



## A Review on the Potentials of Honey as a Suitable Habitat for Probiotics

**Fatemeh taheri**

Department of Food Science and Technology, Gorgan University of Agricultural Sciences and Natural Resources, Gorgan, Iran

**Alireza Sadeghi**

Department of Food Science and Technology, Gorgan University of Agricultural Sciences and Natural Resources,

Gorgan, Iran

**Seid Mahdi Jafari**

Department of Food Materials and Process Design Engineering, Gorgan University of Agricultural Sciences and Natural Resources,

Gorgan, Iran

**Sara Shahryari**

Department of Food Science and Technology, Gorgan University of Agricultural Sciences and Natural Resources, Gorgan, Iran

**Maryam Zarali**

Department of Food Science and Technology, Gorgan University of Agricultural Sciences and Natural Resources, Gorgan, Iran

### Abstract

Honey, as a stressful food ecosystem, possesses high osmotic pressure, low pH, and contains prebiotic polysaccharides, making it a suitable habitat for probiotic microorganisms. Considering the undeniable effects of probiotics on human health and the evolving applications of probiotics, there is a continuous need to isolate different types of probiotics from less studied sources such as honey. In this review article, researches related to probiotic yeasts and bacteria isolated from honey are presented, and some intrinsic characteristics and techno-functional capabilities of these beneficial microorganisms were discussed. Promising characteristics like antimicrobial, antioxidant, anti-aflatoxigenic, cholesterol lowering, anticancer, and immune-modulatory activities of these isolates were also reviewed. Given these emerging capabilities, the isolated probiotic yeasts and bacteria from honey can be potentially used for various purposes in the food and pharmaceutical industries.

**Keywords:** Honey, Probiotic, Yeast, Food Industry.

## Introduction

The probiotic potential of microbial flora isolated from specific and stressful environments has been studied and confirmed. Therefore, stressful ecosystems can be a suitable source for the isolation of probiotic microorganisms. Honey is a viscous, sweet, and highly nutritious liquid produced by honeybees from the nectar of flowers, plant exudates, or secretions of sucking insects. For this purpose, nectar is collected by honeybees and after reducing its moisture and adding enzymes such as invertase and glucose oxidase, it is stored in the hive until it matures [1]. Although the composition of different types of honey varies based on the botanical and geographical origins and bee species, it generally consists of a supersaturated solution of carbohydrates such as fructose and glucose with small amounts of other compounds (flavonoids, enzymes, phenolic compounds, carotenoid compounds, amino acids, organic acids, Maillard reaction products, vitamins, and minerals), which inhibit the growth of many microorganisms [2]. Furthermore, honey is a prebiotic food containing oligosaccharides that are indigestible in the small intestine but digestible by probiotic microorganisms in the large intestine [3]. Thus, the majority of microorganisms in honey are part of the intrinsic flora, originating from pollen, the surrounding environment, or the honeybee's gut, and are introduced into the honey during its production process. Consequently, there is a high likelihood of encountering microorganisms with probiotic potential among them [4].

According to the World Health Organization-Food and Agriculture Organization definition, probiotics are live and active microbial cultures that, when consumed in sufficient amounts, have positive physiological effects on the host [5]. Some of the most important applications of probiotics for humans include nutritional benefits, protective effects against undesirable microorganisms, and maintaining the balance of gut microbiota to prevent gastrointestinal diseases and immune system disorders [6]. Since the probiotic potential of a microorganism is directly related to its ability to survive in the gastrointestinal tract, isolating a microorganism from an unprocessed and stressful environment that has also passed through the gastrointestinal tract increases the chance of encountering resistant strains. Several studies have reported the isolation of some probiotics from honey that are reviewed in the following sections.

## Probiotic Yeasts Isolated from Honey:

In 2012, Saksinchai and colleagues isolated 186 yeast strains from 37 honey samples related to 12 different bee species. After morphological and physiological studies, they successfully identified an ascomycetous yeast named *Zygosaccharomyces siamensis*, a new species of *Zygosaccharomyces* [7]. Additionally, studies by Silva and colleagues in 2020 on microbial isolates from natural honey revealed that the 55 selected isolates belonged to yeasts such as *Papiliotrema flavescens* DMKU-CE139, *Rhodotorula mucilaginosa* SM6-1, *Starmerella meliponinorum* CBS 9117, and *Saccharomyces cerevisiae* sp. Among these, strains of *S. cerevisiae* demonstrated the ability to produce ethanol and glycerol at pH levels of around 4 to 8 and temperatures ranging from 10 to 30 °C [8]. Another study in 2021 by Echeverrigaray and colleagues on honey from 17 different bee species found that yeast populations varied among different honey types, with the highest diversity observed in honey samples with higher water activity. The predominant yeast genera were *Zygosaccharomyces* and *Starmerella*, and the presence of *Wickerhamomyces sydowiorum* in honey was reported for the first time [9]. Additionally, studies conducted by Ziuzia and colleagues on yeasts isolated from lemon honey samples in 2023 in Poland identified 15 selected isolates belonging to species such as *Yarrowia lipolytica*, *Candida magnoliae*, and *Starmerella magnoliae*. According to the screenings, the best producers of beneficial compounds were strains of *Y. lipolytica*, which were capable of producing significant amounts of erythritol and citric acid [10]. Research on three honey samples collected from western Thailand yielded 104 yeast isolates, with 58 showing the ability to grow in acidic pH of 2 to 2.5, resist temperatures of 37 °C, and withstand 3% w/v bile salt concentrations. Genetic analysis using specific primers and sequencing of the internal transcribed spacer (ITS) region revealed that four isolates belonged to *S. cerevisiae*, all of which had the ability to produce acetic acid as an antimicrobial compound. Additionally, 10 isolates belonged to *Meyerozyma guilliermondii* strains, which demonstrated the ability to produce a significant amount of xylitol [11].

A study by Khalafalla and colleagues in 2019 in Egypt on the microbial flora of honeybee guts at different life stages identified 128 microbial isolates, among which yeasts *Zygosaccharomyces mellis* MK005880, *Lachancea thermotolerans* MK000703, and *Wickerhamomyces anomalus* MH997572 showed high potential for use as probiotic cultures. These yeasts were resistant to acidic pH and the presence of bile salts, exhibited suitable antimicrobial activity against one or more human pathogens, lacked hemolytic activity, showed high antibiotic resistance, and demonstrated suitable anti-cancer activity for inhibiting colon cancer [12]. In 2019, Tauber and colleagues, after

isolating *W. anomalus* CBS 262 from the honeybee gut microbiota, studied its effect on modulating the honeybee gut microbiome and their immunity. The researchers observed that gene expression related to immunity in honeybees fed with a sugar solution containing the yeast was reduced compared to the control group fed only with the sugar solution. Furthermore, they reported that *W. anomalus* CBS 262, by producing acid, created conditions favorable for *Lactobacillus* bacteria, thereby reducing the growth of the pathogenic *Nosema ceranae* and modulating the honeybee gut microbiome [13]. In another report, yeasts such as *Starmerella bombicola* CBS 9710, *C. magnoliae* CBS 2677, *Pichia guilliermondii* W1171, *Aureobasidium* sp., *Rhodotorula* sp., *Curvibasidium* sp., and *Metschnikowia* sp. isolated from bee pollen by Chelucci and colleagues in 2023 showed good antioxidant and anti-inflammatory activity, making them potential candidates for probiotic use [14]. Research on palm sap in 2017 yielded 15 yeast isolates, among which *S. cerevisiae* OBS2 and *Pichia kudriavzevii* OBS1 showed suitable viability under simulated gastrointestinal conditions. Additionally, these isolates exhibited antibiotic resistance and good adhesion properties. *S. cerevisiae* OBS2 also demonstrated antioxidant and anti-cancer capabilities [15].

### Probiotic Bacteria Isolated from Honey:

In 2022, Melia and colleagues isolated a group of lactic acid bacteria from natural honey and evaluated them for probiotic characteristics. According to their research, *Lactobacillus plantarum* SN13T showed the highest survival under simulated gastrointestinal conditions. Additionally, this bacterium exhibited suitable antibacterial effects against all studied pathogenic bacteria. Furthermore, *L. plantarum* SN13T had suitable potential for producing probiotic fermented milk [16]. Ebrahimi and colleagues in 2020 isolated *Lactobacillus kunkeei* ENH01 from natural honey and evaluated its probiotic potential. The results showed that the bacterium was resistant to successive acid and bile salt treatments. Moreover, it lacked hemolytic activity and exhibited good antimicrobial effects against *Escherichia coli* and *Aspergillus niger*. Additionally, both live and heat-killed forms of this bacterium showed good anti-aflatoxinigenic potential [17].

Studies on natural honey samples revealed that various strains of *Gluconobacter oxydans* isolated from these samples showed good resistance under simulated gastrointestinal conditions and had high auto-aggregation and hydrophobicity. Furthermore, these isolates were resistant to antibiotics like penicillin and rifampicin. Some strains of this bacterium also had the ability to reduce cholesterol and produce siderophores [4]. Studies conducted by Xie and colleagues in 2021 on honey from different regions of the United States revealed that the number of probiotic bacteria was higher in honey with higher moisture content, and the diversity of bacterial strains was related to the floral origin of the honey [18]. Research on honey samples from different regions of Thailand in 2022 identified *Lactobacillus* and *Bifidobacterium* species as the predominant lactic acid bacteria in these samples. Furthermore, *Lactobacillus johnsonii* CECT 5480 isolated from the honeybee gut exhibited good resistance under simulated gastrointestinal conditions and had suitable potential for adhesion to human gut epithelial cells [19]. A study on the probiotic potential of *L. plantarum* MEP3 and *Fructobacillus fructosus* AREP6 isolated from honey showed that this bacterium was resistant to acidic pH, bile salts, and oxidative stress, making it suitable for probiotic use. Furthermore these bacteria demonstrated antimicrobial activity against pathogens like *E. coli* O157:H7 UFG77, *Staphylococcus aureus* UFG141 and *Listeria monocytogenes* CECT 4031 [20].

### Conclusion:

In conclusion, honey serves as a remarkable habitat for a variety of microorganisms, many of which exhibit probiotic properties. The diversity of probiotic yeasts and bacteria isolated from honey underscores the potential for utilizing these microorganisms in food and pharmaceutical applications. Further research and exploration of honey as a source of probiotics could lead to new opportunities for enhancing human health through the development of novel probiotic products.

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