CHAPTER

26 Biomedical applications of ginsenosides nanoparticles synthesized using microbes

Sri Renuakdevi Balusamy[a], Santhiya Karuppieh[b], Sumathi Venkat[b], Lakshmi Thangavelu[c], Yeon Ju Kim[d], and Haribalan Perumalsamy[d,e]

[a]*Department of Food Science and Biotechnology, Sejong University, Seoul, Republic of Korea,*
[b]*Department of Biochemistry, Biotechnology and Bioinformatics, Avinashilingam Institute for Home Science and Higher Education for Women, Coimbatore, Tamil Nadu, India,*
[c]*Department of Pharmacology, Saveetha Dental College and Hospitals, Saveetha University, SIMATS, Chennai, Tamil Nadu, India,*
[d]*Graduate School of Biotechnology, College of Life Sciences, Kyung Hee University, Yongin, Republic of Korea,*
[e]*Research Institute for Convergence of Basic Science, Hanyang University, Seoul, Republic of Korea*

1 Introduction

Evolution of nanotechnology in the past decade was highly commendable due to its enormous applications in various fields of science. The prominence of nanoscale systems is well portrayed by the nanoparticles, which have the size limit in the range of 1 to 100nm. The promising features of nanomaterials such as the small size with increased surface area to volume ratio promote uniqueness of the former which is different from that of the bulk materials (Singh et al., 2011). Multiple physical, chemical, biological and hybrid methods had been widely adopted for the production of different kinds of nanoparticles (Luechinger et al., 2010; Tiwari et al., 2008; Mohanpuria et al., 2008). However, physical and chemical methods may employ some toxic synthetic chemical components which restrict the major biomedical applications. One of the convenient, non-toxic, environment-friendly modes for the synthesis of nanoparticles involves the use of microorganisms through the biogenic enzymatic process and is considered to have paramount significance. Though other methods would generate a large number of nanoparticles having precise size, shape and within the optimal duration, the subsequent consequences of employed chemicals and other hazardous components could pose an imminent danger to the human health and environment. Expulsion of the energy-intensive chemical methods brings

Agri-Waste and Microbes for Production of Sustainable Nanomaterials. https://doi.org/10.1016/B978-0-12-823575-1.00003-2

forth the replacement with "GREEN" or biogenic method of nanoparticle preparation where the process is benefited by diverse groups of microbes and the optimal growth conditions like temperature, pH and pressure. The synthesized particles are known for their increased catalytic activity, surface area. In addition, a strong and stable contact is established between the microbial enzymes and metal ion which is favored by the presence of bacterial cell-matrix (Bhattacharya and Mukherjee, 2008). There are many categories of nanoparticles including the inorganic types made up of metallic, oxide, sulfide etc. Such nanoparticles are oriented for different applications in the fields of biomedicine, environment, agriculture and many more. For example, gold nanoparticles are involved in specific drug delivery of the anticancer agents such as methotrexate, paclitaxel and doxorubicin. Moreover, gold nanoparticles are utilized in the diagnosis of tumor, genetic disorders, as well as in the techniques of photo-imaging, photo-thermal therapy. The iron oxide nanoparticles are suitably allocated in the treatment of cancer by the phenomenon of magnet-activated drug delivery (Huang et al., 2007). Silver, zinc, and palladium nanoparticles are well known for their exceptional antimicrobial properties. The biological methods of preparation are facile where plants and microorganisms serve as the refined sources accounting for an increased scale-up and reproducibility. These biosynthesized nanoparticles are employed as antibacterial agents, biosensors as well as ameliorate the rate of reactions, separation science and magnetic resonance imaging (MRI). The following content will focus on the production of nanoparticles by microbial factories, related biomedical uses, properties of ginsenosides and how the ginsenoside nanosystems could be used effectively to accelerate their clinical applications.

2 Probiotics

Basic knowledge of the gut microbiome and its relationship with human health is indispensable to understand the intervention of the probiotic concept into the latter. Every human owns an individualistic genome fingerprint of the intestinal microbe consortium which comprise of, at least, 100–1000 species. The compositional count hits to about 10^3 bacteria with the total microbial population being 10^{11}–10^{12}CFU/g. Once the neonate is exposed to a non-sterile environment immediately after birth, colonization of gut bacteria gets commenced. Then the gut microbiota proliferates and diversifies with the help of sophisticated and versatile interactions over the diet, host lifestyle, antibiotic regime followed and the genome nature. Age-related factors could also promote the microbial community shift over the time where a significant decline of the Bacteroidetes/Firmicutes ratio and bifidobacteria is observed in people beyond 60years. This is indicative of the immune system's decreased function. However, the intrinsic core composition of the microbiome remains static throughout adulthood irrespective of the other conditional aspects. Normally, "Barrier effect" of the indigenous colonies is responsible for the resistance exhibited against the ingested pathogens. This phenomenon is found

to play an important role in the digestion, metabolism, immune function and gut-brain interaction. Any deviation from the inherent functions of gut microbial community paves way for diseases that could not be subdued by the typical medicines due to the involvement of antibiotic-resistant strains. To counteract such effects, probiotics are the simple, suitable alternatives given as supplements for supporting and boosting the protective roles of host microflora, especially against enteric pathogens. This is attributed to the ability of probiotic microbes to compete with gut microbes through the intestinal wall adhesion, thereby, enhancing their colonization (Kerry et al., 2018). Probiotics exert a plethora of prospects to human health which includes the maintenance of microbiome and intestinal homeostasis that helps to tackle the problems of obesity, instigation and modulation of the host immune system by activation of specific genes in the localized cellular environment, regulation of gastrointestinal tract (GIT) hormone release, which is required for improving the bioavailability of nutrients, mitigation of lactose intolerance risk, stimulation of new blood vessel formation in the intestine through vascular endothelial growth factor receptor (VEGFR) signaling that regulates the acute and chronic inflammation of mucosal tissues in the intestinal bowel disease (IBD). To obtain the desired activities, selected probiotic strains must be site-specific with efficient attributes of multiplication and colonization. They should not provoke any immunogenic, pathogenic, mutagenic or carcinogenic response against the host body. The probiotic organisms must be of human origin and able to get acclimatized to the in vivo conditions of low pH, high concentrations of conjugated and unconjugated bile acids. In spite of the possible advantages, the expected side effects are supposed to be thoroughly examined before the development and utilization of such supplements to produce new strains with disease-oriented functions (Kerry et al., 2018; Nagpal et al., 2012).

2.1 Versatile clinical applications of the probiotics

The probiotics exhibit dynamic interplay between various physiological environments of the host body such as the intestinal lumen, epithelial and mucosal barrier function and its other components. Interactions of the probiotic microbes with epithelial cells, dendritic cells, monocytes, macrophages, B and T cells are emphasized by their species-specific characteristics and through the secretion of specific protein or metabolite (Nagpal et al., 2012). These nutrient-rich organisms demonstrate potential benefits which are elucidated in the form of their biomedical applications. The antimicrobial activity of probiotic supplements could counteract the harmful effects of antibiotics on a host such as the manipulation of gut microflora population. The lethal effects of the probiotics are observed against pathogenic microbial species which includes the clostridium, salmonella and serovar. One of the reported mechanisms involves the production of short-chain fatty acids like propionic, acetic, lactic, and butyric acids that are known to optimize the abdominal pH responsible for gut bacterial gene expression which in turn facilitates the destruction of foreign or carcinogenic compounds in a lumen. Few secreted peptides, bacteriocin

and hydrogen peroxide are the anti-pathogenic agents capable of compromising the membrane integrity of target cell through the mechanisms of membrane potential depolarization and lipid peroxidation. In addition, nutritional feed of interest might also trigger the host immune system leading to the formation of a defensive and positively charged antimicrobial peptide by the paneth cells of the intestinal epithelium. Urogenital disorders are mostly attributed to abnormal or imbalanced vaginal microflora. This could be reformed by the assistance of lactobacillus based probiotic supplementation. Diabetes is a metabolic disorder which might be influenced by the alteration of gut microbial community. This is evident from the increased number of bacteroidetes and decreased firmicutes population encountered during the type I and II diabetes and obesity. Probiotic regulated release of the gut hormones such as gastric inhibitory polypeptide and glucagon-like peptide-1 exert pronounced effects while dealing with diabetes type II. The process establishes the homeostasis of glucose metabolism by reverting the insulin uptake and the ability of pancreatic β cells to produce insulin. Novel anti-diabetic probiotic supplements namely arabinoxylan and its oligosaccharides are developed for the minimization of adipose tissue. Modulation of the host gut microbial conditions and its environment is interlinked with the excessive storage of body fat. The lipolytic and thermogenic processes mediated by sympathetic nervous system are observed as the probiotic means of proclaiming its anti-obese property. For example, *Lactobacillus gasseri* BNR17 prevents the accumulation of adipose tissue, thereby, opposing the leptin secretion. *L. casei*, *Lactobacillus acidophilus* and *Bifidobacterium longum* are also known to serve as cholesterol-lowering agents. The inflammatory Bowel Diseases (IBD) include the Crohn's disease (CD) and ulcerative colitis (UC) that are manifested by inflamed mucosal, submucosal lining and serosa. The diminished activity of short-chain fatty acids contributes to IBD and levels of the former could be normalized through the diet combo of prebiotics and probiotics as well as from the immune modulators secreted by recombinant probiotic strains for recovering the lost beneficial bacterial commensals of the host body. Species of Lactobacillus, Bifidobacterium, Enterobacter and *E.coli* are denoted to act as the anti-inflammatory agents. Cancer is a group of malicious disorders that had taken a toll on innumerable lives over the decade. Unlike the nanotechnology or biotechnology-based diagnostic and therapeutic options, probiotic supplements confer the maximal curative effects with minimal anticipated side effects. Such anti-cancer activities are demonstrated in vitro through the strains namely *Lactobacillus fermentum* NCIMB-5221 and -8829 that are found to generate short-chain fatty acids like ferulic acid for inhibiting the proliferation of colorectal cancer cells and enhancing the stable epithelial cell growth. Most often, allergic diseases arise due to the cascade of immunological reactions. Deep understanding of the molecular mechanisms underlying the causes of allergies leads to the development of prevention strategy with the help of probiotic-based supplementation. Anti-allergic characteristics pertained to *Lactobacillus plantarum* L67 mitigate the allergic responses by stimulating the production of interleukin-12 and interferon-ϒ from the host cells under in vitro conditions. Angiogenesis can be described as the process of neovascularization or regeneration of the damaged tissues.

The probiotic mediated angiogenesis involves changing the inflammatory cytokine profile, minimal expression of pro-inflammatory pathway, activation of the mechanisms specific to other strains, a decrease of visceral allergic or stress response etc. Mutual exchange of the communication and other signals between GIT and CNS has interacted through the microbial gut-brain axis. This is implicated from the influence of the gut microbiome on the performance of brain activities. Microbial strains such as *L. plantarum*, *Lactobacillus helveticus* R0052 and *B. longum* R0175 promote the desirable outcomes in the conditions of autism, psychological stress and anxiety. In addition, a neurotransmitter named ϒ-aminobutyric acid secreted by the *L. brevis DPC*6108 and *Bifidobacterium dentium*is is reported to alleviate the anxiety and depression in humans (Kerry et al., 2018) (Fig. 1).

2.2 Probiotic mediated nanoparticle synthesis

Prospects of probiotics are attributed to a variety of actions. Some are exerted by the plain viable microbes while others are mediated by the biogenic components of microbial origin. These organisms serve as remarkable sources for both laboratory synthesis and industrial scale-up of the nanoparticles. Such characteristics are due to their non-toxicity, increased rate of growth and proliferation, enormous synthesis of enzymes as well as the ability to thrive in the provided experimental setup. The best-studied example of above qualities is the facultative Lactobacilli species that is capable of synthesizing the nanoparticles under both oxidative and reductive atmospheres. Akin to other microbes,

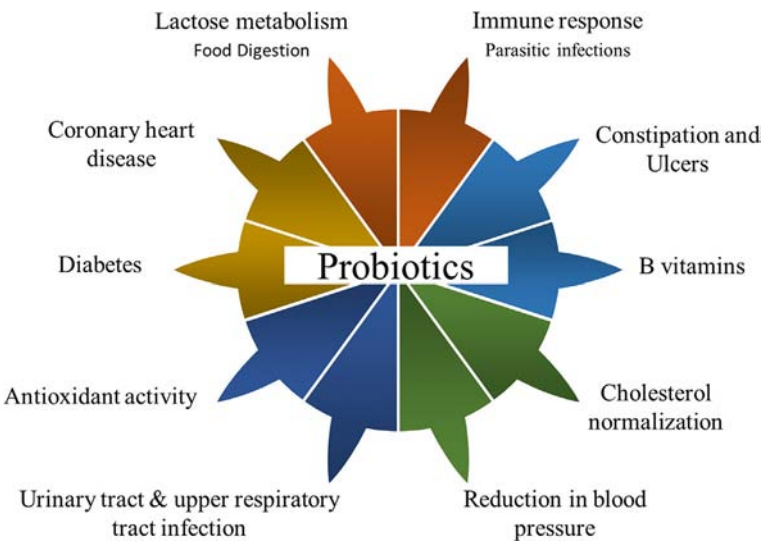

FIG. 1

Potential health benefits of probiotics.

probiotics are known to kindle the process of biosynthesis through the electrostatic attraction between a cationic metal ion and anionic cell membrane. Silver nanoparticles are known to possess antimicrobial and anti-inflammatory properties and are formed by the reduction of Ag^+ by microbial cells. About 200mL of *Lactobacillus bulgaricus* was mixed with 300mL of aqueous 1mM $AgNO_3$ solution and incubated for a period of 24h. This facilitates the reduction of Ag ions and resultant Ag NPs would be indicated by the solution color change from mild yellow to reddish-brown and through the colorimetric measurement. The wound healing, antibacterial, antifungal and UV blocking characteristics of zinc nanoparticles are well explored for use in the commercial products. *Lactobacillus plantarum* VITES07 strain was cultured in the MRS broth and incubated under optimal conditions of 37°C for 24h at a speed of 100 RPM. This microbial culture medium was adjusted to pH6, mixed with $ZnSO_4.H_2O$ solution of 0.1M and heated in the water bath at 80°C for 5–10min. Followed by, 12h was taken for the zinc oxide NPs to settle down at the glassware bottom. ZnO NPs was further dried at 40°C in hot air oven and the optical properties were evaluated by UV-vis spectroscopy. The reaction span (5–10min) plays a vital role in determining the spherical structure and size (about 7nm) of the formed NPs. Glucose is the energy source of culture medium and is responsible for the ionic regulation in pH required for the functioning of membrane-bound oxidoreductases in *L. plantarum* along with the maintenance of oxidation-reduction potential for prominent production of ZnO NPs. Titanium dioxide nanoparticles (TiO_2) perform the role of antimicrobial, antiparasitic and wrinkle-reducing agents. It serves as an excellent drug delivery agent for targeted therapies and therefore suitable for biomedical applications where the titanium pins are available as surgical tools; Ti^{IV} interacts with the transferrin in human serum and is redirected towards the tumor area. This explains their futuristic appeal in the treatment of cancer. Preparation of TiO_2 NPs involves the Lactobacillus culture solution made of 10% sugar with pH strength 2–4 and was incubated overnight. Then the titanium dioxide solution of 0.025M was added and mixed. This process facilitates the release of NPs after 3–4days where the solution is evaporated to dryness in a hot air oven for obtaining the powdered particles that are characterized using TEM and XRD. The pigment-like or anatase form of the Ti nanoparticles was obtained at 300°C from the *Propionibacterium jensenii* KC545833 culture containing $TiO(OH)_2$ as the precursor. The combinatorial culture of Lactobacillus and yeast strains gave rise to different sized TiO_2 NPs in the range of 20–30nm and 10–15nm. Decreased NP size of the yeast cells is primarily attributed to the eukaryotic cellular organization. The antimony trioxide (Sb_2O_3) is a renowned semiconducting agent and a catalyst employed for the manufacture of mineral water packaging plastics. The colloidal mixture of antimony and gluconate elicits the lethal response against kala azar. Similarly, other types of antimony based formulations are effective against various pulmonary diseases and GIT issues like dysentery. The performance of gadolinium oxide (Gd_2O_3) is well demonstrated as phosphors in the tube of color television and

microwave. The culture solution mixed with 0.25M strength gadolinium acetate led to the formation of hazy white light particle precipitate. Cadmium sulfide (CdS) NPs are crucial in the construction of biosensors, photovoltaic, LED and other solar-based sensors. Suspension culture of the lactobacillus isolated from buttermilk is prepared and diluted appropriately. The next step is the cadmium sulfide formation from a mixture of 5mL hydrogen sulfide and 20mL cadmium chloride ($CdCl_2$). The CdS Occurrence is manifested from the development of dual shade orange-yellow solution which is then added to the culture suspension and heated promptly at 60°C for 10–15min to obtain the soft orange yellowy CdS nanoparticles at the flask bottom and are characterized after filtration. Selenium nanoparticles (Se) obtained from the Lactobacillus species vary in their morphological features of size and shape as per the pH range 4 to10 of the culture medium. The organic nanostructured Se is often converted to the form of selenite (SeO_3^{2-}). Such selenium NPs are the worthy precursors of nanosurfacial structures and this role is attributed to the former's instilled properties of uniform particle size distribution and spherical shape. The sodium hydrogen selenite ($NaHSeO_3$) serves as the prerequisite for the production of selenium NPs in the 5% fat milk medium inoculated with the *Lactobacillus casei* strain. Fermentation begins and perpetuates through the pH7–8 and termination of the process is facilitated around the pH3–4. The observed pH alteration assumes significance due to the synthesis of lactic acid by inoculant strain. The entire fermentation is favored through the optimal environment of 37°C from 36 to 48h. Transformation or reduction of the precursor to elemental selenium NP is indicated by red color change of the medium at the decline of the fermentation process. The Se NPs are predominantly synthesized by the lactic acid bacterium intracellularly. Hence the culture solution is centrifuged at 10,000rpm for 10–15min to separate the pellets which are further subjected to the acid hydrolysis using 1.5×37% HCL. This is followed by the ultra-sonication for 10–15min to dislodge the clustered NPs at the bacterial cell wall. At last, the remnants are obtained through vacuum filtration. This step is undertaken for the exclusion of bacterial cell wall and visual or the laser control aids are deployed for the quality control verifications of the synthesized NPs (Khosravi-Darani et al., 2019).

The probiotic facilitated health applications are given in Table 1.

3 Mechanisms of the microbial synthesis of nanoparticles

The intrinsic nature of microorganisms to produce nanoparticles from inorganic precursors is defined by either an intracellular or extracellular pathway. Both are influenced by different enzymes, proteins or other components of the cell. An extracellular method is distinguished for the production of large volumes of nanoparticles along with flexible minimalistic down-streaming steps, simple separation methods and the subsequent commercialization opportunities. Therefore, it is most commonly employed than the intracellular counterpart

Table 1 List of probiotic species and their clinical roles.

S. No	Probiotic organism	Reported health prospects
1.	*Lactobacillus rhamnosus*	• ***L. rhamnosus* CRL1505**—deteriorates the effects of virus-mediated pulmonary diseases (Zelaya et al., 2014) • **Different strains from the fecal samples of human infant**—antidiabetic activity (Panwar et al., 2014) • ***L. rhamnosus* CGMCC1.3724**—promotes weight loss in obese conditions (Sanchez et al., 2013) • *L. rhamnosus* **GG and** *L. rhamnosus* **ATCC 53103**—limits the rate of infection due to rhinovirus in premature infants (Luoto et al., 2013)
2.	*Lactobacillus acidophilus*	• ***L. acidophilus* ATCC-4495**—treats the *C. difficile* mediated diarrhea; antifungal property (Cortés-Zavaleta et al., 2014) • Diminish the symptoms of irritable bowel syndrome (Ortiz et al., 2014) • Lessens the incidence of febrile urinary tract infections (Mohseni et al., 2013) • Prevents and cures the bacterial vaginosis (Homayouni et al., 2014)
3.	*Lactobacillus plantarum*	• ***L. plantarum* NRRL B-4496**—antifungal property (Cortés-Zavaleta et al., 2014) • Diminish the symptoms of irritable bowel syndrome (Ortiz et al., 2014) • Counteract the formation of endotoxin (Lee et al., 2013)
4.	*Lactobacillus casei*	• ***L. casei* Lcr35**—regeneration of vaginal microflora in the patients of bacterial vaginosis (Kovachev and Dobrevski-Vacheva, 2013); therapeutic effects in the adult functional constipation (Chmielewska and Szajewska, 2010) • ***L. casei* Shirota**—immunomodulation (Shida and Nomoto, 2013); therapeutic effects in the adult functional constipation (Chmielewska and Szajewska, 2010); Lowers the antibiotic mediated diarrhea in geriatric patients (Wright et al., 2014). • ***L. casei* 01**—rheumatoid arthritis therapy (Vaghef-Mehrabany et al., 2013) • ***L. casei* CRL-431**—safeguards from the *salmonella* infection (Castillo et al., 2013)
5.	*Lactobacillus delbrueckii* subsp. *bulgaricus*	• ***L. delbrueckii*** subsp. ***bulgaricus* 8481**—improves the systemic immunity in elders (Moro-García et al., 2013) • Lethal effects against *E. coli* (Abedi et al., 2013)

Table 1 List of probiotic species and their clinical roles—cont'd

S. No	Probiotic organism	Reported health prospects
6.	*Lactobacillus brevis*	• ***L. brevis* KB290**—manages the bile salt tolerance (Suzuki et al., 2013)
7.	*Lactobacillus johnsonii*	• ***L. johnsonii* MH-68**—lowers the risk status of *H. pylori* infection and gastritis encounterance (Hsieh et al., 2012) • ***L. johnsonii* F0421**—protection against the *S. sonnei* (Zhang et al., 2012)
8.	*Lactobacillus fermentum*	• ***L. fermentum* NCIMB 5221**—lessens the effects of insulin resistance and hypercholesterolemia (Tomaro-Duchesneau et al., 2014) • ***L. fermentum* RC-14**—prevents and cures the bacterial vaginosis (Homayouni et al., 2014)
9.	*Lactobacillus reuteri*	• ***L. reuteri* NCIMB 30242**—decrease the levels of low-density lipoprotein cholesterol (Dirienzo, 2014)
10.	*Bifidobacterium infantis*	• Diminishes the effects of necrotizing enterocolitis in pre-term infants
11.	*Bifidobacterium animalis* subsp. *lactis*	• *B. animalis* **subsp.** *lactis* **DN-173010**—therapeutic effects in the adult functional constipation (Chmielewska and Szajewska, 2010) • Reduced incidence of the febrile urinary tract infections (Ortiz et al., 2014) • ***B. animalis*** subsp. ***lactis* MB 202/DSMZ 23733**—diminishes the total cholesterol levels (Bordoni et al., 2013)
12.	*Bifidobacterium bifidum*	• Decrease the effects of necrotizing enterocolitis in pre-term infants • ***B. bifidum* MB 109/DSMZ 23731**—diminishes the total cholesterol levels (Bordoni et al., 2013)
13.	*Bifidobacterium longum*	• *B. breve*, *B. infantis*, *B. bifidum*, *B. longum*—Bifidobacterial cocktail is effective against the necrotizing enterocolitis (Janvier et al., 2014)
14.	*Bifidobacterium breve*	• ***B. breve* MB 113/DSMZ 23732**—diminishes the total cholesterol levels (Bordoni et al., 2013) • *B. breve*, *B. infantis*, *B. bifidum*, *B. longum*—Bifidobacterial cocktail is effective against the necrotizing enterocolitis (Janvier et al., 2014)
15.	*Saccharomyces boulardi*	• Therapeutic effects in the moderate ulcerative colitis (Guslandi et al., 2003)

Continued

Table 1 List of probiotic species and their clinical roles—cont'd

S. No	Probiotic organism	Reported health prospects
16.	*Lactococcus lactis* subsp. *lactis*	• ***L. lactis*** subsp. ***lactis*** **ATCC 11454**—probiotic and antimicrobial property (Fernandez et al., 2013)
17.	*Enterococcus durans*	• ***E. durans*** **LAB18s**—attachment based anti-inflammatory activity in the colonic tissue (Raz et al., 2007)
18.	*Enterococcus faecium*	• Used in treating the antibiotic induced diarrhea (Hempel et al., 2012)
19.	*Streptococcus thermophilus*	• Decreases the effects of necrotizing enterocolitis in pre-term infants
20.	*Pediococcus acidilactici*	• ***P. acidilactici*** **UL5**—probiotic and antimicrobial property along with the pediocin production (Fernandez et al., 2013)
21.	*Leuconostoc mesenteroides*	• ***L. mesenteroides*** **B7**—exhibits the probiotic characteristics including the survival at low pH and in the presence of bile salts, pepsin; synthesis of leucoin (Benmechernene et al., 2013)
22.	*Bacillus coagulans*	• Used in treating the antibiotic induced diarrhea (Hempel et al., 2012)
23.	*Escherichia coli* Nissle 1917	• Therapeutic effects in the adult functional constipation (Chmielewska and Szajewska, 2010)

which facilitates the recovery of purified nanoparticles through tedious steps of cell biomass separation by centrifugation and repeated cycles of ultra-sonication for disrupting the cell walls (Markus et al., 2016).

3.1 Intracellular method

This pathway exploits the charges of microbial cell wall and the metallic ions of environment to ensure a smooth ion trafficking through the cell. And this process is mediated by the enzymes and coenzymes of microbe. Polysaccharides or proteins of the cell wall serves as the active site for metal ion binding (Slavin et al., 2017). Generally, the presence of metal ions in the surrounding threatens the microbial survival. Not all, but some microbes are capable of entrapping the metal ions on to its cell wall by means of electrostatic interactions. Positively charged metal ion is attracted to the negatively charged cell wall containing carboxylate groups in the form of specific enzymes, cysteine and polypeptides. Attached metal ions of the cell wall are reduced to their core atom by the transfer of electrons from NADH through the action of NADH dependent reductase enzyme and gets localized in the

plasma membrane. Nuclei part develops as the nanoparticle which is positioned in the cell wall or periplasmic space. These nanoparticles are further stabilized by the proteins or amino acids such as tryptophan, cysteine and tyrosine in the cell (Iravani et al., 2014; Shedbalkar et al., 2014). The synthesis of nanoparticles by *verticillium* sp. is accomplished in three stages namely the trapping, bioreduction and capping where the reactive forces acting upon the metal ions and enzymes of cell wall brings the reduction of former. This leads to the formation of metal atoms and accumulation of metal nanoparticles in the cell wall. The cytoplasmic appearance of some metal nanoparticles is proved by TEM analysis. This is mainly attributed to the diffusion of small nanoparticles across the cell wall. The intracellularly synthesized gold nanoparticles of 5 to 15nm size were found in the cell wall and cytoplasm of *Rhodococcus* species (Ahmad et al., 2003). A new probiotic strain named *Lactobacillus kimchicus* DCY51^{T} had developed the gold nanoparticles of size 5 to 30nm, furnished with a protective capping layer of amino acids that enables the non-toxicity against murine macrophage (RAW 264.7) and human colorectal adenocarcinoma (HT29) cell lines (Figs. 2 and 3) (Markus et al., 2016). Bacterial species such as *L. plantarum* VITES07 and *L. sporogens* are observed to synthesize the ZnO nanoparticles under suitable low pH conditions (Prasad and Jha, 2009).

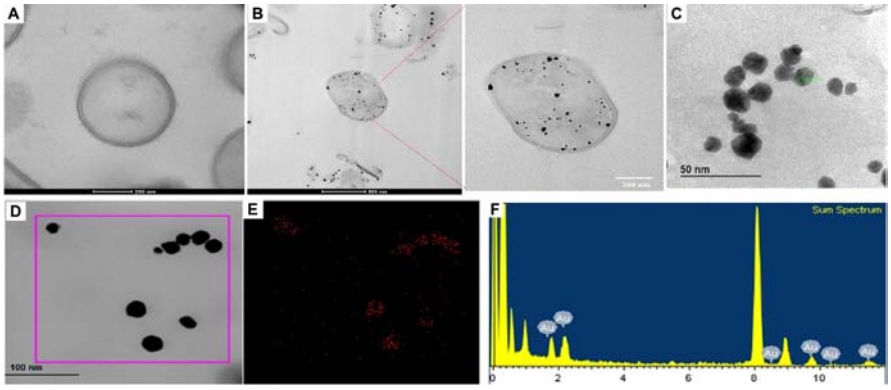

FIG. 2

Intracellular synthesis of BifidoAuNPs CK nanoparticle. (A and B) TEM images confirming intracellular synthesis of Bifi-CKAuNPs; (C and D) Shape and size of the Bifi-CKAuNPs measured by SEM and TEM analysis; and (E and F) Elemental mapping analysis of Bifi-CKAuNPs.

Data from Kim, Y., Perumalsamy, H., Markus, J., Balusamy, S.R., Wang, C., Kang, S.H., Lee, S., Park, S.Y., Kim, S., Castro-Aceituno, V., Kim, S.H., Yang, D.C., 2019. Development of Lactobacillus kimchicus DCY51^{T}-mediated gold nanoparticles for delivery of ginsenoside compound K: in vitro photothermal effects and apoptosis detection in cancer cells. Artif. Cell Nanomed. B 47 (1), 30–44. This is an open-access article distributed under the terms of the Creative Commons CC BY license, with permission from Taylor & Francis.

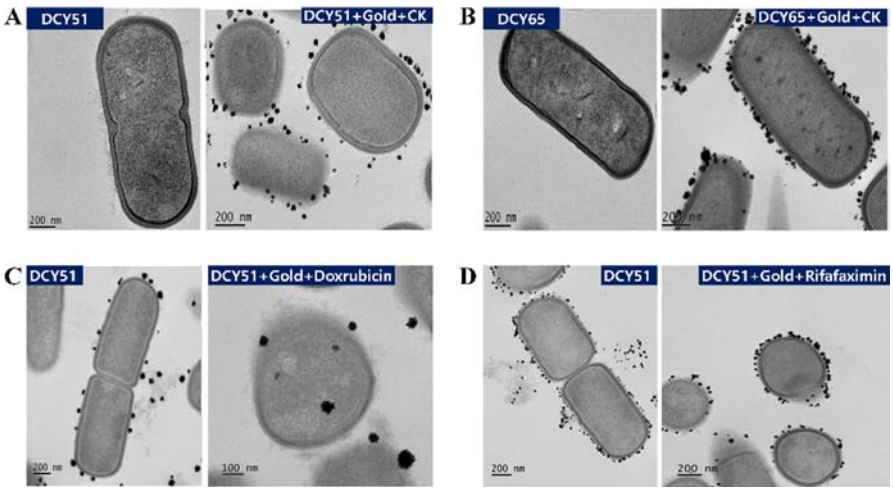

FIG. 3

Intracellular synthesis of DCY51AuNpsCK nanoparticle. HR-TEM analysis of (A) DCY51^T-AuCKNps and (B) DCY65-AuCKNps which were derived from two different *Lactobacillus* strains: *L. kimchicus* DCY51^T and *L. brevis* DCY65, respectively. HR-TEM analysis of model drug compounds derived from *L. kimchicus* DCY51^T. (C) DCY51^T-DoxorubicinNps. (D) DCY51^T-RifaximinNps.

Data from Kim, Y., Perumalsamy, H., Markus, J., Balusamy, S.R., Wang, C., Kang, S.H., Lee, S., Park, S.Y., Kim, S., Castro-Aceituno, V., Kim, S.H., Yang, D.C., 2019. Development of Lactobacillus kimchicus DCY51^T-mediated gold nanoparticles for delivery of ginsenoside compound K: in vitro photothermal effects and apoptosis detection in cancer cells. Artif. Cell Nanomed. B 47 (1), 30–44. This is an open access article distributed under the terms of the Creative Commons CC BY license, with permission from Taylor & Francis.

3.2 Extracellular method

A large number of studies had emphasized the extracellular mode of synthesis as nitrate reductase mediated mechanism involving conversion of the metal ions to metal nanoparticles (Chauhan et al., 2015; Kovachev and Dobrevski-Vacheva, 2013). Generally, extracellular pathway is enzyme-mediated. Enzymes of the cell membrane are released into the growth medium and function as extracellular enzyme. In nitrogen cycle, formation of the nitrite from nitrate is catalyzed by nitrate reductase. During Zn^{2+} bioreduction, an electron is transferred from the NADH by NADH dependent reductase that serves as electron carrier. Subsequently, Zn^{2+} receives the electron and gets reduced to Zn^0, thereby, resulting in the formation of ZnO nanoparticles (Hulkoti and Taranath, 2014). Secreted microbial proteins also play a major role in the synthesis of nanoparticles. Though such proteins are not retained in the native form, they can facilitate the interaction between amino acids and zinc ions for obtaining the zinc oxide nanoparticles. This might be due to the heat influenced disruption of hydrogen and non-polar hydrophobic bonds (Jain et al., 2013). Proteins are employed as capping and reducing agents to ensure the stability and monodispersity

of nanoparticles. Some yeasts use the protein ligands as a capping agent to prevent the aggregation of nanoparticles (Bao et al., 2010).

3.3 Optimization of the microbial nanoparticle synthesis

Size and shape play important role in determining the desired function and properties of the synthesized nanoparticles. Although the biological or microbe mediated synthesis could enable an easy scale-up of nanoparticles, it encounters potential pull-back due to the latter's non-uniform size and morphological features in contrast to those resulting from the usual physical and chemical methods. Therefore, proper measures must be followed for optimizing the physicochemical properties such as pH, temperature and other factors like precursor concentration, microbial age as well as the reaction period to enhance the performance of biosynthesized nanoparticles (Yusof et al., 2019). The pH is known to promote the capping process and hinder the aggregation of nanoparticles (Verma and Mehata, 2016). Biosynthesis of the gold nanoparticles by *Verticillium luteoalbum* was studied under the varying pH conditions. Spherical shaped, 2 to 4nm-sized nanoparticles were observed at pH3 while the differently sized irregular shaped nanoparticles were obtained at the higher pH7 and 9 (Gericke and Pinches, 2006). Temperature is also known to impact the size and yield of the nanoparticles. The nanoparticles produced by *Trichoderma viridae* at 40°C are small in size and exhibit monodispersity. However, lower temperatures gave rise to largely unstable nanoparticles. Similarly, *Sclerotinia sclerotiorum* mediated synthesis of silver nanoparticles at 80°C was studied to have a uniform size range in the order 10–15nm. This implied that higher temperature was able to promote the kinetic energy required for accelerating the rate of synthesis of smaller nanoparticles (Saxena et al., 2016). Sufficient concentration of the precursors is vital for obtaining the nanoparticles having a definite size without being aggregated (Yusof et al., 2019). Appropriate age of the microbial culture is essential to witness the increased yield. According to a study, the highest yield of gold nanoparticles was observed after the 24h of *Verticillium luteoalbum* harvest. This demonstrated that the cells of the early exponential stage were capable of secreting higher amounts of proteins and enzymes that are helpful in the frenetic reduction of metal ions (Gericke and Pinches, 2006).

4 Biomedical applications of nanoparticles

Physical and chemical methods of the nanoparticle production are supported by some agents including the ultraviolet radiation, aerosol spray, laser ablation and toxic chemicals respectively. Still, as a matter of fact, both the former methods owe their flaws. The physical strategy explodes an extensive amount of energy and expense but results in limited NP synthesis while the chemical approach characteristically deals with toxic reagents that impart chemical contamination through the generated by-products. The biological model is best suited as it is an eco-friendly method of synthesis which attributes great yield, in addition to being low cost and non-toxic.

The microorganism derived nanoparticles and its non-hazardous properties have promising roles in targeted delivery of the drugs, treatment of cancer and rheumatology, gene therapy, analyses of DNA, biosensors, diagnostic techniques like magnetic resonance imaging (MRI). Moreover, they are also known to possess the antibacterial, antimicrobial, anti-inflammatory and antioxidant effects (Das et al., 2017). Toxicity evaluation study of the green synthesized ZnO NPs presented dose-dependent results wherein the male and female Sprague Dawley rats were administered with 31.25mg/kg of 100nm ZnO. At this level, the ZnO NPs did not display any adverse symptoms in test groups (Kalpana and Devi Rajeswari, 2018). These bio-based metallic nanostructures resemble the properties of those obtained from that of the chemical process. The *Klebsiella pneumoniae* synthesized CdS quantum dots present in the cell membrane surface is non-corrosive as the harmful features of CdS species are overruled by the photochemical and photo-physical properties of NPs that are also found to function as the bio-semiconductor. On the other hand, CdSe nanoparticles formed by the *Chlorella pyrenoidosa* are used for the sensing of an antibiotic called imatinib. The interaction of human serum with titanium-based surgical instruments could lead to the development of cancer cells. The nano-titanium particles of bacterial origin could render their potential therapeutic applications in cancer chemotherapy and other gene delivery mechanisms (Das et al., 2017). The *Bacillus brevis* based synthesis of the silver nanoparticles shows antimicrobial features against the multidrug-resistant strains of *Staphylococcus aureus and Salmonella typhi*. The cotton fabrics impregnated with the ZnO nanoflowers obtained from *Serratia ureilytica* and CuO NPs of the *Halomonas elongate* demonstrate the suitable antibacterial effects against *S. aureus and E. coli*. Dose-dependent anticancer activity of the iron oxide nanoparticles having superparamagnetic nature and are extracted from the *Bacillus cereus* is observed against the MCF 7 and 3T3 cell lines. Spherical gold nanoparticles of specific sizes isolated from the bacterial species such as *Klebsiella pneumonia* (10–15nm) and *Pseudomonas fluorescens* (5–50nm) exerted their antibacterial effects. *Streptomyces griseoplanus* based production of the spherical Ag NPs of 19.5–20.9nm size is known for their antifungal characteristics. In case of fungal species, Ag NPs synthesized from the *Ganoderma sessiliforme* were reported to showcase antibacterial, antioxidant and anticancer effects at the spherical shape with 45nm size. While same Ag NPs synthesized by the fungi namely *Rhodotorula glutinis* and *Rhodotorula mucilaginosa* could elicit the distinguishable antifungal, dye degradation, cytotoxic activities. The well-established native strain of *Penicillium chrysogenum* could solicit renowned medicinal properties by synthesizing the Pt NPs which is cytotoxic. The AgCl nanoparticles synthesized by *Macrophomina phaseolina* are employed for their antibacterial features. The Nostoc species generated silver nanoparticles procured effective cytotoxicity against the MCF 7 breast cancer cell lines. Among the algal group**,** studies about the ZnO NPs obtained from *Sargassum muticum* indicated the reduction of angiogenesis and stimulation of apoptosis in the HepG2 liver carcinoma cells. The 7nm sized CuO NPs from the algae *Cystoseira trinodis* demonstrated the significant antibacterial and antioxidant properties. Members of the strain *Cystoseira baccata* are reported to enhance the anticancer activities of

the intrinsically synthesized Au nanoparticles. Antibacterial attributes of the ZrO_2 NPs synthesized from *Chlorella pyrenoidosa* play a crucial role in the clinical applications (Ovais et al., 2018). The large surface area of the nanoparticles is optimized as the functional unit to bring about the multimodal detection or bioimaging of different objects. Doped GdZnO quantum dot (QD) nanosystem is purposed for the magnetic resonance imaging (MRI) and optical imaging. The Fe_3O_4-ZnO fabricated magnetic quantum dot core-shell is potentiated for visualizing the tumor therapeutic response. The drug delivery applications of ZnO are facilitated by interactional surface chemistry, phototoxic effects and enlarged surface area of the nanosystem. Blue fluorescent ZnO QDs are bound to the folate conjugated chitosan via electrostatic attachment and doxorubicin (DOX) utilizing the hydrogen bonding, thereby promoting the intrinsic entrapment of the latter drug. The drug is released out of the NP based carrier at physiological pH7.4. And this drug delivery system is commonly employed in cancer chemotherapy. In the cancer immunotherapy, Fe_3O_4-ZnO core-shell NP delivers the carcinoembryonic antigens to dendritic cells. Exciting gene delivery applications include the release of green fluorescent protein gene named pEGFPN1 DNA from the 3D tetrapod-like zinc nanostructures; Low cytotoxicity and increased plasmid DNA encapsulation efficiency of the ZnO is enhanced upon the positively charged polymer poly(2-(dimethylamino)ethyl methacrylate) [PDMAEMA] coating. Biosensing quality of the ZnO NPs is accredited to its high adsorption property, strong catalytic roles with an increased isoelectric point (IEP) where such characteristics complement the electric charge based reactions with antibodies and enzymes that are having less IEPs. As a result, ZnO based cholesterol, glucose and urea detectable biosensors are market commercialized in recent times (Kalpana and Devi Rajeswari, 2018). 1,2-dinitrochlorobenzene induced contact dermatitis in the swine model leads to the development of pro-inflammatory cytokines such as tumor necrosis factor-α (TNF-α) and transforming growth factor β (TNF-β) that are altered by silver nanosystems, thus, preventing the initiation of the inflammatory cascade. Human clinical studies had emphasized the antimicrobial activity of Ag nanoparticles coated biofilm where the response is enhanced by the reduction of inflammatory response which is brought about by the decreased cytokine outflow, matrix metalloproteinase levels, lymphocyte and mast cell infiltration. These hallmarks revealed the anti-inflammatory action of silver nanosystems.

5 Bacteria based nanoparticles

By using the Zn and ZnS, magnetite-ZnO nanostructures of the size order 16.35 and 22nm were produced by *Pseudomonas stutzeri* and *Brevundimonas diminuta* respectively. Magnetite was formed from the amalgamation of the given NPs with siderophore molecule or other Fe compounds. ZnO is obtained from the *Brevundimonas diminuta* at 23°C and pH7 during light conditions. Since the bioreactor production of ammonia by *Paenibacillus* is restricted after 2.5h, enzyme function of the strain is stabilized through the adsorption of cell-free extract by TiO_2 nanostructures.

This process is prompted by continuous shaking for 2h carried out at 4°C. The adsorptive enzyme NP conjugate promoted the synthesis of NH_3 until 17h (Ghashghaei and Emtiazi, 2015). The buttermilk sourced Lactobacillus strains are capable of intracellularly growing the hexagonal 20–50nm-sized Au-Ag alloys. The crystal formation did not hinder bacterial survival and sustenance. This specific feature opens door for the recovery of metal ions and other clinical applications (Nair and Pradeep, 2002).

6 Ginseng

The immemorial glory of the traditional oriental medicine is well depicted by one of the splendid herbs called *Panax ginseng* that is either used raw or formulated with other synergistic herbs by the Chinese medicine practitioners. The genus name "Panax" which means "all-healing" had originated from the Greek word. This term was first coined by the Russian Botanist Carl A. Meyer. Ginseng species belongs to the genus Panax of Araliaceae family. The Panax is estimated to contain at least seventeen different species. Of which, *Panax ginseng* C.A. Meyer (Korean ginseng), *Panax quinquefolium* (*Xiyangshen*, American ginseng), *Panax notoginseng* (*Sanqi*) and *Panax japonicus* (Japanese ginseng) are the popularly used medicinal herbs (Leung and Wong, 2010; Choi, 2008; Mohanan et al., 2018).

6.1 Ginsenosides and its types

Ginsenosides are the active components of ginseng and was first isolated in the year 1963 (Shibata et al., 1963). These triterpene saponins are composed of the dammarane structure based skeleton where sugar moieties such as glucose, xylose, arabinose and rhamnose are linked to the C-3, C-20 or C-12 positions (Matsuura et al., 1984; De Smet, 2002; Mohanan et al., 2018). Ginsenosides are often denoted as "Rx" where "R" indicates the root letter while "x" indicates the chromatographic polarity letter given in terms of alphabetical order (Shibata et al., 1963). For instance, Ra is given to the compound with least polarity while Rb is better polar natured than the Ra. 150 different types of ginsenoside saponins are primarily classified into two groups namely: (1) the 20(*S*)-protopanaxadiol (PPD) (Rb1, Rb2, Rb3, Rc, Rd, Rg3, Rh2, Rs1) and (2) the 20(*S*)-protopanaxatriol (PPT) (Re, Rf, Rg1, Rg2, Rh1). The PPT is differentiated from PPD using the carboxyl group seen at C-6 position of the PPD (Matsuura et al., 1984; De Smet, 2002); Other ginsenoside saponins include the ocotillol saponin F11 (24-R-pseudoginsenoside) (Namba et al., 1986) and the pentacyclic oleanane saponin Ro (3,28-O-bisdesmoside) (Sanada et al., 1974). The ginsenoside Compositional range is found to vary in many different ginsengs and is dependent upon factors including the type of species, age and part of the plant, harvest season, adopted storage and cultivation methods (Lim et al., 2005). Generally, Rf ginsenoside is specific to the Asian ginseng while the F11 is useful in identifying the American ginseng (Liu et al., 2000). The ginseng roots are steam dried at 100°C from two to four hours for obtaining the darker shade red ginseng that has

the distinctive saponin content of Rg6, Rk1, Rs1, Rs2, Ra1, Ra2, Ra3, Rf2, Rg4, Rg5. This derivatization is enabled by the heat transformation and deglycosylation of original ginsenosides (Kaneko and Nakanishi, 2004). Another processed ginsenoside steamed at 120°C is called the Sun ginseng with remarkable percentages of anti-tumor ginsenosides such as Rg3, Rg5 and Rk1 (Lee et al., 1997; Liu et al., 2000; Xu et al., 2007).

6.2 Multifaceted roles of the ginsenosides

Incorporation of the methyl jasmonate to the Ginseng in vitro cultures markedly increased the production of ginsenosides which possess the antimicrobial, antifungal and antifeedant properties. These properties enable the protection of ginseng plants from biotic stress attacks (Choi et al., 2005; Sung and Lee, 2008; Katerere et al., 2003; Mallavadhani et al., 2003). Ginsenosides are known for exhibiting diverse pharmacological actions that are furnished with the inherent steroid analogue structures which would ensure the interaction between cell membrane-bound ion channels and the extracellular, intracellular receptors. This kind of association can produce a desired modification in the transcriptional stage (Mohanan et al., 2018). Binding of ginsenosides to the suitable receptors is known to modulate the expressions and functions of the latter group that comprise of the receptor tyrosine kinase (RTK), serotonin (5-HT), NMDA and nicotinic acetylcholine receptors (AChR). Direct attachment of the ginsenosides to the ligand-binding sites of steroid hormone receptors is well depicted through various experiments (Leung and Wong, 2010). The Ginsenosides of Rg1 and Re (Leung et al., 2006; Leung and Yue, 2007) classes act as the functional ligands of glucocorticoid receptors (GR) while the Rh1 and Rb1 active components bind well with estrogen receptors (ER), especially in the case of ERβ isoform for Rb1 (Leung and Yue, 2007). The anti-stress and adaptogenic features of Rg1 and Re ginsenosides are due to partial agonist action of the latter compounds as they are involved in inhibiting the binding of synthetic glucocorticoid hormone analogue dexamethasone to GR and exerting limited steroidal stimulation during the low threshold binding of the ligand with a receptor (Leung et al., 2006; Leung and Yue, 2007). Most ginsenoside compounds are capable of binding to numerous steroidal hormone receptors and elicit a coordinated response. Apart from GR, ginsenoside Rg is observed to interact with the ER as well as promote the cross-talk with insulin-like growth factor-1 receptor (IGF-IR) that are seen in the neuronal cells. The Synchronized effects of progesterone, androgen and ER α isoform receptors prospect the action of ginsenoside Re upon the cardiac myocytes (Furukawa et al., 2006). These multi-targeted actions attribute the multi- functional roles of ginsenosides.

Supportive observations from several in vivo and in vitro studies had stated the pharmacological effects of Rg1, Rg3 and Rb1 upon the cardiovascular, neuronal, immune systems whereby the active ginseng components serve as standardized neuroprotective, anticancer and antidiabetic agents. The Rg1, Rg3 and Rb1 are found abundantly in the crude fractions of protopanaxadiol (PPD), protopanaxatriol (PPT) and Korean Red Ginseng extract respectively. An intense insight about

their involvement in different signal transduction mechanisms and related molecular activities is required to assess their respective efficacies during the various pathophysiological conditions including cancer, neuronal diseases, diabetes mellitus and cardiovascular diseases (Mohanan et al., 2018).

6.3 Need of the ginsenoside nanosystems

Despite their tremendous pharmacological potentials and other therapeutic roles in multiple maladies, ginsenosides suffer from the limitations of having decreased oral bioavailability which is evident from the rat models as below 5%. The complete systemic utility of herbal products is suppressed due to the physicochemical factors of poor water solubility, low membrane permeability, instability in the gastrointestinal fluid and accelerated body metabolism. To improve the drug-delivering activity and tap into the other holistic benefits of ginsenosides, some in vivo properties of the active component must be considered before the development of nanocarriers. The big dammarane skeleton of ginsenosides often affects the passage of an active substance through the cell membrane and therefore, the gastrointestinal energy-dependent transporter namely sodium-dependent glucose co-transporter 1 assist the cellular uptake of ginsenosides. The oral bioavailability is also coordinated by the p-glycoprotein efflux system that revokes the entry of ginsenosides. The amount of sugar units' present is directly linked to the solubility of different ginsenoside classes. This is endorsed by the poor cellular uptake and processing of the anticancer ginsenosides as they are aglycone in nature. Acid hydrolysis and enzymatic digestion of the ginsenosides by intestinal microflora could lead to the loss of the former's therapeutic efficiency. The selected delivery system must ensure proper release of the ginsenosides at a targeted site. In addition, it should be biocompatible, biodegradable, safe, non-toxic and inert with the drug compound. The Ginsenosides' dissolution, protection from the GIT fluids and enzymes, cell permeation are all influenced by the suitable drug carrier or delivery agent that would overcome the poor absorption and bioavailability of ginsenosides. The composition, type of pharmaceutical components, size and shape of the delivery system are responsible for the optimal targeted release of the compound with reduced systemic toxicity. When higher dosage of the ginsenosides is administered for long period, some of the encountered toxic effects include hypertension, anxiety, insomnia, diarrhea and vomiting which are all could be prevented ().

6.4 Ginsenosides-based micro/nanocarriers

The nanosized delivery vehicles of ginsenosides are attributed for promoting the bioavailability rate. They are detailed as follows:

6.4.1 Emulsion systems

Generally, the emulsions are made up of two phases namely oil and water. They are classified into the oil in water (O/W) type or water in oil (W/O) type appropriately.

6.4.1.1 Microemulsions

The main advantage of microemulsion system is the thermodynamic stability which is enhanced with the use of an appropriate amount of oil, water, surfactant, and co-surfactant. Smaller droplet size with large surface area and good drug dissolution rates of the microemulsion makes it readily available for increasing the in vivo absorption rates and bioavailability of the low soluble drugs such as ginsenosides. The nanometer-sized microemulsions possess better efficiency than the micrometer sized emulsion systems. Also, the route of drug administration plays a crucial role in facilitating the improved target organ uptake along with soaring therapeutic potential. The nasal route is desired for drug entry into the brain where the active medicinal ingredient surpasses the blood-brain barrier and reaches cerebrospinal fluid (CSF). Other significant prospects of the former mode include first-pass metabolism bypass, reduced enzyme activity, accelerated therapeutic response and high systemic absorption. As Tao et al reported, during the nasal mode of entry, the ginsenosides Rg1 and Rb1 loaded on to O/W microemulsion shows relatively high absorption rates with peak concentrations of the active ginsenosides in the brain to about 126.31% and 147.48% than the control ginsenoside solution alone. Therefore, the ginsensoside microemulsion carriers could be delivered in brain for facilitating the potentiating levels of bioavailability when administered intranasally. Intraduodenal administration of the ginsenoside Rg1 to rats was studied to have improved the dissolution and intestinal uptake rates of the compound which had increased from 268% to 1270% (Kim et al., 2018).

6.4.1.2 Nanoemulsions

Overall compositional features are similar to that of the microemulsion systems. The dispersive effect of the nanoemulsion is optimized by the mechanical forces generated from the high-pressure homogenizer and ultrasound generator. Generally, the nanoemulsions are 100nm with low surfactant levels in the structure. These characteristics exert pronounced effects on drug absorption and bioavailability. The low oral bioavailability of the *Panax notoginseng* saponins (PNS) is remarked by the actions of glycosidase, gastric acid and microflora of the GIT. Nanoemulsion system of the PNS is made up of oil phase, a mixture of surfactants and other co-surfactants. Then this PNS solution was stirred well and subjected to the high-pressure homogenization. The method usually procures the colorless solution of PNS nanoemulsions. A rapid test evaluated the stability and shelf life of the former solution to be 6months in the absence of turbidity. Controlled and sustained release of the PNS out from nanoemulsions had occurred within 48h while active substances of the plain PNS solution were absorbed into the systemic circulation at the 10h stretch. The pharmacokinetic activities of PNS nanoemulsions in rats had revealed the overall absorption rate and bioavailability as 5 times and 2.58 times greater than that of the raw PNS. This might be due to the highly permeable nature of the PNS dispersed phase enclosed in the small droplets of nanoemulsion (Kim et al., 2018).

6.4.2 Polymer microparticles

The commonly encountered size ranges from 1 to 1000μm. The polymer-based microparticles demonstrate the elevated drug absorption rates in GIT as well as the constant bioavailability through the regulated release of drugs. The Rg-1 bound gelatin microspheres (GMS) crosslinked with genipin were administered in rats through the intramuscular mode. These structures were then observed for their angiogenic effect and left ventricular activity. The crosslinked GMS was much stable in contrast to the non-crosslinked GMS which had distorted in the aqueous environment. Also, active Rg-1 released out of GMS had promoted the cardiac angiogenesis along with inhibition of the left ventricular hypertrophy and myocardial fibrosis. The chitosan and collagen (CC) scaffold coated with Rg-1 loaded GMS lead to new cell proliferation and movement including the formation of tube human umbilical vein endothelial cells which is the signatory feature of skin tissue regeneration. Rg-3 holding capacity of the chitosan microspheres is evaluated by following the nasal administration where the prolonged accumulation of any drug is prevented due to the rapid clearance response. However, chitosan microspheres containing active ginsenosides are known to protect the latter from enzymatic digestion and thereby contribute to the prolonged Rg-3 stay in the nasal cavity. Another experiment had suggested the antifatigue role of Rg-3 through the investigation of swimming time taken by the weight loaded mice. It was observed that the duration exceeded by 12.5% after injecting the Rg-3 contained chitosan microspheres in comparison to the usual time with Rg-3 aqueous solution. The enteric coated and mucoadhesive polymer-based microparticles are developed using the anionic polymer Eudragit L100-55 that solubilize at pH5.5 to 7 and the chitosan employed is responsible for the mucoadhesive property. This microparticle system protected the ginsenoside degradation from the acidic environment of the stomach and enabled monitored release of the compound. At pH1.2, the drug release is restricted while the pH6.8 promoted sustained release rates of the ginsenosides (Kim et al., 2018).

6.4.3 Polymer nanoparticles

The polymeric nanoparticles are extremely small of the order 10 to 1000nm. Improved dissolution, uptake, release rate and protection of the drug from in vivo factors combined with the facile target site deposition are the important set of advantages offered by these nanovectors. Voruganti et al reported the anticancer property of novel ginsenoside 25-OCH3-PPD (GS25) by the negative regulation of P53 tumor suppressor gene in the rat models. Although the oral bioavailability of GS25 is low due to existing hydrophobicity, administration of the GS25 bound polyethylene gly*co*l (PEG)-poly(lactic-co-glycolic acid) (PLGA) nanoparticles (GS25NP) had elevated the former peaks of oral bioavailability. PLGA had increased the rate of drug dissolution and bioavailability in the above case. However, the systemic presence of drug is rapidly eliminated. To circumvent this, PEG is coated as a hydrophilic polymer for ensuring the systemic drug stability over time. The encapsulated GS25NP had exhibited great anticancer activities with both in vitro and in vivo models. By using the double emulsion/solvent evaporation method, salvianolic acid B, tanshinone IIA

and PNS were mixed with the PLGA nanoparticles and administered through the intratymphanic route in guinea pigs. Ginsenoside R1 contained in the PLGA nanoparticles was released after 72h of injection. And later it was destined to the brain, inner ear and cerebrospinal fluid (CSF). The active component was able to attenuate the oxidation activities and thus, preventing the brain cerebral ischemia through the expression of superoxide dismutase enzyme in serum and brain. This observation had signified the role of PLGA nanoparticles in the treatment of brain diseases. The hydrophobic ginsenoside compound K (CK) was lined along the hydrophobic polymer backbone of glycol chitosan (GC), thereby leading to the self-assembly of nanoparticles at the buffer physiological pH7.4. This nanostructure was observed to collapse at a slightly higher acidic environment of the tumor and inflammatory areas. Hence, it could serve as the pH-dependent delivery system of ginsenosides. The ginsenoside Rb1 is coupled with betulinic acid, dihydroartemisinin, and hydroxycamptothec to form the stable self-assembled nanoparticles of 100nm that has a higher systemic half-life and less toxic than other free drugs. Thus, from the cited examples, it is clear that the self-assembled ginsenoside based nanocarriers serve as an effective drug delivery system for cancer treatment (Kim et al., 2018). The ginsenoside compound K (CK)-polymer conjugates can kill HT29 cancer cells while allowing cell survival and proliferation in the murine macrophage RAW264.7 cell lines. These reports are deviating from the effects of CK alone. Individualistic effects of the Rg-3 and Rg-3-PEG-PLGA on lewis lung cancer mice model had demonstrated the downregulation of cancer-related proteins by the latter nanoconjugate system, though, not significantly distinguished from that of the former's effects (Mathiyalagan and Yang, 2017).

6.4.4 Liposomes

These are the small phospholipid bilayer vesicles that can encapsulate both hydrophobic and hydrophilic drugs and protect them from the degradative action of gastric acids and enzymes. Liposomes are biocompatible and biodegradable with an enlarged surface area that facilitates the high drug absorption rates, especially, of the low aqueous soluble molecules and hikes the drug bioavailability nature. The Rg-3 entrapped liposomal system was scrutinized for its potential anticancer and pharmacokinetic properties after the treatment in wistar rats. Good pharmacokinetic C_{max} and area under the curve (AUC) factors were observed to correspond with the better dissolution and cellular permeability rates of Rg-3 along with the remarkable tumor reduction properties. In the aqueous suspension, liposomes are shown to form aggregates with one another between the internal spaces, which might lead to drug spillage. Sometimes, even the lipid bilayer compound undergoes oxidation or hydrolysis. Thus, the overall stability and integrity of the liposomes become liable to disaggregation. Therefore, methods such as freeze-drying, vacuum drying, fluidized bed drying are adopted to evaporate the aqueous part of liposomes. This results in the formation of solid-state proliposomes. Employing the lyoprotective agents such as glucose and mannitol for freeze-drying of liposomes is known to maintain the biological efficacy and retention of the drugs or active components, in addition to generating the uniform-sized carrier particles. This method impairs the anticancer

effects of Rg-3 loaded liposomal vehicle. Ginseng fruit saponin (GFS) loaded on to the proliposome (GFS-P) was mixed with the bile salt named sodium deoxycholate to promote the better absorption of medicinal component in GIT. At pH1.2 and 6.8, the GFS was released out of GFS-P at a controlled and sustained rate. The drug encapsulation efficiency and particle size distribution of the proliposome remained same even after three months at the room temperature 37°C. This ensures the stability of the drug delivery system. Biomembrane permeability of the ginsenoside Re through the GIT is excellent in the presence of bile salt. The significant pharmacokinetic C_{max}, increased AUC (area under the curve) and higher half-life was projected by the GFS-P in rats (Kim et al., 2018). The liposomal Rg-3 and raw Rg-3 solutions were tested in human liver cells (HepG2) and lung cancer cell lines (A549) for their anticancer efficacy where maximal cytotoxicity is mediated by the drug nanocarrier group than the plain drug group (Mathiyalagan and Yang, 2017).

6.4.5 Ethosomes and transfersomes

Though incorporated with the anti-allergic and anti-inflammatory properties, traditional ginsenosides do not possess the deep dermal penetrative properties upon topical application. To overcome this, higher level liposomes such as ethosomes and transfersomes are employed. Ethosomes are composed of the phospholipid, ethanol and water while the transfersomes are made up of phospholipids and the edge activators or surfactants in the lipid bilayer. Both are known to confer the demanded lipid layer flexibility through ethanol and surfactants that further disrupt the skin component organization. Transfersome-based topical application of the ginsenoside Rh-1 is found to be greatly reliable for the well dermal intrusive and drug entrapment efficiency. The next range of efficacy was demonstrated by ethosomes and liposomes in order. Flexible liposomal delivery systems are suited for topical applications of the ginsenosides (Kim et al., 2018).

6.4.6 Niosomes

Niosomes are the amphiphilic vesicles layered with non-ionic surfactant molecules. They can entrap drugs such as ginsenosides that have a wide range of water solubility. They are constructed as the pH-responsive double-coated mixed micelles containing ginsenoside Rh-1 and the surface agent named Pluronic F-68. This Rh-1 loaded multicore niosomal structure is called Rh-1 MCN. It acts as anticancer agents that are carefully accumulated at the tumor sites and diminishes the cancer cell count after two weeks. The pH based drug release study demonstrated the Rh-1 outflow at pH5 and leak-free stiff niosomal compartment at pH7.4 (Kim et al., 2018).

7 Microbial synthesis of the ginsenoside nanoparticles and applications

A direct intracellular membrane-bound synthesis of gold nanoparticles from the Korean Kimchi derived Probiotic *Lactobacillus kimchicus* DCY51^T was proposed by Markus et al which is elaborated as follows: 100mL MRS broth

containing the aforementioned probiotic is incubated at 37°C for 24h in the shaking rpm of 100 ×g. About 25mL of the culture broth is centrifuged at 6300×g for 5min. The cell biomass recovered after centrifugation is rinsed and dissolved in 15mL sterile distilled water. Followed by the incorporation of filter-sterilized gold salts of 1mM concentration, the cell suspension is incubated at 37°C with shaking speed of 150 ×g in the dark. Intracellular formation of the AuNP was monitored by color change of the reaction mixture. The ultrasonication and centrifugation at 2500× g are performed repeatedly at regular intervals of 5min. Each time the cell pellet was recovered carefully. At high speed of 28,000 ×g for 10min, the AuNP is harvested and purified with 80% methanol. The formed AuNP was utilized as a dried powder after the overnight incubation. At 540nm, Ultraviolet-visible spectrophotometry had revealed the biological reduction of HAuCl4 to AuNP that are of uniform spherical shapes with a size range between 5 and 30nm. No toxicity is rendered upon the murine macrophage (RAW264.7) and human colorectal adenocarcinoma (HT29) cell lines. This is attributed to the surrounding amino acid residues and surface-bound proteins of AuNP that serve as capping agents. Free radical scavenging activity of the AuNP is enormous against DPPH when compared to the corresponding gold salt. In addition to this, prolonged stability in the physiological buffers and biological media ensure the promising role of probiotic mediated drug carriers in the targeted therapies of cancer, diagnosis, photothermal therapy, biosensing and imaging (Markus et al., 2016).

In the concurrent study, an exciting drug delivery system is explored using the AuNP which is non-covalently loaded with ginsenoside compound K (AuCKNps) through the one-pot biosynthesis process facilitated via *Lactobacillus kimchicus* DCY51^{T}. The DCY51^{T}-AuNps are prepared by following the protocol of Markus et al., with slight modifications. The cell biomass recovered by centrifugation is subjected to the 10mM PBS wash and suspended in 25mL sterile distilled water. About 1mM AuNp and 1mg of ginsenoside compound K were supplied to the cell pellet and the resulting culture suspension is incubated at 37°C with the shaking at 150 ×g under dark conditions. Visual perception helps in detecting the formation of DCY51^{T}-AuCKNps in this environment. Then the dark purple reaction mixture is subjected to the centrifugal spinning at 2500× g for 5min and the supernatant containing unbound nanoparticles as well as the drugs should be discarded. Drug carrying nanoparticles are then harvested from bacterial cells through the series of ultrasonication and centrifugation at 2500× g for 5min. At last, the integrated DCY51^{T}-AuCKNps are collected by centrifugation at 28,000 ×g for 10min. Furthermore, the DCY51^{T}-AuNps and DCY51^{T}-AuCKNps are stored at 4°C as water-based solutions for future use. The powdered form of the above combination is obtained by air-drying overnight. Then the ginsenoside bound AuNPs are leveraged for different analytical and spectroscopic studies including the field emission transmission electron microscopy (FE-TEM), energy-dispersive X-ray (EDX) spectroscopy, elemental mapping, X-ray powder diffraction (XRD), selected area electron

diffraction (SAED), Fourier-transform infrared (FTIR) spectroscopy and the dynamic light scattering (DLS). Also, the drug-binding capacity of nanoparticles was evaluated using liquid chromatography-mass spectrometry (LC-MS). No mere accumulation of the DCY51^T-AuNps and DCY51^T-AuCKNps upon each other was observed during the induction of pH change. On separately treating the A549 cells (human lung adenocarcinoma cell line) and HT29 (human colorectal adenocarcinoma cell line) with DCY51^T-AuCKNps and ginsenoside compound K solution, the former had slightly increased cytotoxicity than the latter. A combined laser directed treatment with DCY51^T-AuCKNps had increased the cellular apoptosis in A549, HT29 and AGS cells (human stomach gastric adenocarcinoma cell line) in contrast to the cells treated with DCY51^T-AuCKNps alone. Hence the study demonstrated the synergistic anticancer activity of DCY51^T-AuCKNps which could serve as potential photothermal and chemotherapeutic agents (Kim et al., 2019).

8 Futuristic views

Biological or green synthesis of nanoparticles is the massive mechanism with exciting scale-up possibilities, reduced cost and eco-friendly approach. However, as the technique is still in its infancy stage, the ignition issues like maintenance of shape, size, stability and aggregation-based regulation of the nanostructure growth hurdle the futuristic outlook. Henceforth, prime enhancement of the green synthesis should facilitate the focus on finding out the mechanistic approaches involved in nanoparticle synthesis, associated impact of the enzymes and proteins, elucidation of conservative properties of the bio-based nanosystems, exploration of the novel and systematic methods of nanoparticle purification without chemical use, the establishment of the non-toxic microbial nanofactories with efficient optimization of the size and shape, manufacture feasibility, cost deployment by utilizing recyclable materials and quick mode of nanoparticle synthesis (Das et al., 2017).

9 Conclusion

The Colossal clinical aspects of ginsenosides and its nanoderivatives had gained considerable attention in the present era due to a simple method of synthesis and sophisticated therapeutic efficiency as elucidated above. The microbial modes of green nanoparticle generation and operable drug delivery systems are of crucial importance in the enhancement of targeted diagnostics and therapies. This also helps in visualizing the chemical-free future environmental trends. Therefore, this technology should be optimized for commercial uses by eliminating the currently employed energy-intensive and toxic methods. The revolutionary synthesis of probiotic DCY51^T based ginsenoside compound K loaded gold nanoparticles serves as a suitable delivery vehicle for distressing the cancer region specifically, thereby, promoting the destruction.

References

Abedi, D., Feizizadeh, S., Akbari, V., Jafarian-Dehkordi, A., 2013. *In vitro* anti-bacterial and anti-adherence effects of *Lactobacillus delbrueckii* subsp *bulgaricus* on *Escherichia coli*. Res. Pharm. Sci. 8, 260–268.

Ahmad, A., Senapati, S., Khan, M.I., Kumar, R., Ramani, R., Srinivas, V., Sastry, M., 2003. Intracellular synthesis of gold nanoparticles by a novel alkalotolerant actinomycete, *Rhodococcus* species. Nanotechnology. 14 (7), 824.

Bao, H., Hao, H., Yang, Y., Zhao, D., 2010. Biosynthesis of biocompatible cadmium telluride quantum dots using yeast cells. Nano Res. 3 (7), 481–489.

Benmechernene, Z., Chentouf, H.F., Yahia, B., Fatima, G., Quintela-Baluja, M., Calo-Mata, P., Barros-Velázquez, J., 2013. Technological aptitude and applications of *Leuconostoc mesenteroides* bioactive strains isolated from algerian raw camel milk. Biomed. Res. Int. 2013. https://doi.org/10.1155/2013/418132, 418132.

Bhattacharya, R., Mukherjee, P., 2008. Biological properties of "naked" metal nanoparticles. Adv. Drug Deliv. Rev. 60 (11), 1289–1306.

Bordoni, A., Amaretti, A., Leonardi, A., Boschetti, E., Danesi, F., Matteuzzi, D., Roncaglia, L., Raimondi, S., Rossi, M., 2013. Cholesterol-lowering probiotics: *In vitro* selection and *in vivo* testing of bifidobacteria. Appl. Microbiol. Biotechnol. 9, 8273–8281.

Castillo, N.A., de Moreno de LeBlanc, A., Galdeano, C.M., Perdigón, G., 2013. Comparative study of the protective capacity against *Salmonella* infection between probiotic and nonprobiotic *Lactobacilli*. J. Appl. Microbiol. 114, 861–876.

Chauhan, R., Reddy, A., Abraham, J., 2015. Biosynthesis of silver and zinc oxide nanoparticles using *Pichia fermentans JA2* and their antimicrobial property. Appl. Nanosci. 5 (1), 63–71.

Chmielewska, A., Szajewska, H., 2010. Systematic review of randomised controlled trials: probiotics for functional constipation. World J. Gastroenterol. 16, 69–75.

Choi, K.-T., 2008. Botanical characteristics, pharmacological effects and medicinal components of Korean *Panax ginseng* C A Meyer. Acta Pharmacol. Sin. 29 (9), 1109–1118.

Choi, D.-W., Jung, J.D., Ha, Y.I., Park, H.-W., et al., 2005. Analysis of transcripts in methyl jasmonate-treated ginseng hairy roots to identify genes involved in the biosynthesis of ginsenosides and other secondary metabolites. Plant Cell Rep. 23 (8), 557–566.

Cortés-Zavaleta, O., López-Malo, A., Hernández-Mendoza, A., García, H.S., 2014. Antifungal activity of *Lactobacilli* and its relationship with 3-phenyllactic acid production. Int. J. Food Microbiol. 173, 30–35.

Das, R.K., Pachapur, V.L., Lonappan, L., Naghdi, M., Pulicharla, R., Maiti, S., Cledon, M., Dalila, L.M.A., Sarma, S.J., Brar, S.K., 2017. Biological synthesis of metallic nanoparticles: plants, animals and microbial aspects. Nanotechnol. Environ. Eng. 2 (18). https://doi.org/10.1007/s41204-017-0029-4.

De Smet, P.A.G.M., 2002. Herbal remedies. N. Engl. J. Med. 347 (25), 2046–2056.

Dirienzo, D.B., 2014. Effect of probiotics on biomarkers of cardiovascular disease: implications for heart-healthy diets. Nutr. Rev. 72, 18–29.

Fernandez, B., Hammami, R., Savard, P., Jean, J., Fliss, I., 2013. *Pediococcus acidilactici* UL5 and *Lactococcus lactis* ATCC 11454 are able to survive and express their bacteriocin genes under simulated gastrointestinal conditions. J. Appl. Microbiol. 116 (3), 677–688.

Furukawa, T., Bai, C.-X., Kaihara, A., Ozaki, E., et al., 2006. Ginsenoside Re, a main phytosterol of *Panax ginseng*, activates cardiac potassium channels via a nongenomic pathway of sex hormones. Mol. Pharmacol. 70 (6), 1916–1924.

Gericke, M., Pinches, A., 2006. Biological synthesis of metal nanoparticles. Hydrometallurgy. 83 (1–4), 132–140.

Ghashghaei, S., Emtiazi, G., 2015. The methods of nanoparticle synthesis using bacteria as biological nanofactories, their mechanisms and major applications. Curr. Bionanotechnol. 1 (1), 3–17.

Guslandi, M., Giollo, P., Testoni, P.A., 2003. A pilot trial of *Saccharomyces boulardii* in ulcerative colitis. Eur. J. Gastroenterol. Hepatol. 15, 697–698.

Hempel, S., Newberry, S.J., Maher, A.R., Wang, Z., Miles, J.N., Shanman, R., Johnsen, B., Shekelle, P.G., 2012. Probiotics for the prevention and treatment of antibiotic- associated diarrhea: a systematic review and meta-analysis. JAMA 307, 1959–1969.

Homayouni, A., Bastani, P., Ziyadi, S., Mohammad-Alizadeh-Charandabi, S., Ghalibaf, M., Mortazavian, A.M., Mehrabany, E.V., 2014. Effects of probiotics on the recurrence of bacterial vaginosis: a review. J. Low Genit. Tract. Dis. 18, 79–86.

Hsieh, P.S., Tsai, Y.C., Chen, Y.C., The, S.F., Ou, C.M., King, V.A., 2012. Eradication of *Helicobacter pylori* infection by the probiotic strains *Lactobacillus johnsonii* MH-68 and *L. salivarius*ssp. *Salicinius* AP-32. Helicobacter 17, 466–477.

Huang, X., Jain, P.K., El-Sayed, I.H., El-Sayed, M.A., 2007. Gold nanoparticles: interesting optical properties and recent applications in cancer diagnostics and therapy. Nanomedicine (London) 2, 681–693.

Hulkoti, N.I., Taranath, T.C., 2014. Biosynthesis of nanoparticles using microbes—a review. Colloids Surf. B Biointerfaces. 121, 474–483.

Iravani, S., Korbekandi, H., Mirmohammadi, S.V., Zolfaghari, B., 2014. Synthesis of silver nanoparticles: chemical, physical, and biological methods. Res. Pharm. Sci. 9 (6), 385.

Jain, A., Roy, N., Gaur, A., Bhattacharya, S., Rani, V., 2013. Green synthesis of silver nanoparticles: an approach to overcome toxicity. Environ. Toxicol. Pharmacol. 36 (3), 807–812.

Janvier, A., Malo, J., Barrington, K.J., 2014. Cohort study of probiotics in a North American neonatal intensive care unit. J. Pediatr. 164 (5), 980–985.

Kalpana, V.N., Devi Rajeswari, V.M., 2018. A review on green synthesis, biomedical applications, and toxicity studies of ZnO NPs. Bioinorg. Chem Appl. 2018. https://doi.org/10.1155/2018/3569758, 3569758.

Kaneko, H., Nakanishi, K., 2004. Proof of the mysterious efficacy of ginseng: basic and clinical trials: clinical effects of medical ginseng, Korean red ginseng: specifically, its anti-stress action for prevention of disease. J. Pharm. Sci. 95 (2), 158–162.

Katerere, D.R., Gray, A.I., Nash, R.J., Waigh, R.D., 2003. Antimicrobial activity of pentacyclic triterpenes isolated from African Combretaceae. Phytochemistry. 63 (1), 81–88.

Kerry, R.G., Patra, J.K., Gouda, S., Park, Y., Shin, H.S., Das, G., 2018. Benefaction of probiotics for human health: a review. J. Food Drug Anal. 26, 927–939.

Khosravi-Darani, K., Cruz, A.G., Mozafari, M.R., Abdi, Z., Ahmadi, N., 2019. Biosynthesis of metal nanoparticles by probiotic bacteria. Lett. Appl. NanoBioSci. 8 (3), 619–626.

Kim, H., Lee, J.H., Kim, J.E., Kim, Y.S., Ryu, C.H., Lee, H.J., Kim, H.M., Jeon, H., Won, H., Lee, J., Lee, J., 2018. Micro-/nano-sized delivery systems of ginsenosides for improved systemic bioavailability. J. Ginseng Res. 42, 361–369.

Kim, Y., Perumalsamy, H., Markus, J., Balusamy, S.R., Wang, C., Kang, S.H., Lee, S., Park, S.Y., Kim, S., Castro-Aceituno, V., Kim, S.H., Yang, D.C., 2019. Development of lactobacillus kimchicus DCY51^{T}-mediated gold nanoparticles for delivery of ginsenoside compound K: *in vitro* photothermal effects and apoptosis detection in cancer cells. Artif. Cell. Nanomed. B 47 (1), 30–44.

Kovachev, S., Dobrevski-Vacheva, R., 2013. Effect of *Lactobacillus casei*var. *Rhamnosus* (Gynophilus) in restoring the vaginal flora by female patients with bacterial vaginosis-randomized, open clinical trial. Akush. Ginekol. 52 (1), 48–53.

Lee, Y.J., Chung, E., Lee, K.Y., Lee, Y.H., Huh, B., Lee, S.K., 1997. Ginsenoside-Rg1, one of the major active molecules from *Panax ginseng*, is a functional ligand of glucocorticoid receptor. Mol. Cell. Endocrinol. 133 (2), 135–140.

Lee, S.J., Bose, S., Seo, J.G., Chung, W.S., Lim, C.Y., Kim, H., 2013. The effects of co-administration of probiotics with herbal medicine on obesity, metabolic endotoxemia and dysbiosis: a randomized double-blind controlled clinical trial. Clin. Nutr. 33 (6), 973–981.

Leung, K.W., Yue, P.Y.K., et al., 2007. Pharmacogenomics and the Yin/Yang actions of ginseng: anti-tumor, angiomodulating and steroid-like activities of ginsenosides. Chin. Med. 2 (1), 1–21.

Leung, A.W.N., Cho, W.C.S., Chung, W.-S., et al., 2006. Ginsenoside Re of *Panax ginseng* possesses significant antioxidant and antihyperlipidemic efficacies in streptozotocin-induced diabetic rats. Eur. J. Pharmacol. 550 (1–3), 173–179.

Leung, K.W., Wong, A.S.-T., 2010. Pharmacology of ginsenosides: a literature review. Chin. Med. 5 (1), 1–7.

Lim, W., Mudge, K.W., Vermeylen, F., 2005. Effects of population, age, and cultivation methods on ginsenoside content of wild American ginseng (*Panax quinquefolium*). J. Agric. Food Chem. 53 (22), 8498–8505.

Liu, W.K., Xu, S.X., Che, C.T., 2000. Anti-proliferative effect of ginseng saponins on human prostate cancer cell line. Life Sci. 67 (11), 1297–1306.

Luechinger, N.A., Grass, R.N., Athanassiou, E.K., Stark, W.J., 2010. Bottom-up fabrication of metal/metal nanocomposites from nanoparticles of immiscible metals. Chem. Mater. 22 (1), 155–160.

Luoto, R., Ruuskanen, O., Waris, M., Kalliomäki, M., Salminen, S., Isolauri, E., 2013. Prebiotic and probiotic supplementation prevents rhinovirus infections in preterm infants: a randomized, placebo-controlled trial. J. Allergy Clin. Immunol. 133, 405–413.

Mallavadhani, U.V., Mahapatra, A., Raja, S.S., Manjula, C., 2003. Antifeedant activity of some pentacyclic triterpene acids and their fatty acid ester analogues. J. Agric. Food Chem. 51 (7), 1952–1955.

Markus, J., Mathiyalagan, R., Kim, Y., Abbai, R., Singh, P., Ahn, S., Perez, Z.E.J., Hurh, J., Yang, D.C., 2016. Intracellular synthesis of gold nanoparticles with antioxidant activity by probiotic *Lactobacillus kimchicus* DCY51^{T} isolated from Korean Kimchi. Enzym. Microb. Technol. 95, 85–93.

Mathiyalagan, R., Yang, D.C., 2017. Ginseng nanoparticles: a budding tool for cancer treatment. Nanomedicine (London) 12 (10), 1091–1094.

Matsuura, H., Kasai, R., Tanaka, O., 1984. Further studies on dammarane-saponins of ginseng roots. Chem. Pharm. Bull. 32 (3), 1188–1192.

Mohanan, P., Subramaniyam, S., Mathiyalagan, R., Yang, D.-C., 2018. Molecular signaling of ginsenosides Rb1, Rg1, and Rg3 and their mode of actions. J. Ginseng Res. 42 (2), 123–132.

Mohanpuria, P., Rana, N.K., Yadav, S.K., 2008. Biosynthesis of nanoparticles: technological concepts and future applications. J. Nanopart. Res. 10 (3), 507–517.

Mohseni, M.J., Aryan, Z., Emamzadeh-Fard, S., Paydary, K., Mofid, V., Joudaki, H., Kajbafzadeh, A.M., 2013. Combination of probiotics and antibiotics in the prevention of recurrent urinary tract infection in children. Iran. J. Pediatr. 23, 430–438.

Moro-García, M.A., Alonso-Arias, R., Baltadjieva, M., Fernández Benítez, C., Fernández Barrial, M.A., Díaz Ruisánchez, E., Alonso Santos, R., Alvarez Sánchez, M., Saavedra Miján, J., López-Larrea, C., 2013. Oral supplementation with *Lactobacillus delbrueckii*subsp.*bulgaricus* 8481 enhances systemic immunity in elderly subjects. Age (Dordr.) 35, 1311–1326.

Nagpal, R., Kumar, A., Kumar, M., Behare, P.V., Jain, S., Yadav, H., 2012. Probiotics, their health benefits and applications for developing healthier foods: a review. FEMS Microbiol. Lett. 334, 1–15.

Nair, B., Pradeep, T., 2002. Coalescence of nanoclusters and formation of submicron crystallites assisted by lactobacillus strains. Cryst. Growth Des. 2 (4), 293–298.

Namba, T., Matsushige, K., Morita, T., 1986. Saponins of plants of *Panax* species collected in central Nepal and their chemotaxonomical significance. I. Chem. Pharm. Bull. 34 (2), 730–738.

Ortiz, L., Ruiz, F., Pascual, L., Barberis, L., 2014. Effect of two probiotic strains of *Lactobacillus* on *in vitro* adherence of *Listeria monocytogenes*, *Streptococcus agalactiae*, and *Staphylococcus aureus* to vaginal epithelial cells. Curr. Microbiol. 68 (6), 679–684.

Ovais, M., Khalil, A.T., Ayaz, M., Ahmad, I., Nethi, S.K., Mukherjee, S., 2018. Biosynthesis of metal nanoparticles via microbial enzymes: a mechanistic approach. Int. J. Mol. Sci. 19, 4100.

Panwar, H., Calderwood, D., Grant, I.R., Grover, S., Green, B.D., 2014. *Lactobacillus* strains isolated from infant faeces possess potent inhibitory activity against intestinal alpha- and beta-glucosidases suggesting anti-diabetic potential. Eur. J. Nutr. 53 (7), 1465–1474.

Prasad, K., Jha, A.K., 2009. Zinc nanoparticles: synthesis and adsorption study. Nat. Sci. 1, 129–135.

Raz, I., Gollop, N., Polak-Charcon, S., Schwartz, B., 2007. Isolation and characterisation of new putative probiotic bacteria from human colonic flora. Br. J. Nutr. 97, 725–734.

Sanada, S., Kondo, N., Shoji, J., Tanaka, O., 1974. Studies on the saponins of ginseng. I. Structures of ginsenoside-Ro, -Rb_1, -Rb_2, -Rc and -Rd. Chem. Pharm. Bull. 22 (2), 421–428.

Sanchez, M., Darimont, C., Drapeau, V., Emady-Azar, S., Lepage, M., Rezzonico, E., Ngom-Bru, C., Berger, B., Philippe, L., Ammon-Zuffrey, C., et al., 2013. Effect of *Lactobacillus rhamnosus* CGMCC1.3724 supplementation on weight loss and maintenance in obese men and women. Br. J. Nutr. 111, 1507–1519.

Saxena, J., Sharma, P.K., Sharma, M.M., Singh, A., 2016. Process optimization for green synthesis of silver nanoparticles by *Sclerotinia sclerotiorum* MTCC 8785 and evaluation of its antibacterial properties. Springerplus. 5 (1), 1–10.

Shedbalkar, U., Singh, R., Wadhwani, S., Gaidhani, S., Chopade, B.A., 2014. Microbial synthesis of gold nanoparticles: current status and future prospects. Adv. Colloid Interface Sci. 209, 40–48.

Shibata, S., Tanaka, O., Naga, I.M., Ishit, T., 1963. Studies on the constituents of Japanese and Chinese crude drugs. XII. Panaxadiol, a sapogenin of ginseng roots. Chem. Pharm. Bull. 11 (6), 759–761.

Shida, K., Nomoto, K., 2013. Probiotics as efficient immunopotentiators: translational role in cancer prevention. Indian J. Med. Res. 138, 808–814.

Singh, R.P., Shukla, V.K., Yadav, R.S., Sharma, P.K., Singh, P.K., Pandey, A.C., 2011. Biological approach of zinc oxide nanoparticles formation and its characterization. Adv. Mater. Lett. 2, 313–317.

Slavin, Y.N., Asnis, J., Hafeli, U.O., Bach, H., 2017. Metal nanoparticles: understanding the mechanisms behind antibacterial activity. J. Nanobiotechnol. 15 (1), 1–20.

Sung, W.S., Lee, D.G., 2008. In vitro candidacidal action of Korean red ginseng saponins against *Candida albicans*. Biol. Pharm. Bull. 31 (1), 139–142.

Suzuki, S., Yakabe, T., Suganuma, H., Fukao, M., Saito, T., Yajima, N., 2013. Cell-bound exopolysaccharides of *Lactobacillus brevis* KB290: protective role and monosaccharide composition. Can. J. Microbiol. 59, 549–555.

Tiwari, D.K., Behari, J., Sen, P., 2008. Time and dose-dependent antimicrobial potential of Ag nanoparticles synthesized by top-down approach. Curr. Sci. 95 (5), 647–655.

Tomaro-Duchesneau, C., Saha, S., Malhotra, M., Jones, M.L., Labbé, A., Rodes, L., Kahouli, I., Prakash, S., 2014. Effect of orally administered *L. fermentum* NCIMB 5221 on markers of metabolic syndrome: an *in vivo* analysis using ZDF rats. Appl. Microbiol. Biotechnol. 98, 115–126.

Vaghef-Mehrabany, E., Alipour, B., Homayouni-Rad, A., Sharif, S.K., Asghari-Jafarabadi, M., Zavvari, S., 2013. Probiotic supplementation improves inflammatory status in patients with rheumatoid arthritis. Nutrition 30, 430–435.

Verma, A., Mehata, M.S., 2016. Controllable synthesis of silver nanoparticles using Neem leaves and their antimicrobial activity. J. Radiat. Res. Appl. Sci. 9 (1), 109–115.

Wright, K., Wright, H., Murray, M., 2014. Probiotic treatment for the prevention of antibiotic-associated diarrhoea in geriatric patients: a multicentre randomised controlled pilot study. Australias. J. Ageing. 34 (1), 38–42.

Xu, T.M., Xin, Y., Cui, M.H., Jiang, X., Gu, L., 2007. Inhibitory effect of ginsenoside Rg3 combined with cyclophosphamide on growth and angiogenesis of ovarian cancer. Chin. Med. 120 (7), 584–588.

Yusof, H.M., Mohamad, R., Zaidan, U.H., Rahman, N.A.A., 2019. Microbial synthesis of zinc oxide nanoparticles and their potential application as an antimicrobial agent and a feed supplement in animal industry: a review. J. Animal Sci. Biotechnol. 10 (1), 1–22.

Zelaya, H., Tsukida, K., Chiba, E., Marranzino, G., Alvarez, S., Kitazawa, H., Agüero, G., Villena, J., 2014. Immunobiotic Lactobacilli reduce viral-associated pulmonary damage through the modulation of inflammation-coagulation interactions. Int. Immunopharmacol. 19, 161–173.

Zhang, Y.C., Zhang, L.W., Ma, W., Yi, H.X., Yang, X., Du, M., Shan, Y.J., Han, X., Zhang, L.L., 2012. Screening of probiotic *Lactobacilli* for inhibition of *Shigella sonnei* and the macromolecules involved in inhibition. Anaerobe 18, 498–503.