

## **EXPLORING THE NANOCARRIER POTENTIAL OF SURFACE LAYER PROTEINS FROM LACTOBACILLUS SPECIES**

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**ABSTRACT:** Surface layer protein (SLP) is the outboard structure of bacteria which controls their adaptability and adherence to other bacteria and biological surfaces. SLP could maintain its 3D structure and has a self-assembly mechanism, even after being isolated from the host microorganism. This protein is potential to be integrated into drug nanocarriers because it has size in nanometer, protective features, self-assembly capacity, and high compatibility either with biological systems or chemical substances. It has been used as a surface coating for liposomes, emulsomes, polymeric SLP, drug delivery, significantly improved the physicochemical properties of the drug delivery system (higher stability and longer half-nanomedicine, Lactobacillus, life) and synergic bioactivities (increasing intestinal absorption; protecting against the acidic gastric environment; drug carrier. enhancing immune response, antibiofilm, and antibacterial activity). Additionally, the integration of SLP into a drug delivery system is simple and sustainable. In this review, references were searched in Scopus, PubMed and Google Scholar engines by using the terms “surface layer protein,” “SLP,” “drug carrier,” “drug delivery,” “Lactobacillus,” and “nanomedicine.” To the best of our knowledge, this work is the first review of the applications of the SLP as drug nanocarriers.

**Key words:** nanoparticles (NPs), metal NPs/nanocomposites, or as a direct nanocarrier of small molecules. Moreover, SLP

### **INTRODUCTION**

Surface layer protein (SLP) is an array of crystalline proteins or glycoproteins found on the outermost cell exterior of unicellular microorganisms and a predominant protein secreted by bacteria or archaea [1, 2]. It consists of monomeric subunits aligned in two-dimensional lattice symmetries [3]. The SLP was found in different locations depending on the host, for instance, on the peptidoglycan of Gram-positive bacteria, on the lipopolysaccharide of Gram-negative bacteria, or on the pseudomurein or other biopolymers—layers substituting peptidoglycan of the archaea [4]. The SLP directly interacts with the environment; thus, it offers protective, adhesive, antifouling, and adaptability towards extreme conditions, also plays a role in the virulence of pathogenic bacteria [4, 5]. In addition, because of the presence of the pores (approximately 2–8 nm) which are formed by

the array structures, the SLP is responsible in ion transport [2,4,6]. The percentage of the pores was 70% of the total SLP surface area [7]. The SLP was observed for the first time in *Spirillum serpens*, a Gram-negative bacterium [2]. It is visualized as hexagonal structure formed from monomeric subunit proteins. In the 1970s, it was reported that the protein could be effortlessly isolated using a high concentration (2 M) of guanidine hydrochloride because of its non-covalent binding with the cell wall [6,8]. However, the 2D hexagonal conformation is not retained by the detached SLP, yet it could be reconstituted in an appropriate substrate [8]. In correlation with the protection and adaptive function, SLP is a non-conserved protein that has undergone evolution, even being diverse at the same species level [7]. This diversity includes variety in the level of gene and protein; and phenotypic structure and function. Previously, these variances were not considered correlated with taxonomic identification; however, some evidences have been found in some species that might contribute to taxonomic classification [7]. Bharat and colleagues reported that SLP consists of 400–2,500 amino acid residues with low sequence similarity, which are predominantly hydrophobic and acidic [9]. The protein structure is generally the same in certain domains; for example, an anchoring domain is commonly built by trimers, pseudotrimers, or  $\alpha$ -helix folds and a combination of beta-sandwich, beta-roll, beta-helix, and coiled-coil structures [9]. Interestingly, among those of either bacteria's or archaea's SLPs, SLPs from *Lactobacillus* sp show unique features, such as relatively higher pI value and smaller molecular size [2,10]. Heretofore, the smallest SLP is found in *Lactobacillus* sp. (approximately 23–71 kDa), whereas most of the SLP is 40–200 kDa [11,12]. The exploration of SLP from *Lactobacillus* sp. has been reported in the last three decades, exhibiting potential in medicine and nanotechnology [3]. The position of SLP among other outer proteins of *Lactobacillus* sp. is depicted in Figure 1. In this review, we focus on prospective applications of SLP from *Lactobacillus* sp., particularly as a drug nanocarrier.

## **METHODOLOGY**

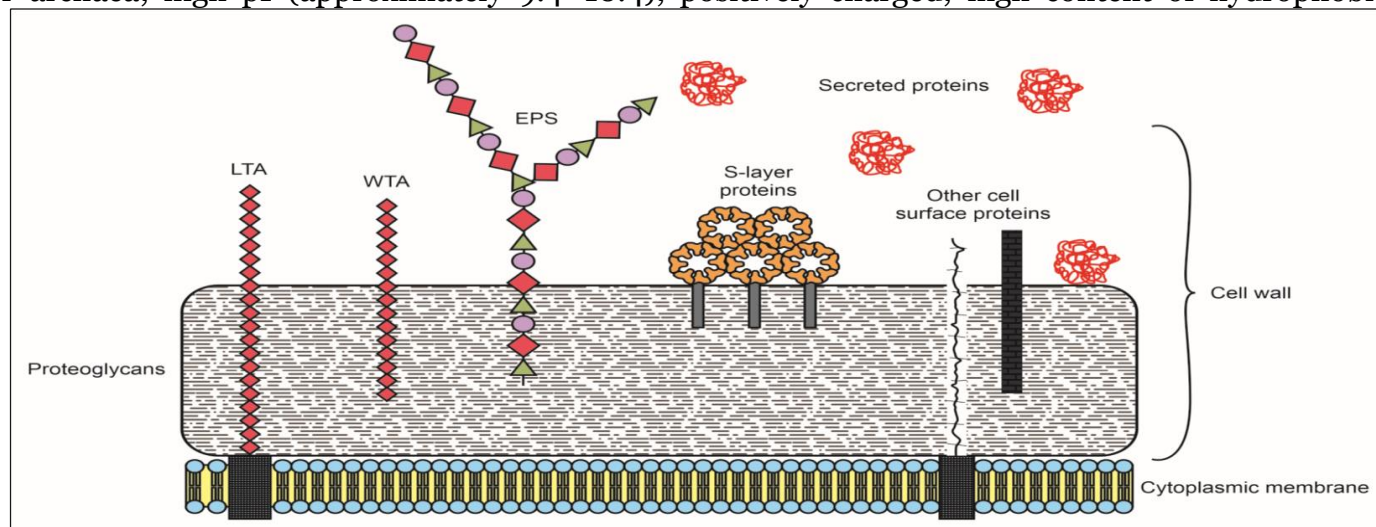
Publications that focused on SLP; *Lactobacillus* sp.; and nanomedicine or drug delivery were collected from search engines (Google Scholar, Scopus, and PubMed) until July 2024. The related publications were selected and reviewed.

## **RESULTS AND DISCUSSION**

### **Features of SLP from *Lactobacillus* sp.**

*Lactobacillus* is a genus of lactic acid bacteria (LAB), rod or coccobacilli-shaped, Gram-positive, low pH tolerant, facultatively anaerobic, non-sporing, and the largest genus among the other genera in the LAB [13–15]. The main characteristic of *Lactobacillus* sp. is the production of lactic acid (up to 85%) during carbohydrate fermentation [16]. These bacteria are commonly identified in healthy digestive and female genitourinary tracts [17]. *Lactobacillus* sp. is commonly found in plants and overripe foods [10]. Adhesion of the cell surface to its natural habitat is important for *Lactobacillus* sp. [3]. The characteristics of *Lactobacillus* SLP are the smallest size among SLP from other bacteria

or archaea; high pI (approximately 9.4–10.4), positively charged; high content of hydrophobic



residues and low content of sulfuric acid residues, mostly in oblique or hexagonal shape, and nonglycosylated [10,18]. Only *L. kefir* possesses glycosylated SLP, which was identified as O- and N-glycosylated [19,20]. Unlike other genera that display a correlation between the SLP genetic order and taxonomy classification, *Lactobacillus* has undetectable homogeneity [9]. However, SLP-encoding genes show similarity at the strain level [3]. Extracted SLP from *Lactobacillus brevis* KM3 dan *L. brevis* KM7 showed similar molecular mass, secondary structure (predominantly formed of  $\beta$ -sheet and less  $\alpha$ -helix), and thermal analysis profile [21]. An alignment analysis of the amino acid residue sequences of *Lactobacillus acidophilus*, *Lactobacillus casei*, *Lactobacillus helveticus*, and *Lactobacillus crispatus* recognized a conserved domain at the C-terminus; meanwhile, the N-terminal was more variable. In particular, C-terminal SLPA\_III shows conserved amino acid sequences in *Lactobacillus*, whereas the N-terminal domains SLPA\_I and SLPA\_II are responsible for the self-

Figure 1. Structure of *Lactobacillus* sp outer protein. LTA: lipoteichoic acid; WTA: wall teichoic acid; EPS: extracellular polymeric substances. S-layer proteins are attached on the outer part of the bacterial cell wall. 034 Yuliawati et al. / Journal of Applied Pharmaceutical Science 15 (01); 2025: 032-042 assembly process [22]. The  $\beta$ -sheet and  $\alpha$ -helix structures were identified at the N-terminal domain of the SLP of *L. acidophilus* ATCC 4356, but only the  $\alpha$ -helix structure was observed at the C-terminal domain [23]. Only the N-terminal domain is exposed to the nearby environment; however, the C-terminus plays a role in anchoring the bacterial cell wall [23]. This anchoring function is useful for surface antigen display for vaccine delivery [24]. Genetic variation in the *Lactobacillus* sp led to secondary structural diversification among the strains. A comprehensive study of the SLP-encoding gene from numerous strains of *Lactobacillus* sp has been written by Hynönen et al. [3]. In general, SLPA-, SLPB-, SLPC-, and SLPD-encoding genes are commonly found in *Lactobacillus* sp. However, the expression of these genes varied depending on growth conditions. For instance, under an aerobic condition, SLP B and D were expressed in *L. brevis* ATCC 14869, whereas only SLP B was found under an anaerobic condition [18]. The secondary structure of SLP isolated from *Lactobacillus kefir* HBA20 was dominant in the  $\beta$ -sheet conformation compared to the  $\alpha$ -helix content [25]. SLPA is a major SLP found in *Lactobacillus* family. All of *Lactobacillus* species reported by Palomino et al. [24] own the SLPA gene, while only seven of 13 species harboring the SLPX gene, and only two of 13 species have the SLPB gene. *L. acidophilus* La14 was a species having all of these SLP-encoding genes [24]. The SLPX was more than 50-fold upregulated in *L. acidophilus* in gastrointestinal stimulated growth conditions. The overexpression of the SLPX corresponded to a survival rate of up to 93% [26]. Several strains of *L. brevis* showed diverse expression levels of SLP. The *L. brevis* B144 from traditional fermented buffalo milk had the highest SLP concentration among the other strains. The amino acid sequence of this strain showed almost 50% peptide fragment coverage with *L. brevis* KB290 [27]. *Lactobacillus* sp. has been widely used as a probiotic and provides a number of health benefits, such as stimulation of the immune system and defence mechanisms against pathogens [28]. It competes with pathogens by occupying the binding sites on the gastrointestinal tissue via cell surface proteins, mucus-binding proteins, or moonlighting proteins [29]. SLP is one of the proteins that acts on mucus binding mechanisms and stimulates protection against viruses via a dendritic cell-specific intercellular adhesion molecule-3-grabbing non-integrin (DCSIGN) interaction [29,30]. SLP of *L. amylovorus* is crucial for diarrhea recovery in post-weaning piglets [31]. In addition, SLPA and SLPB from several mutants of *L. acidophilus* were responsible for bacterial growth under different environmental stress conditions (salt, detergent, and alcohol) and enhanced human immunity by binding to uromodulin and DC-SIGN [32]. SLPA from *L. helveticus* showed anti-inflammatory activity by repressing inflammatory cytokines and biomarkers [33]. SLP of *L. casei* f05 protects intestinal tissue from *Escherichia coli* and *Salmonella enterica* by competing for mucus



binding and inducing pathogen apoptosis [34]. A list of genes and molecules that are responsible for the protection and probiotic function of *Lactobacillus* sp was reviewed by Lebeer et al. [35].

Importantly, SLP maintains its bioactivity, even after being extracted from the host. Isolated SLP of *L. plantarum* L-91 decreased the adhesion percentage of pathogenic *E. coli* by more than 50% in an in vitro immobilized collagen assay [36]. The SLP which was isolated from *L. helveticus* R0052 successfully protected pathogenic *E. coli*-infected human cancer cells [37]. The extracted SLP of *L. crispatus* KT-11 combats rotavirus infection by inhibiting the release of viralspecific proteins in the Caco2 cell line [38]. In addition, SLP from *L. acidophilus* also acts in an autoimmune mechanism, for example, in inflammatory bowel disease (IBD), by maintaining gut homeostasis through the SLPA/SIGNR3 interaction [39]. *Lactobacillus crispatus* 2029 inhibited the interaction of *Candida albicans* on HeLa cells and protected the epithelial cells from pathogen infection [40]. Wang et al. [41] reported that SLP isolated from *L. acidophilus* NCFM® inhibited the growth of HTC116, a colon cancer cell line. The isolated SLP of *L. acidophilus* is recommended as an oral drug nanocarrier because of its impressive stability in stimulated gastric fluid with and without pepsin [42]. The development of recombinant *Lactobacillus* SLP was then considered to more efficiently exploit the advantages of using whole cells. The recombinant SLPA of *L. brevis* KCTC3102 expressed in *E. coli*

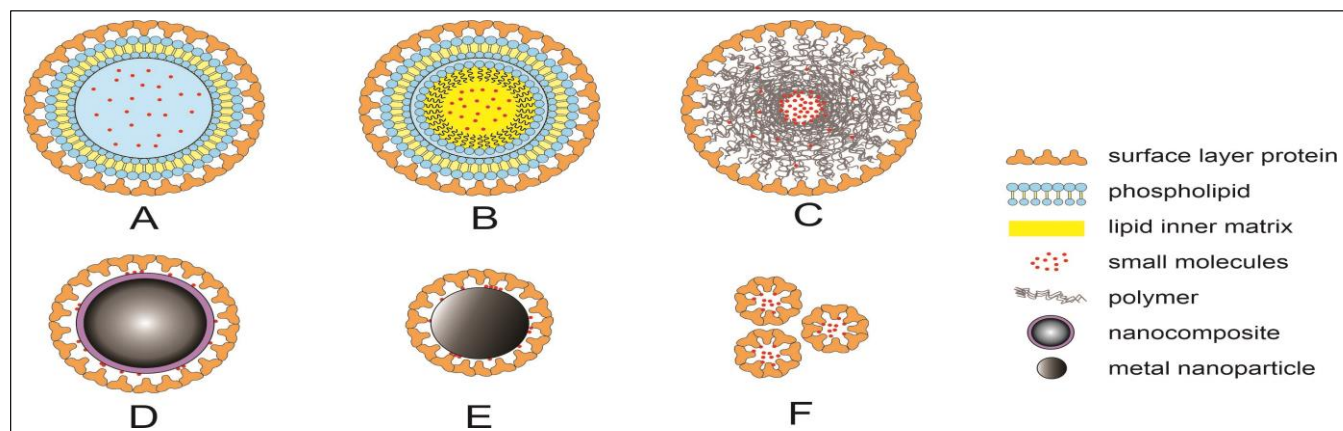


Figure 2. *Lactobacillus* sp. SLP coatings on various types of drug carriers. (a) SLP-coated liposome. (b) SLP-coated emulsome. (c) SLP-coated polymeric nanoparticle. (d) SLP-coated nanocomposite. (e) SLP-coated metal nanoparticle. (f) small molecule loaded-SLP based carrier.

BL21(DE3), was used to treat neonatal calves with diarrhea [43]. A SLP-encoding gene (including N- and C- fragments) was constructed with pHSAN, pHGFPSAC, and pHGFP expression plasmids, and then expressed in *E. coli* M15 [23]. A list of SLP promoters, secretion signal peptides, and other genetic analyses, which will be crucial for further recombinant SLP studies, has been reviewed [44,45]. The combination properties of maintaining activities after isolation (as native or a recombinant protein), selfassembly, regular and predictable structure, ability to form particles of

nanosize, and high stability of SLP make it interesting to include this material in drug nanocarrier development [46]. Sagmeister et al. [22] observed that selfassembled Lactobacillus sp. SLP, forming lattice p2 symmetry with small pores. This structure is a potential characteristic of drug nanocarriers because it offers protection from harsh environments [22]. The interaction of each protein forming SLP remains unclear. However, although the diversity of the SLP amino acid sequences is high, the functional domains and their interactions, as well as their predicted domain folds, are similar. This even applies to SLP with some supplementary domains [22]. With regard to the properties required as a drug nanocarrier, SLP has certain regions with positive and negative charges, which in some cases are important for drug loading or entrapment. SLP (predominantly SLPA) maintains the mechanism of moonlighting protein once applied as a drug nanocarrier. It is beneficial for an active targeting delivery system, for instance, by specifically adhering to the intestinal or colon cells via target molecules (fibronectin, collagen I and IV; mucin; laminin; DC-SIGN) [24,47,48]. SLP can also precisely bind to the target cell via certain enzymes, such as triose phosphate isomerase, GPI, glucose-6 phosphate isomerase, or phosphoglycerate kinase [48,49]. Compilation of the features of Lactobacillus SLP is described in Table 1.

**SLP as drug nanocarriers**

The SLP matrix is composed of protein or glycoprotein molecules that form regular and typical arrangements [4]. They can assemble in liquid phases, solid phases, and at the air-liquid contact via an entropy-driven process [4,48,50]. The arrangement of the SLP matrix depends on the sequence of its amino acid residues. The SLP protein consists mostly of nonpolar amino acids, therefore, the early step of the assembly process mainly involves hydrophobic interactions [4,51]. The array is formed by the interaction of negatively charged carboxylic groups with positively charged amino groups at certain amino acid residues, without any covalent bonds [4]. Stabilization of the SLP matrix was enhanced by the bonding of acidic residues and cations (Ca<sup>2+</sup> and Mg<sup>2+</sup>) [4,52,53]. In addition, after the SLP confirmation was completed, each monomer remained in its position because of the low free energy. Therefore, it could be used in nanoparticles (NPs) for therapeutic applications and

**Table 1.** Characteristics of SLP from Lactobacillus sp.

No.	Characteristics	Ref
1	Secreted by both bacteria and archaea, up to 15 % of the total secreted proteins.	[2]
2	Important in adaptability in extreme conditions and in the virulence of pathogenic bacteria.	[4,5]
3	Playing roles in ion transport	[4–6]
4	Small size (25 and 71 kDa) and high pI (9.4–10.4)	[10,18]

- |   |   |      |
|---|---|------|
| 5 | The encoding genes of SLP are similar in strain level                             | [3]  |
| 6 | The anchoring function is useful for surface antigen display for vaccine delivery | [22] |
| 7 | Applicable in drug nanocarrier development  | [46] |
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is considered a building block of bionanomimetic NPs (Fig. 2) [54,55]. Table 2 shows a list of SLP applications for many types of drug nanocarriers.

SLP-coated lipid membrane liposomes and emulsome

Solid lipid NPs have been developed and used in various drug delivery systems. The delivery mode is particularly used to formulate poorly water-soluble drugs by incorporate the substance in the core of the nanocarrier that was formed in the multilamellar or unilamellar layer [56]. The lamellar system was formed through the ability of the lipids component that able to self-assemble into bilayers to form vesicle, this formation was affected by the charge and geometry of the phospholipids components. The spherical bilayer shape will encapsulate the drug substances and form vesicles with sizes ranging from 50 nm to 1  $\mu$ m. Liposomes are spherical vesicles consisting of a phospholipid bilayer shell with an aqueous core. It has been shown that liposomes are excellent drug vehicles because they can load and transport hydrophobic substances in their lipidic shells and hydrophilic compounds in their aqueous core [56]. Coating liposomes with SLP offers several advantages such as stabilizing lipid membrane, preventing aggregation, giving protection against the gastrointestinal tract environment, improving encapsulation efficiency, permeability, and bioavailability profile, and improving the release profile of the drug. SLP-coated liposomes are also comparable to the “artificial virus-like particles” or “artificial cell envelopes,” as they increase mucosal immunity of the vaccine preparation [57]. SLP lattices also stabilize the lipid membranes [58]. There are studies supporting the positive effects of proteinliposome interactions, including SLP protein from various *Lactobacillus* sp. [59–61]. The SLP coating effectively prevents the aggregation of positively charged liposomes, as it provides an immense barrier because of its bulky structure [3]. Additionally, SLP reduced the charge repulsion between stearyl amine molecules, leading to an increase in chain packaging and membrane rigidity [62].

The presence of SLP neutralizes the charge at the membrane interface and increases the entrapment efficiency and permeability [63]. The membrane rigidity of liposomes was also improved when coated with SLP. Carboxyl groups in the interior of the SLP array electrostatically bind to the zwitterionic-lipidic parts of the liposome. Not all of the lipid molecules of the liposome bilayer had contact with the SLP, but only a maximum of three molecules had a protein-bilayer interaction. Therefore, it produces semi-fluidic membrane characteristics because unbound lipid molecules are allowed to freely diffuse in the liposomal bilayers [58]. The attachment of SLP did not only affect the hydrophobic lipid acyl chains, but also provided supporting scaffolding to the lipid membranes [64,65]. Glycosylated SLP has a better affinity to liposomes, but there was no difference in the

stabilizing capacity of glycosylated and unglycosylated SLP in SLP-coated liposomes [60,62]. The interaction of SLP with the lipid monolayer of liposomes depends on the materials that determine the hydration state of the lipid interface. If the surface pressure was high, the membrane became more rigid, and surface polarity increased at high cholesterol ratios. Meanwhile, the membrane structure was more relaxed at low pressure because the monolayer was less packed and more hydrated. This indicated that protein insertion could modify the hydration state of the interface [60]. The SLP-coated liposome protects the drug substance within the micelles against the gastric environment condition, e.g., quick changes and variations in pH and temperature, gastric grinding process, and digestive enzymes. The crosslinking of the proteins and glutaraldehyde further enhances the effect [62]. SLP can adhere to the gastrointestinal mucosa, which increases drug retention time in the gastrointestinal tract and promotes drug internalization [50]. Their adhesive and immunomodulatory properties also account for their impressive potential as vaccine delivery platforms [47]. The interaction between SLP-coated calcein-loaded liposomes and human colon adenocarcinoma Caco-2 cells was evaluated under various conditions [60]. The SLPs used in this formulation were extracted from *L. kefir* JCM 5818. After incubation at 37°C for 30 minutes, the concentration of calcein internalized by Caco-2 cells from SLP-coated liposomes was significantly higher than that of the control liposomes. In the calcein dequenching analysis, a group of SLP-coated liposomes delivered up to 40% higher calcein than the group without the SLP coating [60]. SLP-coated liposomes can be developed as drug vehicles for oral administration, particularly for macromolecular substances [60]. Streptavidin and enhanced green fluorescent protein (EGFP) are macromolecular compounds that have been reported to be successfully entrapped by SLP-coated liposomes [66,67]. SLP-coated liposomes were also able to attach to ferritin via covalent bonds [64]. Biotinylation of SLP-coated liposomes has been reported to enhance the binding efficiency of streptavidin [67]. The SLP of *L. acidophilus* CICC6074 was applied to coat cholesterol-lowering peptide (LQPE)-loaded liposomes for increasing intestinal absorption. Even though SLP noncovalently binds to the lipid heads of liposomes, it could produce a firm and stable fusion. SLP facilitated liposome absorption across the intestinal mucosa by lowering the hydrophobicity and electrostatic interaction between them [68]. It contrasting with the study reported by Tan et al, SLPB-coated liposome reported that the uptake of the vesicles in the intestinal was not improved. While, it improved the stability of the preparation against harsh environments in the gastrointestinal tract, and facilitated specific uptake of vesicles into Peyer's patches, and ultimately increasing the bioavailability of the drug by 427.65-fold [61]. Additionally, coating liposomes with SLP from *L. buchneri* reported to be able to increase the stability, maintain sustained release, and added antibacterial activity to carvacrol (Car)/ $\beta$ -cyclodextrin ( $\beta$ -CD)-loaded liposomes [69]. SLP from *L. buchneri* and *L. kefir* has been used to coat cationic liposomes and reduced the negativity of the



liposome's zeta potential. The SLP coating reduced the liposomes leakage percentage against high temperatures (50°C), light, and pH (3,7, and 9) [70].

### **Emulsomes**

Emulsomes are liposome-like particle delivery systems with a solid lipid in the inner part that forms a colloidal solution as the final product [57,71,72]. The solid core of the emulsome can entrap a greater concentration of poorly watersoluble drug compounds and gives a prolonged release profile [71–73]. In the case of curcumin, a poorly water-soluble compound, the solubility increased 10,000-fold, from 0.07 to 0.11 mg/ml to  $\approx 11$  ng/ml [71]. Methods that have been utilized to prepare emulsomes involves a dehydration-rehydration process followed by temperature-controlled extrusion [71]. Ucisik et al. [71] studied the characteristics of SLP-coated emulsomes. It showed that the binding and crystallization of SLP on emulsomes also require intermolecular electrostatic interactions between the amine groups of the bilayers and the carboxyl groups inside the SLP matrix, Similar to SLP-coated liposome production [71]. SLP coating decreased the cytotoxicity of positively charged emulsomes. Dipalmitoylphosphatidylcholine in emulsomes is toxic to the human liver carcinoma cell line (HepG2). Compared with the SLP-coated emulsomes at similar concentrations of dipalmitoylphosphatidylcholine, toxic effects were only observed in the group without SLP coating. The safety of SLPcoated emulsomes was still indicated even at 2.5 times higher concentration. They had solid electrostatic attraction with the negatively charged cell membrane (CM), which was assumed to be due to their high toxicity. The recrystallization of SLP on the surface of the emulsomes caused significant shifts in the positively charged vesicles to nearly zero or to a highly negative zeta potential [57,74]. According to visualization using a transmission electron microscope (TEM), it was observed that the SLP-coated emulsomes had already contact with the CM before being internalized by the cells. Therefore, SLP modifies the lipid surface of emulsomes and enhances their biocompatibility [57]. The S-layer fusion protein was shown to form a uniform monomolecular lattice on the surface of the emulsomes and the CurcuEmulsomes, altering the surface characteristics of the lipid-based nanocarrier and bestowing IgG binding functionality on the nanocarrier. Entrapped curcumin at a concentration of  $30 \mu\text{g}\cdot\text{ml}^{-1}$  did not influence the self-assembly characteristics of the S-layer protein. This study indicates that S-layer fusion technology is a highly effective approach for the immobilization of foreign proteins such as protein G domains on emulsomes. The distinct advantage of using S-layer proteins is that they can be recrystallized in an oriented fashion on a variety of supports including spherical surfaces covered by phospholipids [46]. Previous studies have also shown that mixtures of native S-layer proteins [47] and S-layer fusion proteins incorporating different functional domains [46] assemble into coherent monomolecular layers on different surfaces including liposomes [48]. Ucisik et al. [74] reported that SLP fusion protein (in this research SLP was fused with two protein G domain) able to form a uniform monomolecular lattice in the emulsome, but it alters the characteristics of the outward layer of the lipid nanoparticle while still

able to entrapped the active compound. However, a high curcumin concentration (110 µg/ ml) disturbed the self-assembly process of the SLP, in which SLP recrystallization was not detected [74]. It was assumed that the nonspecific absorption of curcumin on the outermost layer of the emulsome disturbed the recrystallization process of the SLPs or because the incorporation of a high concentration of curcumin influenced the rigidity or irregularity of the outermost phospholipid bilayer of the nanovesicle. SLP and SbpA are smooth cytophobic forms that exterminate the adsorption of human plasma proteins at basic pH [75] and cell adhesion (e.g., HepG2) [76]. Therefore, coating with SbpA might reduce the adhesion of opsonins and increase the half-life of the SLP-coated emulsome, resulting in antifouling characteristics. This study also showed that SLP is a valuable method for immobilizing foreign proteins, such as protein G domains, on emulsome. SLP-coated small molecules drug As previously mentioned, SLP can be attached directly to an active pharmaceutical ingredient without any other nanocarriers. Qamsari et al. encapsulated omeprazole, a proton pump inhibitor, with extracted SLP from *L. acidophilus* ATCC 4356 using a simple incubation method (25°C, 2 h, 100 rpm). SLP encapsulation reduced omeprazole instability and protected against gastric acid degradation [77]. SLP-coated polymeric NPs Polymeric NPs are the most developed drug delivery systems. Various types of polymers have been applied to the system [76]. Natural or synthetically modified biodegradable polymers are preferred because of their low toxicity, side effects, high biocompatibility, and low immunogenicity [78]. Even so, a non-biodegradable polymer such as chemically synthesized dextran-methacrylate acid (dex-MA) has also been studied for a certain purpose of delivery with extreme barriers, such as the intracellular delivery of cytotoxic drugs [79]. Another advanced characteristic of polymeric NPs is that they are easily modified for a specific drug release-triggering factor, such as pH, temperature, oxidative stress level, or light [79–82]. Several polymeric NPs were coated with *Lactobacilli*'s SLP to enhance their intrinsic bioactivity [83,84]. SLP isolated from *Lactobacillus* sp. was combined with doxorubicin (DOX)-loaded mouse melanoma B16F10 CM/polyethyleneimine-modified (2-hydroxylpropyl)- $\gamma$ -cyclodextrin (HPAD) NPs to increase protection of the antigen and enhance the immune response. The addition of SLP to the outer part of the DOX/HPAD/CM NPs effectively and efficiently inhibited tumor growth and metastasis by improving the targeted microenvironment immune system [84]. SLP-coated polymeric NPs have also been used in dental care. Triclosan-loaded PLGA NPs were coated with SLP extracted from *L. acidophilus*. The presence of *L. acidophilus* SLP remarkably eliminated the *Staphylococcus mutans* biofilm and hindered the pathogenic formation of SpragueDawley rat teeth by sustaining the release of triclosan. In addition, taking advantage of the safety of *L. acidophilus* or generally recognized as safe (GRAS), the SLP-coated PLGA NPs are undoubtedly safe for dental treatment [84]. SLP-coated metal NPs/nanocomposites Metal NPs (MNPs) of gold (Au), silver (Ag), titanium (Ti), palladium (Pd), copper (Pb), and zinc (Zn) are frequently studied as drug nanocarriers [85]. MNPs have a high loading capacity, easily conjugate

with specific ligands through hydrogen bonds, covalent bonds, or electrostatic interactions, extend the half-life of the loaded drug in blood circulation, and avoid early renal elimination [86]. The application of SLP on MNPs could involve different functions, such as a capping agent, boosting bioactivity, or as a synthesis-supporting system [87–89]. Capping agents in NPs, specifically MNPs, are crucial for ensuring colloidal stability by preventing particle agglomeration [90]. MNPs stabilization is achieved by many interactions with the capping agent, for example, electrostatic, steric, van der Waals, or hydration forces [91]. Some capping agents also synergize with MNPs for several bioactivities such as anti-inflammatory, anticancer, antibacterial, antidiabetic, and wound healing activities [92]. A number of materials appear to be prospective MNPs capping agents, including polymeric materials (chitosan, polyethylene glycol, and polyvinyl alcohol), chelating agents (ethylenediaminetetraacetic acid or EDTA), plant extracts, and proteins (bovine serum albumin or BSA) [90]. SLP is considered for use in MNPs, nanocomposite coatings, or capping because appropriate interactions exist among these materials. SLP of *L. buchneri* was used to cap the AgNPs via electrostatic interactions and hydrogen bonding. The SLP-capped AgNPs resulted in satisfactory antibiofilm and antibacterial activity against *S. enterica* and *Staphylococcus aureus* because of increased permeability to the bacterial CM and lowered activity of respiratory chain dehydrogenase [87]. SLP of *L. helveticus* was applied to silver NPs (AgNPs) to enhance its antibacterial effect on pathogenic *Pseudomonas aeruginosa* PAO1. It was reacted with  $\text{AgNO}_3$  as a precursor, and the AgNPs were synthesized in an eco-friendly manner using an aqueous extract of *Juglans regia* green husk [88]. Isolated SLPs from *L. kefir* CIDCA 8348 and 83111 were used as support systems for AgNPs. The SLPs were mixed with an  $\text{AgNO}_3$  solution before chemical reduction. As a supporting system, the SLP is low-cost, simple, and “greener” to use, and has an incredible bio-dimensional matrix, yet notably, no additional stabilizer is needed because it prevents the AgNPs aggregation and improves the particle catalytic activity [92]. In other studies, similar SLPs were used as a supporting system in combination with acrylic particles to synthesize platinum NPs (PtNPs), resulting in multiple reused catalytic MNPs [93, 94].

## **CONCLUSION**

As a food-grade and potential probiotic organism, *Lactobacillus* genus is an excellent source of SLP that will be developed as one of the raw materials in the drug formulation. SLP is a natural material with a typical structure and stability. SLP can be used directly as drug nanocarriers, antigens, drug substances, or other medically important molecules. It can also be integrated with other well-developed drug nanocarriers such as liposomes, emulsomes, polymeric NPs, and metal NPs/nanocomposites. The integration of SLP into the surface layer of a drug nanocarrier can improve its physical, chemical, and biological properties.

All data generated and analyzed are included in this research article.

Nanoparticles; NPs: nanoparticles; N-terminal: amino terminal; Pd: palladium; pH: power of hydrogen; pI: isoelectric point; PLGA: polylacticco-glycolic acid; PtNPs: platinum nanoparticles; SLP: surface layer protein; TEM: transmission electron microscopy; Ti: titanium.

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