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Bacteria, Viruses, Membrane-Enclosed Microentities and Fungi as the Environmental Evolutionary Entities Coexisting in Non-Human Mammalian Milk §

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Abstract

The revival of studying symbiotic bacteria in the human body in the 1990s and the application of culture-independent approaches in research on microorganisms of non-human animals have greatly enriched our understanding on the biological, ecological and evolutiological relationship between macroorganisms and microorganisms. In the theoretical UPOEE model, a novel concept called "Evolutionary Background Entities (EBE)" was proposed for referring to those entities of lower evolutionary levels that are the evolutionary "background entities" of the entities at higher evolutionary levels.[1] In addition, in the recent paper,[2] the following notion has been proposed: (i) animals are not only inhabitants of natural habitats, but also are the "niches" or "habitats" of evolutionary micro-entities including bacteria, viruses, and fungi; and (ii) the interaction between an evolutionary entity and its environment is actually the interaction between the entity and its environmental evolutionary entities at the same and/or different evolutionary levels. As a natural food, milk is one of the primary environmental factors that newborns of mammals have to contact; and therefore, from the evolutionary perspective, it is necessary to answer the question: what are the environmental evolutionary entities in non-human mammalian milk? Here, I briefly review prokaryotic bacterial cells, viruses, membrane-enclosed microentities, eukaryotic fungal cells as the environmental evolutionary entities coexisting in milk of non-human mammals.

Key words: Mammals; Milk; Breastmilk; Breastfeeding; Bacteria, Viruses; Membrane-enclosed microentities; Exosomes; Microvesicles; Fungi; Eukaryote; Prokaryote; Immune; Evolution; Symbiotic bacteria; Bacterial diversity; Pasteurization

1. Background

Since the revival of studying symbiotic bacteria in the human body in the 1990s, increasing evidence has revealed that bacterial communities exist not only in normal human milk,[3-7] but also naturally in milk of non-human mammals including goats or ovine,[8-10] cow or buffalo,[10-16] and camel.[10]

Traditionally, some types of non-human mammalian milk have been chosen as the best substitute of human milk to nourish human neonates,[17] or as an ingredient used in various nutritional foods for children and adults.[15] Existing evidence has revealed that mammalian milk, such as cow milk, sheep milk, and donkey milk, normally contains not only various eukaryotic cells but also harbors many prokaryotic cells and subcellular micro-entities such as bacteria, viruses, and microvesicles.

During the past several months, the following novel theoretical identifications have been proposed: (i) evolutionary entities of lower evolutionary levels that are the evolutionary background entities of the entities at higher evolutionary levels;[1] (ii) animals are not only inhabitants of natural habitats, but also are the niches or habitats of evolutionary micro-entities including bacteria, viruses/phages, and fungi;[2] and (iii) the interaction between an evolutionary entity and its environment is actually the interaction between the entity and its environmental evolutionary entities at the same and/or different evolutionary levels.[2]

Clearly, our traditionally held nutritional knowledge cannot account for the complex interaction between the human body and non-human mammalian milk at different evolutionary levels. In fact, accumulating evidence has indicated that non-human mammalian milk and its relevant dairy foods supply us not only with molecular compounds including carbohydrates, fats, and proteins, but also subcellular and cellular entities. For instance, ruminal bacteria are normal inhabitants in the rumen of ruminant animals, including the cow, [18] and are detectable in cow milk;[15] and however, these ruminococcal species were even detected in human neonates' feces by culture-independent molecular approaches once they consumed cow milk or its relevant food products.[19]

It has been demonstrated that dendritic cells can penetrate the gut epithelium to take up noninvasive bacteria directly from the gut lumen.[20,21] Therefore, live bacteria, including both noninvasive and pathogenic, and even viruses can spread to other locations, such as the lactating mammary gland through the circulation of lymphocytes within the mucosal-associated lymphoid tissue and blood. Nevertheless, the phenomenon described by Favier and colleagues suggested that the most possible migration route for ruminococcal species may be from cow's rumen to cow's milk, then to the human baby's gut.

Moreover, the composition of the bacterial community in non-human mammalian milk, such as cow milk, was shown to have biogeographic characteristics,[15] which suggested that as an environmental factor of a suckling progeny, milk itself is also an evolutionary entity that is inevitably affected by its external factors within the maternal host body at the molecular, subcellular, and cellular levels.

The consumption of raw non-human mammalian milk without pasteurization is strongly discouraged from a medical perspective over worries about the possible existence of pathogenic bacteria in unpasteurized milk.[22] However, in most pasteurized milk, some bacterial microorganisms in milk remain alive. Therefore, in fact, microorganisms in pasteurized milk are a mixture of living and dead. For example, approximately 2% of pasteurized cow milk samples tested culture-positive for Mycobacterium paratuberculosis [23] and Listeria monocytogenes that were still alive in post-pasteurization processing environments.[24]

Despite the fact that modern humans are more dependent on non-human mammalian milk than ever before, as the advantage and disadvantage of human milk and breastfeeding have been gradually elucidated in biology, ecology, and evolutionary biology during the past decades, there are still many unknowns in non-human mammals' milk, which require more attention. On the other hand, each mammal species produces its own milk for its suckling progeny. Milk, as a natural food, is one of primary environmental factors that newborns of mammals have to contact; and therefore, from the evolutionary perspective, it is necessary to answer the question: What are the environmental evolutionary entities in non-human mammalian milk? In this paper, based on accessible literature, I briefly review bacterial cells, viruses, membrane-enclosed microentities, and fungal cells as the environmental evolutionary entities in milk of several selected non-human mammal species.

2. Cow Milk

2.1 Bacterial microorganisms

Traditionally, the presence of bacteria in raw milk was attributed to external contamination from air, milking equipment, feed, soil, feces and grass.[13] However, it is now an undisputed fact that diverse bacterial species, both pathogenic and non-pathogenic, naturally exist in raw cow milk. This has been conformed by conventional bacterial culture analysis and culture-independent molecular approaches.[11-13,15,16,25]

Recently, Oikonomou and colleagues showed that four bacterial genera: Faecalibacterium, Lachnospiraceae, Propionibacterium and Aeribacillus were the core bacterial species because they were detected in every milk sample.[25] In fact, commercial cow milk is usually pasteurized and according to European Commission legislation, raw cow milk requires minimum pasteurization at 72°C for 15 seconds.[23] Low pasteurization is at 60 °C for 30 min.[26] and heat treatment at 120 °C for 20 min is the classic sterilization.[26] Lindstrom and colleagues pointed out that "the standard milk pasteurization treatment does not eliminate spores, and the intrinsic factors of many dairy products allow botulinal growth and toxin production."[27]

Staphylococcus

Many staphylococci species in cow milk have been isolated, among which, Staphylococcus aureus and coagulase-negative staphylococci are predominant.[28-31] Pulsed-field gel electrophoresis (PFGE) showed that many different types of Staphylococcus epidermidis exist in cow milk samples.[32] Zadoks and colleagues found that Staphylococcus aureus from bovine teat skin and human skin were the same pulsotypes, and were not an important source of intramammary Staphylococcus aureus infections in dairy cows.[33] Pangallo and colleagues reported that Staphylococcus aureus, Staphylococcus epidermidis, Staphylococcus felis, Staphylococcus pasteuri, Staphylococcus sciuri, and Staphylococcus Xylosus were detected in May bryndza cheese.[34] Methicillin-resistant Staphylococcus epidermidis isolates were also found in raw bovine milk.[35] And more recently, methicillin-resistant Staphylococcus aureus (MRSA) strains were isolated from bovine milk with mastitis, and were shown to be multi-drug resistant to erythromycin, clindamycin, chloramphenicol, gentamicin, tetracyclin, ciprofloxacin and vancomycin.[36] Methicillin-resistant Staphylococcus aureus strains from bovine milk were identical or similar in geno- and serotypes to those of human MRSA isolates in Japan.[37] In addition, methicillin-resistant coagulase-negative staphylococci (MR-CNS) were also detected in 46.4% of cow milk samples.[38]

Streptococcus

Streptococcus dysgalactiae, Streptococcus uberis, Streptococcus macedonicus, and Streptococcus thermophilus are the predominant streptococci in cow milk and cheese.[28,39-40] Streptococcus equi subspecies zooepidemicus, a group C streptococcus, is very rare in humans but commonly found in cow milk.[41] Streptococcus parauberis and Streptococcus thermophilus were detected in May bryndza cheese.[34]

Streptococci and Staphylococci: the Seasonal Effect

Concentrations of many milk components, such as total protein, fat, casein and whey protein, were found to have seasonal and lactational characteristics.[42] The seasonal effect of bacteria in cow milk was first described by Osteras and colleagues in 2006.[28] They found that while the prevalence of Staphylococcus aureus in milk decreased throughout days, the prevalence of Streptococcus dysgalactiae increased.[28] In April and May, Streptococcus dysgalactiae and coagulase-negative staphylococci in cow milk were shown to have the highest prevalence; in contrast, in June and July, bacterial species with the highest prevalence in cow milk were Staphylococcus aureus and Streptococcus uberis.[28]

Mycobacterium

Mycobacteria are intracellular microorganism. Mycobacterium species including Mycobacterium bovis, Mycobacterium africanum, and atypical mycobacteria were isolated from unpasteurised cow milk samples in north-central Nigeria [43] and in the Southern Highlands to Tanzania.[44] The number of Mycobacterium avium subsp. paratuberculosis cells in cow milk can be as high as 10^6 to 10^7 CFU/ml.[45]. Mycobacterium avium subsp. paratuberculosis was also detected in meat and drinking water.[46] Grant and colleagues showed that even at 73°C for 15 or 25 seconds, M. paratuberculosis was still capable of surviving if the bacterium was in sufficient numbers before heat treatment.[23] Therefore, in fact, Mycobacterium paratuberculosis was still detectable in some pasteurized cow milk.[23,47] Mycobacterium avium subspecies paratuberculosis is believed to be the etiologic pathogen of Johne's disease (JD), a systemic infection and chronic inflammation of the intestine in animals, and is closely associated with Crohn's disease, a systemic disorder mainly characterized by chronic inflammation of the intestine in humans.[48,49]

Lactobacillus

Lactobacillus plantarum, Lactobacillus paracasei, Lactobacillus fermentum, Lactobacillus curvatus, Lactococcus lactis, Lactobacillus casei, Lactobacillus helveticus, and Lactobacillus rhamnosus were found in cow milk and/or cheese.[31,34,39,40,50] Some strains of Lactobacillus casei, Lactobacillus reuteri, and Lactobacillus plantarum in cow milks from the summer/fall period were shown to have their antifungal activity against Penicillium expansum, Mucor plumbeus, Kluyveromyces lactis, and Pichia anomala.[41]

Escherichia coli

Escherichia coli were detected in bovine milk and cheese.[31,34,54] Shiga toxin-producing Escherichia coli (STEC) O26: H11, O103: H2, O111: H8, O145: H28 and O157: H7 were detected in raw milk and cheeses.[14,53,54] Verocytotoxin-producing Escherichia coli O26 was detected in raw water buffalo (Bubalus bubalis) milk;[14] and this strain was associated with outbreaks of gastroenteritis, hemorrhagic colitis, and hemolytic uremic syndrome in the United States and Europe.[55]

Other bacteria

Many other bacterial species belonging to different genera were also detected in raw milk and cheeses. These genera included Acinetobacter, Alcalignes, Arcobacter, Bacillus, Bifidobacterium, Brevibacterium, Brucella, Citrobacter, Clostridium, Corynebacterium, Coxiella, Enterobacter, Enterococcus, Haemophilus, Hafnia, Helicobacter, Leuconostoc, Lactococcus, Mannheimia, Pediococcus, Proteus, Pseudomonas, Psychrobacter, Raoultella, Salmonella, Vagococcus, Variovorax, and Weissella.[10,13,31,34,39,40,56-63]

2.2 Fungi

Recently, studies on fungal species have revealed that there is a diverse group of fungal species in cow milk and cheese. Fungal species, including yeast species and mold species in cow milk and cheese, belong to different genera which included Alternaria, Aspergillus, Beauveria, Candida, Chrysosporium, Cladosporium, Cryptococcus, Debaryomyces, Engyodontium, Fusarium, Geotrichum, Galactomyces, Gymnoascus, Issatchenkia, Kazachstania, Kluyveromyces, Malassezia, Penicillium, Pichia, Rhodotorula, Saccharomyces, Torrubiella, Torulaspora, Trichosporon and Yarrowia.[34,39,64,65]

2.3 Viruses

Torque teno viruses (TTV) were detected in the milk of water buffaloes (Bubalus bubalis) in spite of the authors' belief that its presence could be due to human contamination.[66]

2.4 Microvesicles

Microvesicles with approximately 100 nm in diameter were isolated from bovine milk.[67-69] Exosomal microRNA-21 in cow milk may promote metabolic processes by affecting mechanistic target of rapamycin complex 1 (mTORC1) signaling.[70]

3. Goat or Sheep Milk

3.1 Bacterial microorganisms

Staphylococcus

Staphylococci including Staphylococcus aureus, Staphylococcus epidermidis, Staphylococcus simulans, Staphylococcus caprae, Staphylococcus equorum, and Staphylococcus saprophyticus were isolated from goat and/or sheep’s milk and cheeses.[9,30,71-74]

Streptococcus

Streptococcus equi subspecies zooepidemicus, a group C streptococcus is very rare in humans and is generally present in goat cheese.[41,75]

Lactobacillus

Lactobacillus plantarum and Lactobacillus paracasei were found in Spanish farmhouse goat's milk cheeses.[8,76] Some strains of Lactobacillus casei, Lactobacillus reuteri, and Lactobacillus plantarum in goat milks from the summer/fall period have been shown to have antifungal activity against Penicillium expansum, Mucor plumbeus, Kluyveromyces lactis and Pichia anomala.[51]

Lactococcus

Lactococcus species including Lactococcus lactis and Lactococcus garvieae were isolated from goat milk.[8,9,77] Several strains of Lactococcus in raw goat milk were found to have antimicrobial activity against Listeria monocytogenes ATCC 7644.[78]

Enterococci

Enterococci including Enterococcus faecalis, Enterococcus faecium, Enterococcus hirae, Enterococcus casseliflavus and Enterococcus durans, were isolated from goat milk samples.[77,79,80] Several strains of Enterococcus in raw goat milk have been found to have antimicrobial activity against Listeria monocytogenes ATCC 7644.[78]

Helicobacter pylori

Helicobacter pylori were detected in raw sheep milk.[10,81] Dore and colleagues suggested that Helicobacter pylori might be a commensal species in sheep.[81,82]

Coxiella burnetii

As an obligate intracellular gram-negative bacterium, the Coxiella burnetii strain genotype CbNL01, identified as the causative agent of Q fever, a zoonosis in the Netherlands, was detected in goat milk.[83] After vaccination with a completely inactivated vaccine in dairy goats, vaccine-derived Coxiella burnetii DNA has been detected in goat milk.[84,85]

Other bacteria

Many other bacterial species, belonging to different genera were identified in sheep and goat milk and/or cheeses. The bacterial genera included Acinetobacter, Aerococcus, Arthrobacter, Bifidobacterium, Brachybacterium, Brevibacterium, Brucella, Chryseobacterium, Clostridium, Corynebacterium, Dietza, Escherichia coli, Enterococcus, Exiguobacterium, Hafnia, Hahella, Janthinobacterium, Jeotgalicoccus, Kocuria, Kosmotoga, Lactococcus, Leuconostoc, Macrococcus, Mannheimia, Megasphaera, Micrococcus, Ornithinicoccus, Pantoea, Pediococcus, Petrotoga, Pseudomonas, Rothia, Salinicoccus, Serratia, Thermoanerobacterium, Vagococcus, and Weissella.[8,9,30,58]

Seasonal Change of Bacterial Community

The seasonal effect on bacterial community was also described in goat milk by Callon and colleagues.[9] They found that during a lactation year, bacterial species in winter milk were mainly from the genera of Lactococcus and Pseudomonas; those in summer milk were mainly Pseudomonas agglomerans and Klebsiella; and in contrast, Chryseobacterium indologenes, Acinetobacter baumanii, Staphylococcus, Corynebacteria and yeasts were remarkable in autumn milk.[9]

3.2 Fungal community

Fungal species in sheep or goat milk and/or cheeses were diverse and distributed in different fungal genera including Aspergillus, Candida, Cladosporium, Cryptococcus, Debaryomyces, Engyodontium, Fusarium, Geotrichum, Kluyveromyces, Malassezia, Mucor, Penicillium, Pichia, Torrubiella, Trichosporon, and Rhodotorula.[9,44,65]

3.3 Virus

Small Ruminant Lentiviruses

Antibodies and proviral DNA of Small Ruminant Lentiviruses were detected in milk samples from sheep and goats.[87]

Catarrhal fever virus

Sheep-associated malignant catarrhal fever virus was detectable in all colostrum and milk samples and DNA fragment from peripheral blood lymphocytes from healthy adult sheep.[88]

Tick-borne encephalitis virus (TBEV)

Balogh and colleagues showed that after experimental infection with live tick-borne encephalitis virus (TBEV), the milking goats did not show any clinical signs or fever compared to uninfected ones; and moreover, infectious virions were detected in the milk samples from the infected goats without previous immunization, whereas, no detectable TBEV in the milk sample from the infected goats with previous immunization.[89]

Louping-ill virus

Reid and colleagues showed that louping-ill virus was detected in milk of all lactating goats after the experimental inoculation with louping-ill virus and becoming viremic. Some kids sucking these goats became infected with marked clinical signs and one died.[90]

Powassan virus

Woodall and Roz showed that after a lactating goat with a 74-day-old kid was inoculated with Powassan virus, although the inoculated goat did not show any clinical sign of illness, virus was detectable in the lactating goat's milk on postinoculation days 7 through 15.[91] More interestingly, although the offspring was not inoculated, it developed neutralizing antibodies by day 22, which was believed to be the consequence of active viral infection from maternal milk;[91] however, the recently proposed novel mechanisms in transferring immunity-associated eukaryotic cells among fetus, the maternal body, and suckling offspring cannot be ruled out.[92,93]

4. Camel Milk

4.1 Bacterial microorganisms

Leuconostoc mesenteroides

Benmechernene and colleagues reported that Leuconostoc mesenteroides strains were isolated from raw camel milk in two southwest Algerian arid zones.[94,95]

Streptococcus-like strains

Streptococcus salivarius subsp. thermophilus was isolated from raw dromedary milk in Morocco using various cultured media.[96] Kadri and colleagues identified two novel Streptococcus species named Streptococcus moroccensis sp. nov. and Streptococcus rifensis sp. nov. from raw camel milk in Morocco.[97]

Brucella species

Brucella species were identified in camel milk samples.[58] Brucella melitensis is a pathogenic bacterium that can cause human, camel and cattle's brucellosis.[98,99] Shimol and colleagues showed that Brucella melitensis was cultivable in the milk samples of infected camel.[98]

Camelimonas lactis

Novel strains of Camelimonas lactis were isolated from the milk of camels in the United Arab Emirates.[100]

Lactobacillus and Lactococcus

Lactobacillus helveticus, Lactobacillus casei, Lactobacillus plantarum, and Lactococcus lactis strains were isolated from raw dromedary's milk.[96,101]

Bifidobacteria species

Bifidobacteria species such as Bifidobacterium angulatum, Bifidobacterium longum, Bifidobacterium bifidum, and Bifidobacterium breve were identified in whole camel milk.[57]

Other bacteria

Many other bacterial species belonging to different genera were identified in sheep and goat milk and/or cheeses. These bacteria, including Staphylococcus aureus, Streptococcus equi subsp. zooepidemicus, Escherichia coli, and Helicobacter pylori were identified from raw camel milk samples.[10,52,102,103]

4.2 Viruses

Torque teno virus (TTV) was detected in the milk of camel, and a further study on the similarity between isolates from camels and humans revealed that camels and humans shared a common source of TTV infection in the United Arab Emirates.[104]

5. Sow Milk

5.1 Bacterial microorganisms

Lactobacillus

Lactobacillus reuteri, Lactobacillus salivarius, Lactobacillus plantarum, Lactobacillus paraplantarum, and Lactobacillus brevis were identified in sow milk, and some strains of them displayed probiotic potential.[105]

Enterococcus

Enterococci, including Enterococcus faecalis, Enterococcus faecium, Enterococcus hirae, Enterococcus casseliflavus, and Enterococcus durans were identified from porcine milk samples.[79]

Weissella paramesenteroides

Weissella paramesenteroides was identified in sow milk.[105]

5.2 Viruses

Swine torque teno virus (TTV)

Recently, Martinez-Guino and colleagues first reported that swine torque teno virus (TTV) was detected in swine colostrum,[106] and further suggested that the sow-to-piglet transmission route of swine TTV via breastmilk and breastfeeding may co-exist with the trans-placental/intra-uterine and piglet-to-piglet transmission routes in the pig population.[106,107]

6. Feline Milk

6.1 Bacterial microorganisms

Enterococcus

Enterococci, including Enterococcus faecalis, Enterococcus faecium, Enterococcus hirae, Enterococcus casseliflavus and Enterococcus durans were identified from feline milk samples.[79]

6.2 Viruses

Feline leukemia virus

Feline leukemia virus, an RNA virus associated with lymphosarcoma, is present in the excretions and blood of viremic animals and can be transferred horizontally to healthy cats.[108] Pacitti, Jarrett, and Hay once reported in 1986 that feline leukaemia virus (both FeLV antigen and infectious virus) was detectable in milk sample of cat, which was believed by the authors to be one of mechanisms associated with the transmission of feline leukaemia virus to previously non-viraemic kittens.[109]

7. Canine Milk

Bacterial microorganisms

Lactobacillus

Lactobacilli species such as Lactobacillus reuteri, Lactobacillus fermentum, Lactobacillus murinus, and Lactobacillus animalis were identified in milk of bitches, and some strains of them showed probiotic characteristics.[110]

Enterococcus

Enterococci, including Enterococcus faecalis, Enterococcus faecium, Enterococcus hirae, Enterococcus casseliflavus, and Enterococcus durans were identified from canine milk samples.[79]

8. Donkey Milk

Bacterial microorganisms

Donkey milk is believed by some scholars to be the best substitute for human milk due to its immunological properties, and its content of lactose, proteins, minerals, and omega-3 fatty acids;[111] and therefore, consuming donkey milk as a source of nutrition for humans is attracting more attention.[112] However, the study on microorganisms including bacteria, viruses, and fungi in donkey milk is just at its infantile stage.

Escherichia coli

Diverse Escherichia coli strains, including Shiga toxin-producing Escherichia coli, were isolated from donkey raw milk and traditional dairy products in Iran.[113]

Lactic acid bacteria

Recently, Lee and colleagues detected some uncultivable and cultivable bacterial species from donkey milk powder.[114] Murua and colleagues identified bacteriocinogenic Lactobacillus plantarum from donkey milk.[115] Moreover, Carminati and colleagues further revealed the diversity of cultivable lactic acid bacteria (LAB) species in donkey milk, which included diverse bacterial species in the genera of Enterococcus, Streptococcus, and Pediococcus.[112]

9. Monkey Milk

Simian immunodeficiency virus (SIV)

The simian immunodeficiency virus (SIV)-specific CD8(+) T lymphocyte was detected in breastmilk of rhesus monkeys after simian immunodeficiency virus infection.[116,117] Permar and colleagues also revealed that a fraction of milk SIV viruses produced by locally-infected cells existed in milk despite host immune pressures.[118]

10. Equine Milk

Bacterial microorganisms

Lactobacillus rhamnosus strains and Lactobacillus fermentum stains were isolated from a traditional fermented mare milk of Indonesia.[119] A bacteriocin-producing Leuconostoc mesenteroides strain was isolated from Mongolian fermented mare's milk.[120]

11. Mice Milk

Breastmilk of mice was shown to be a source of murine cytomegalovirus (MCMV), and there is a vertical transmission of viruses between breastmilk and exposed suckling offspring.[121]

12. Concluding Remarks

Milk is one of the primary environmental factors that newborns of mammals must come into contact with. The novel notion that “the interaction between an evolutionary entity and its environment is actually the interaction between the entity and its environmental evolutionary entities at the same and/or different evolutionary levels”[2] reminds us that the interaction between suckling and milk is far beyond the scope of nutrient absorption and immune protection. The coexistence of fungal eukaryotic entities, bacterial prokaryotic entities, viral subcellular entities, and microvesicle subcellular entities in mammalian milk reflects the different evolutionary signatures at the cellular and subcellular evolutionary levels; and they constitute the components of the environmental evolutionary entities in non-human mammalian milk, from which, it will be helpful to elucidate the role played by milk as the environmental factor in evolution.

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