

Microbial Diversity of Human Milk and Infants

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Abstract

A wide variety of microbial communities found in human milk have positive benefits on health, including colonisation and gut development in young children. In this study, we described the bacterial communities in the Egyptian mother-infant pairs throughout the first year of life while they were breastfed normally. Forty-one isolates were chosen for probiotic potential out of one hundred isolates. The physical and biochemical profiles of the chosen isolates were created. Based on the 16S rRNA gene sequence and phylogenetic trees between the isolates' sequence and the closest sequences in the database, the taxonomic evidence of these isolates was examined. The isolates were identified as belonging to three genera: *Lactobacillus*, *Enterococcus* and *Lactococcus* based on taxonomic and biochemical evidence. With a high incidence of its many species, the genus *Lactobacillus* was the most prevalent one in samples of human milk and faeces (*Lactocaseibacillus paracasei*, *Lactobacillus delbrueckii*, *Lactiplantibacillus plantarum*, *Lactobacillus gasseri* and *Lactocaseibacillus casei*). Interestingly, BlastN and Jalview alignment findings showed that six isolates had poor identity ratios with database sequences (less than 95%). The distinct physiological, biochemical and probiotic characteristics of these isolates substantiated this differentiation. The isolate *L. delbrueckii*, ASO 100, which had the best probiotic and antibacterial characteristics and the lowest identity ratio, is highly likely to be a new species. Probiotic tests and ultrastructural analysis were performed on nine isolates and the results showed that these isolates had a high potential for use as preventative and therapeutic agents in the treatment of intestinal pathogens due to their antibiotic resistance and antibacterial activity as well as their high probiotic characteristics.

Keywords: Human milk • Probiotic bacteria • 16S rRNA gene

Introduction

The prophylactic and therapeutic benefits of human milk have been demonstrated by the American Academy of Pediatrics in the development of infants' immune systems and in protecting them from a wide range of infectious diseases, including gastrointestinal, respiratory, inflammatory bowel and allergic diseases [1,2]. The coordinated action of a number of bioactive substances, including oligosaccharides, fatty acids, immunoglobulins, cytokines, immune cells, lactoferrin, immunomodulating factors and healthy microbial communities is what gives breast milk its protective properties [3].

When an infant is delivered vaginally, breast milk serves as the second major source of infant germs after the birth canal [4]. 800 mL of breast milk is thought to provide a newborn with between 10⁵ and 10⁷ commensal bacteria each day. The neonatal gut microbiota begins to form, develops and is composed in a unique way thanks to human breast milk. A wide variety of bacterial species, including helpful, commensal and perhaps probiotic bacteria, can be found in the human milk microbiome [5].

According to the Food and Agriculture Organization (FAO) and the World Health Organization (WHO), probiotics are live bacteria that, when taken in suitable amounts, boost the host's health [6]. The ability to colonise and predominate in the neonatal gut, resistance to stomach acid and bile salts, adherence to the intestinal mucosa, induction of anti-inflammatory responses, inhibition of pathogens by the production of antimicrobial constituents and enhancement of the immune system are just a few of the many advantageous

traits of probiotic bacteria [7,8]. Probiotics and their purified molecules will be used as a sensible, secure substitute for medication and other treatments to manage health imbalances and diseases in humans and animals. This will usher in a new era of therapeutics. Accordingly, a number of studies have documented the benefits of probiotics in the prevention and management of inflammatory bowel disease, food hypersensitivity, cardiometabolic diseases and anticancer activity.

Previous research has demonstrated the abundance of new probiotics in human milk and stool samples. According to the inquiry and report, Korean mother-infant couples' milk and excrement samples contained unique strains of *Lactobacillus gasseri* E.J.L and *Bifidobacterium breve* J.T.L. In a recent intriguing study, novel LAB bacterial strains from Chinese infants were found to have potential probiotic properties against inflammatory and oxidative stress-related human diseases. These strains belonged to the genera *Lactobacillus gasseri*, *Lactiplantibacillus plantarum* and *Lactocaseibacillus rhamnosus*.

Infants' gut microbiota and, by extension, the microbial diversity of human milk are influenced by environmental, demographic and genetic factors, as well as maternal lifestyle. To gain fresh insights into customised therapy, it is crucial to bioprospect the gut microbiota and choose potential probiotic candidates. We search for such healthy probiotics from Egyptian groups with distinctive immune systems as part of the current inquiry into potential future approaches to treating chronic diseases. A morphological, biochemical and ultrastructural profile of selected bacterial isolates was created. Additionally taken into account were probiotic tests, antibiotic sensitivity and antibacterial activity. The 16S rRNA gene sequence and phylogenetic tree analyses were used to show that these isolates have taxonomic evidence.

Isolation and identification of lactic acid bacteria

Only 41 gram-positive, catalase-negative and non-endospore forming lactic acid bacterial isolates were selected from a total of 100 bacterial isolates. All of the cocci isolates tested positive for microaerophilic or facultative anaerobic conditions. Only 14 of the isolates were determined to be cocci-shaped bacteria under microscopic examination, while 39 were determined to be rod-shaped bacteria.

Molecular identification

The selected 41 isolates' 16S rRNA gene amplified PCR products were

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sequenced and added to the NCBI database. The isolates' accession numbers show how closely related their sequences are to those already deposited in the database. Three genera were identified through pairwise sequence alignment: *Lactobacillus*, *Enterococcus* and *Lactococcus*. Human milk and faeces included large concentrations of the *Lactobacillus* species *L. paracasei*, *L. delbrueckii*, *L. plantarum*, *L. gasserii* and *L. casei*. Four isolates belonged to *L. delbrueckii*, while fifteen separate isolates belonged to *L. paracasei*. Only two isolates of each species of *L. plantarum*, *L. gasserii* and one strain of *L. casei* were found, though. Twelve isolates from the species *E. faecium*, *E. faecalis* and *E. lactis* showed that the genus *Enterococcus* also had a significant incidence.

Probiotic characteristics

For the purpose of focusing on their probiotic and ultrastructural traits, nine isolates from various species were chosen. All of the tested isolates exhibited acid tolerance, thrived well and were unaffected by a pH drop from 6 to 3 for 4 hours following incubation. All isolates maintained viable cell counts more than 6-7 Log CFU mL⁻¹. It was discovered that *L. lactis* ASO 26's cell count slightly decreased at pH 3. For 4 hours at pH 3, relative log CFU mL⁻¹ growth for *L. delbrueckii* ASO100, *L. paracasei* ASO32 and *L. plantarum* ASO50 were measured. This test revealed that all isolates, with the exception of *L. lactis* ASO 26, were resistant to low pH.

All isolates grew well in the presence of 0.5% bile salts during incubation at 4 h, with the exception of *L. lactis* ASO 26, which showed a decreased profile during the incubation duration, according to the bile salt (ox-gall bile salts 0.5%) tolerance test. The maximum bile salts tolerance, however, was shown by *L. delbrueckii* ASO100, *L. paracasei* ASO32 and *L. plantarum* ASO50, respectively.

Discussion

In this study, we look for fresh, powerful probiotics in Egyptian mothers' breast milk and infants' stool samples. The novel components of our study include the first investigation of the diversity of the human milk microbiota from Egyptian samples and the isolation of novel probiotics from Egyptian infants' faeces with a distinct immune system as preventative and therapeutic agents for managing chronic diseases. Lactic acid bacteria should be able to endure harsh conditions in the human body (intestinal juice, low pH and salivary enzymes) to maintain gut microbiota balance, immune homeostasis and monitor beneficial physiological roles in human health, just as they can withstand pathogens and reactive oxygen species (ROS) associated with gut diseases. In this study, we look for fresh, powerful probiotics in Egyptian mothers' breast milk and infants' stool samples. The novel components of our study include the first investigation of the diversity of the human milk microbiota from Egyptian samples and the isolation of novel probiotics from Egyptian infants' faeces with a distinct immune system as preventative and therapeutic agents for managing chronic diseases.

Lactic acid bacteria should be able to endure harsh conditions in the human body (intestinal juice, low pH and salivary enzymes) to maintain gut microbiota balance, immune homeostasis and monitor beneficial physiological roles in human health, just as they can withstand pathogens and reactive oxygen species (ROS) associated with gut diseases. These findings were consistent with a previous study, which isolated 27 gram-positive, catalase-negative bacteria from healthy infant faeces and demonstrated that they had a negative profile toward mannitol and sorbitol. ASO57, ASO55, ASO5 and ASO100, interestingly, did not produce acid from xylose, raffinose, or trehalose like the other isolates, indicating that these isolates had distinct profiles and perhaps different probiotic properties.

The newly discovered LAB isolates could be used as effective and competitive probiotic tablets for controlling intestinal pathogens and human disorders because they showed high acid and bile salt tolerance, antibiotic resistance and antibacterial activity. Interestingly, the results of pairwise sequence alignment showed that six isolates had low identity ratios (less than 95%) and a high likelihood of being new species. The isolate *L. delbrueckii*, ASO 100 will be subject to complete proteomic analysis to stand for its probiotic

determinants, as it revealed the most brilliant probiotic and antibacterial features. Additionally, another in vivo experiment will be conducted to test the prophylactic and therapeutic ability of this isolate to modulate gut-brain axis microbiota in an A-type transgenic mouse.

Conclusion

In this work, we searched for novel Probiotic isolates by screening human breast milk and newborn stool samples from Egyptian sources. Through physiological and biochemical tests, 41 isolates were submitted to the gene bank database, categorised and identified. Antibiotic resistance, antibacterial activity and strong probiotic characteristics were found in representative samples from the various species. Six of our isolates showed average nucleotide identities to other sequences deposited in the database that were less than 95%. With the lowest identity ratio and promising probiotic and antibacterial characteristics, the isolate *L. delbrueckii*, ASO 100 strongly suggests that it is a novel probiotic species.

Acknowledgement

None.

Conflict of Interest

None.

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