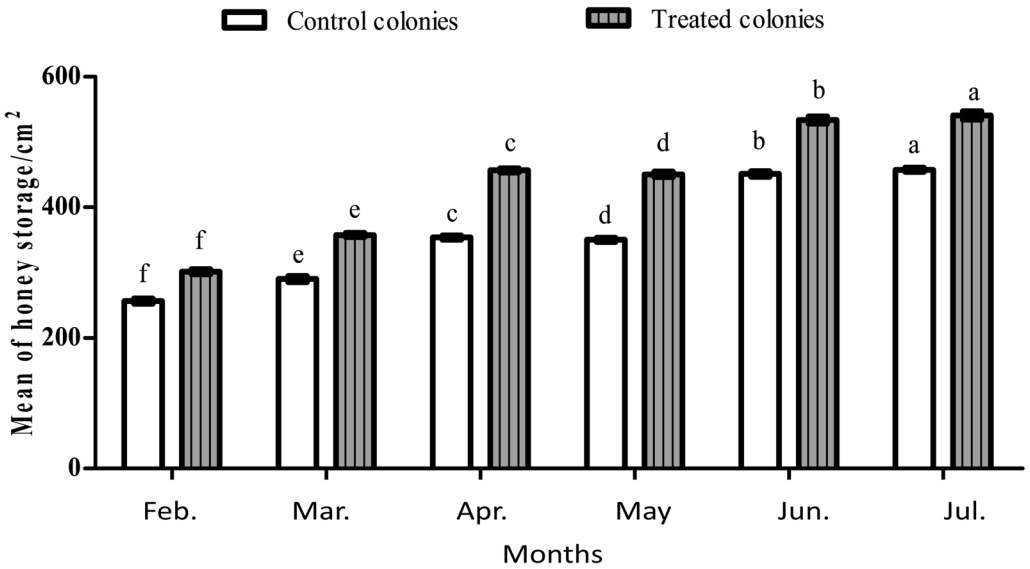

Fig. 3 The average (± SE) of stored honey /cm^2 in control and treated colonies

February, March, April, May, June, and July, respectively, while treated bee colonies scored higher values, 98.53 ± 3.25, 114.75 ± 4.01, 135.22 ± 3.43, 160.10 ± 4.21, 132.10 ± 4.23, and 143.30 ± 5.33 cm^2 in February, March, April, May, June, and July, respectively (Fig. 5).

The average no. of combs covered with bees (population density/colony)

In addition to the effect of Hbs-LAB in significantly improving the stored pollen and bee bread they also led to important modifications in the mean no. of combs covered with bees. In the treated hives, a greater mean no. of combs covered with bees was scored in May and June, 10.25 ± 0.09 and 10.33 ± 0.00, respectively. Whereas a greater mean no. of combs covered with bees in untreated bee colonies recorded 8.53 ± 0.14 and 7.30 ± 0.17 in the same months, respectively.

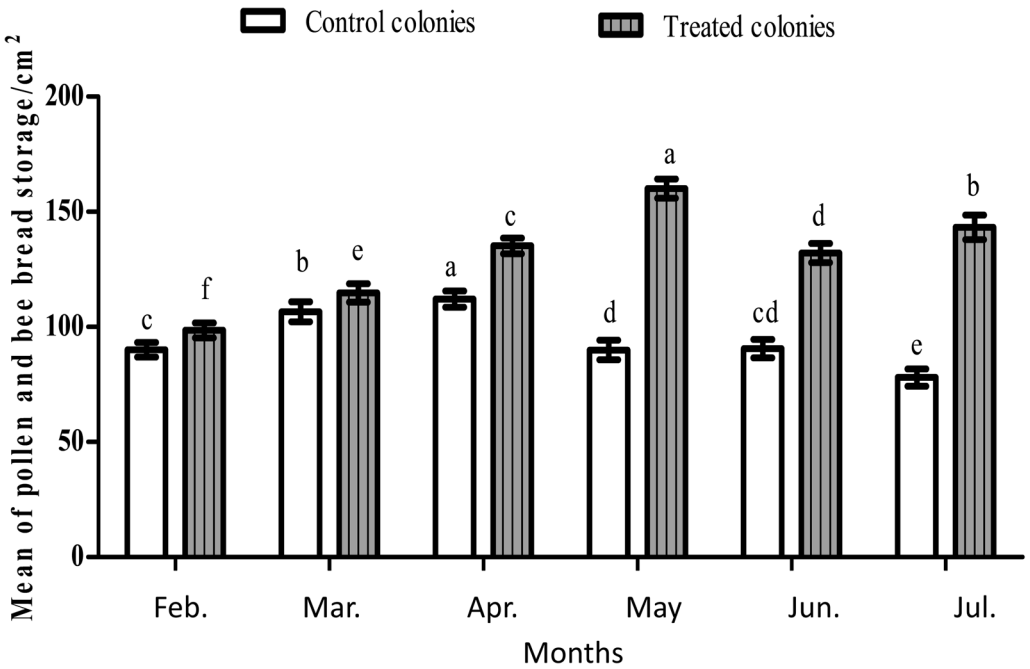

Fig. 4 The average (± SE) of stored pollen and bee bread/cm^2 in control and treated colonies

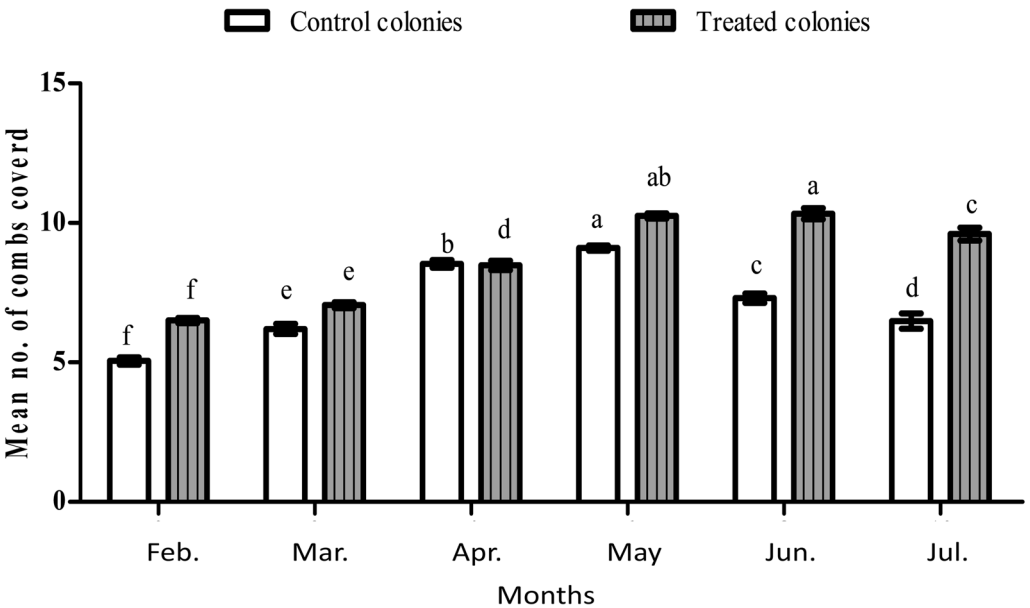

Fig. 5 The average (± SE) no. of combs covered with bees in control and treated colonies

SDS polyacrylamide gel electrophoresis

As shown in Fig. 6, there were clear differences in the hemolymph protein pattern between untreated and treated colonies; higher numbers of the total protein bands were recorded in the hemolymph of treated worker bees. The number of total protein bands was recorded in the hemolymph of untreated workers 13 bands, while in treated bee workers no. of protein band reached 16 bands.

The data in Table 1 showed that the untreated worker samples' hemolymph molecular low weight protein bands (KDa) were 70.990.3 and their high weight protein bands were 171.570.2 KDa. The hemolymph molecular low weight protein bands in treated worker samples were

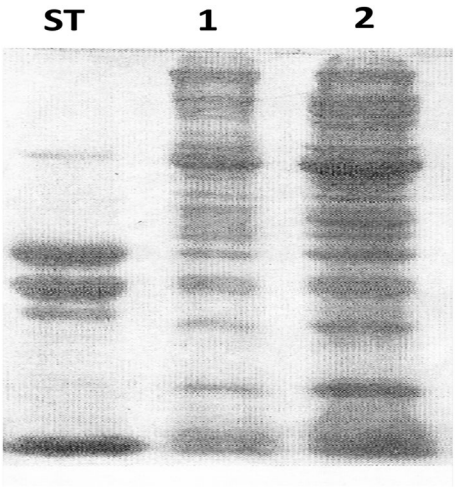

Fig. 6 Electrophoretic of total proteins concentration in untreated and treated honey bee workers hemolymph samples. St: protein standard, 1: control colonies, 2: treated colonies

99.240.3 KDa, whereas the high weight protein bands were 183.870.3 KDa.

Discussion

The use of probiotics has become need of the hour. Numerous studies insist the use for probiotics to improve workers as well as entire colony health because it is environment friendly methodology for rehabilitation of the host (Vergalito et al. 2020; Olofsson et al. 2014; Kwong and Moran 2016). The purpose of study was to evaluate the contribution of probiotics previously isolated from honeybee intestinal tract in the bee development under controlled experimental conditions. The degree of development of honeybees was judged by taking several measurements. To use a microorganism as a probiotic, it is important to determine with certainty its genus, species and subspecies, if applicable (Mustar and Ibrahim 2022; Zahoor et al. 2021). In the current study, the bacterial strains *Lb.brevis*, *Lb. casei,* and *En. faecalis* isolated from foragers bee worker intestinal tracts were used as natural supplements to beehives (Audisio 2017; Olofsson et al. 2016; Schwarz et al. 2016). Obtained data were similar with (Bonilla-Rosso and Engel 2018; Bemmo et al. 2021; Martinson et al. 2011) stated that when the bacterium was administered to the hives, the brood area was larger after 2nd month onward after application when compared with untreated hives. Honey includes a large amount of live and active LAB, which is applied to

Table 1 The hemolymph protein bands differentiation of control and treated bee workers in honeybee colonies

No. of protein bands	Molecular weight of the hemolymph protein bands (KDa)		
	Protein standard	Control bee workers	Treated bee workers
1		118.81 ± 0.3	
2		171.57 ± 0.2	183.87 ± 0.3
3		157.67 ± 0.3	156.85 ± 0.2
4			146.63 ± 0.4
5		134.92 ± 0.1	137.84 ± 0.3
6		126.58 ± 0.1	126.22 ± 0.4
7	116.00 ± 0.4	114.42 ± 0.2	135.24 ± 0.4
8			110.30 ± 0.2
9		103.93 ± 0.4	122.92 ± 0.3
10	96.73 ± 0.3	98.79 ± 0.3	110.57 ± 0.2
11		95.33 ± 0.3	122.37 ± 0.1
12			112.53 ± 0.3
13		87.44 ± 0.2	115.63 ± 0.3
14	97.18 ± 0.3	90.72 ± 0.2	125.78 ± 0.2
15		73.15 ± 0.1	106.83 ± 0.4
16	66.41 ± 0.2	70.99 ± 0.3	112.06 ± 0.2
17			99.24 ± 0.3
Total of protein bands		13	16

KDa, Unit of kilo Dalton

wounds. This is a good explanation on why honey is used to treat wounds (Heyndrickx et al. 1996; Adeniyi et al. 2015). Addition of probiotic products to feed improves colony health through intestinal colonization with beneficial bacterial (*Lb. acidophilus*, *Bifidobacterium lactis*, *Lb. casei*) compared with untreated colonies (BorgesD and Goodwin 2021). Numerous studies have shown that rising intestine secretory activity may account for the improved bee colony formation, as seen by the increased number of brood cells discovered. Furthermore, it has been demonstrated that decreasing intestinal pH results in a decline in the quantity of potentially harmful spores, colonization of the intestine with helpful bacteria, and an improvement in the efficiency of nutritional absorption from pollen and nectar. This clearly demonstrates a connection to the large rise in brood comb discovered following probiotic feeding. Given that intestinal villus development increases the surface area available for nutrient absorption and consequently improves digestive assimilation, the chance to promote its development in apicultural practice may be especially valuable. This may also improve the effectiveness of other pharmaceutical therapies added as feeding syrup additives (Borges and Goodwin 2021).The concentrations of proteins were increased in the EM 5% experimental group, 15 days after the experiment in control laboratory conditions started (Gajger et al. 2020). The results of this study are in line with past works on the effects of dietary sterols on several fitness indicators in insects (Chakrabarti et al. 2020). Treated of Hbs-LAB in bees feeding caused clear changes in the amino acids including their numbers and molecular weight, some amino acids were not found and others new were recorded in treated worker bee's hemolymph samples (Glavinic et al. 2017). One means of improving colony health involves the addition of prebiotic and probiotic products to supplementary feeds (Martinson et al. 2011; Mudroňová et al. 2011).

Conclusions

The incorporation of probiotics Hbs-LAB in honeybee's sugar syrup proves good for pollinator's overall health. It is good and environment-friendly methodology to improve the efficiency of honeybee's immunity and reproductivity. Our findings of the present study will be helpful to obtain higher storing honey and pollen yields. Future studies on the assessment of various other probiotic in colonies from other locations or other subspecies at pilot and commercial scales are needed for obtaining exact quantitative results at higher scales.

Abbreviations

Hbs-LAB Honeybee-specific lactic acid bacteria
KDa Unit of kilo Dalton
St Protein standard

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Author contributions

All authors contributed to the study conception and design. Material preparation, data collection and analysis were performed by AH and YE. The first draft of the manuscript was written by AH. All authors read and approved the final manuscript.

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Availability of data and materials

The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

Declarations

Ethics approval and consent to participate

This article does not contain any studies with human participants or animals performed by any of the authors.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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References

Adeniyi BA, Adetoye A, Ayeni FA (2015) Antibacterial activities of lactic acid bacteria isolated from cow faeces against potential enteric pathogens. Afri Health Sci 15(3):888–895. https://doi.org/10.4314/ahs.v15i3.24

Alberoni D, Gaggía F, Baffoni L, Di Gioia D (2016) Beneficial microorganisms for honey bees: problems and progresses. Appl Microbiol Biotechnol 100:9469–9482

Al-Ghamdi A, Khan KA, Ansari MJ, Almasaudi SB, Al-Kahtani S (2018) Effect of gut bacterial isolates from *Apis mellifera* jemenitica on *Paenibacillus larvae* infected bee larvae. Saudi J Biol Sci 25:383–387

Andreeva AV, Nikolaeva ON, Ismagilova ER, Tuktarov VR, Fazlaev RG, Ivanov AI, Khakimova AZ (2018) Effect of probiotic preparations on the intestinal microbiome. J Eng Appl Sci 13(S8):6467–6472

Audisio MC (2017) Gram-positive bacteria with probiotic potential for the *Apis mellifera* L. honey bee: the experience in the northwest of Argentina. Probiot Antimicrob Proteins 9:22–31

Bemmo UL, Kenfack CH, Bindzi JM, Barry RB, Ngoufack FZ (2021) Viability and in vivo hypocholesterolemic effect of *Lactobacillus plantarum* 29V in local honey. J Adv Biol Biotechnol 24:24–33

Bonilla-Rosso G, Engel P (2018) Functional roles and metabolic niches in the honey bee gut microbiota. Curr Opin Microbiol 43:69–76

BorgesD G-N, Goodwin HP (2021) Effects of prebiotics and probiotics on honey bees (*Apis mellifera*) infected with the microsporidian parasite *Nosema ceranae*. Microorganisms 9(39):481. https://doi.org/10.3390/microorganisms9030481

Budge GE, Adams I, Thwaites R, Pietravalle S, Drew GC, Hurst GGD, Tomkies V, Boonham N, Brown M (2016) Identifying bacterial predictors of honey bee health. J Invertebr Pathol 141:41–44

Chakrabarti P, Lucas HM, Sagili RR (2020) Evaluating e_ects of a critical micronutritient (24-methylenecholesterol) on honeybee physiology. Ann Entomol Soc Am 113:176–182
Chernitskiy A, Shabunin S, Kuchmenko T, Safonov V (2019) On-farm diagnosis of latent respiratory failure in calves. Turkish J Vet Anim Sci 43(6):707–715
Daisley BA, Pitek AP, Chmiel JA, Al KF, Chernyshova AM, Faragalla KM, Reid G (2020) Novel probiotic approach to counter *Paenibacillus larvae* infection in honey bees. ISME J 14(2):476–491
Elzeini HM, Ali AA, Nasr NF, Elenany YE, Hassan AA (2021) Isolation and identification of lactic acid bacteria from intestinal tract of honey bee *Apis mellifera* L. in Egypt. J Apicult Res 60(2):349–357
Endo A, Salminen S (2013) Honeybees and beehives are rich sources for fructophilic lactic acid bacteria. System Appl Microbiol 36:444–448
Fathy HM, Laila El-Batran L, Ali MAA (2017) Influence of bee hives position on brood rearing activity and stored pollenin honey bee colonies (*Apismellifera* L.). J Plant Protect Pathol Mansoura Univ 8(3):115–118
Gajger IT, Vlainic J, Šoštaric P, Janez Prešern J, Jernej Bubnič J, Škerl MIS (2020) Effects on some therapeutical, biochemical, and immunological parameters of honey bee (*Apis mellifera*) Exposed to probiotic treatments, in field and laboratory conditions. InSects 11:638. https://doi.org/10.3390/insects11090638
GlaviniciU SB, Draskovic V, Stevanovic J, Lakic N, Stanimirovic Z (2017) Dietary amino acid and vitamin complex protects honey bee from immune suppression caused by *Nosema ceranae*. PLoS ONE 12:e0187726
Gomaa EZ, Rushdy AA (2014) Improvement of *Lactobacillus brevis* NM101-1 grown on sugarcane molasses for mannitol, lactic and acetic acid production. Ann Microbiol 64(3):983–990. https://doi.org/10.1007/s13213-013-0733-7
Heyndrickx M, Vandemeulebroecke K, Hoste B, Janssen P, Kersters K, De Vos P, Logan NA, Ali N, Berkeley RC (1996) Reclassification of Paenibacillus (formerly *Bacillus pulvifaciens* (Nakamura 1984) Ash et al 1994, a later subjective synonym of Paenibacillus (formerly Bacillus) larvae (White 1906) Ash et al 1994, as a subspecies of P. larvae, with emended descriptions of P. larvae as P. larvae subsp larvae and P. larvae subsppulvifaciens. Int J System Evolut Microbiol 46:270–279
Kešnerová L, Emery O, Troilo M, Liberti J, Erkosar B, Engel P (2020) Gut microbiota structure differs between honeybees in winter and summer. ISME J 14(3):801–814
Kwong WK, Moran NA (2016) Gut microbial communities of social bees. Nat Rev Microbiol 14:374–384
Laemmli UK (1970) Cleavage of structural proteins during assembly of head of bacteriophage T4. Nature 227:680–685
Martinson VG, Danforth BN, Minckley RL, Rueppell O, Tingek S, Moran NA (2011) A simple and distinctive microbiota associated with honey bees and bumble bees. Mol Ecol 20:285–295
Mishukovskaya G, Giniyatullin M, Tuktarov V, Khabirov A, Khaziahmetov F, Naurazbaeva A (2020) Effect of probiotic feed additives on honeybee colonies overwintering. Am J Anim Vet Sci 15(4):284–290
MSTAT (1991) Version 2.10. Michigan State University
Mudroňová D, Toporčák J, Nemcová R, Gancarčíková S, Hajdučková V, Rumanovská K (2011) *Lactobacillus* sp. as a potential probiotic for the prevention of *Paenibacillus larvae* infection in honey bees. J Apicult Res 50(4):323–324
Mustar S, Ibrahim N (2022) A sweeter pill to swallow: a review of honey bees and honey as a source of probiotic and prebiotic products. Food 11(14):2102. https://doi.org/10.3390/foods11142102
Olofsson TC, Alsterfjord M, Nilson B, Butler E, Vásquez A (2014) *Lactobacillus apinorum* sp. nov., *Lactobacillus mellifer* sp. nov., *Lactobacillus mellis* sp. nov., *Lactobacillus melliventris* sp. nov., *Lactobacillus kimbladii* sp. nov., *Lactobacillus helsingborgensis* sp. nov. and *Lactobacillus kullabergensis* sp. nov., isolated from the honey stomach of the honeybee *Apis mellifera*. Int J Syst Evol Microbiol 64:3109–3119
Olofsson TC, Butler E, Lindholm C, Nilson B, Michanek P, Vásquez A (2016) Fighting off wound pathogens in horses with honey bee lactic acid bacteria. Curr Microbiol 73(4):463–473. https://doi.org/10.1007/s00284-016-1080-2
Pătruică S, Dumitrescu G, Stancu A, Bura M, Dunea IB (2012) The effect of prebiotic and probiotic feed supplementationon the wax glands of worker bees (*Apis mellifera*). Anim Sci Biotechnol 45(2):268–271
Raymann K, Moran NA (2018) The role of the gut microbiome in health and disease of adult honey bee workers. Curr Opin Insect Sci 26:97–104
Safonov V (2020) Assessment of heavy metals in milk produced by black-and-white holstein cows from Moscow. Curr Res Nutr Food Sci J 8(2):410–415
Schmidt K, Engel P (2016) Probiotic treatment with a gut symbiont leads to parasite susceptibility in honey bees. Trends Parasitol 32:914–916
Schwarz RS, Moran NA, Evans JD (2016) Early gut colonizers shape parasite susceptibility and microbiota composition in honey bee workers. Proc Natl Acad Sci 113:9345–9350
Vergalito F, Testa B, Cozzolino A, Letizia F, Succi M, Lombardi SJ, Tremonte P, Pannella G, Di Marco R, Sorrentino E (2020) Potential application of Apilactobacilluskunkeei for human use: evaluation of probiotic and functional properties. Foods 9:1535
Yowell K, Flurkey WH (2006) Effect of Freezing and microwave heating on proteins from codfish fillets: analysis by SDS polyacrylamide gel electrophoresis. J Food Sci 51(2):508–509
Zahoor F, Sooklim C, Songdech P, Duangpakdee O, Soontorngun N (2021) Selection of potential yeast probiotics and a cell factory for xylitol or acid production from honeybee samples. Metabolites 11:312

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