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# *Lactobacillus* surface layers and their applications

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**Abstract**

Surface (S-) layers are crystalline arrays of proteinaceous subunits present as the outermost component of cell wall in several species of the genus *Lactobacillus*, as well as in many other bacteria and Archaea. Despite the high similarity of the amino acid composition of all known S-layer proteins, the overall sequence similarity is, however, surprisingly small even between the *Lactobacillus* S-layer proteins. In addition, the typical characteristics of *Lactobacillus* S-layer proteins, distinguishing them from other S-layer proteins, are small size and high-predicted p*I* value. Several lactobacilli possess multiple S-layer protein genes, which can be differentially or simultaneously expressed. To date, the characterized functions of *Lactobacillus* S-layers are involved in mediating adhesion to different host tissues. A few applications for the S-layer proteins of lactobacilli already exist, including their use as antigen delivery vehicles.


*Keywords:* S-layer; S-layer protein; S-layer gene; *Lactobacillus*; Adhesion

**Contents**



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## 1. Introduction

Surface layers (S-layers) are monomolecular crystalline arrays composed of protein or glycoprotein subunits with molecular masses ranging from 40 to 200 kDa [1,2]. S-layers have been identified as the outermost structure of cell envelope in numerous organisms from the domains Bacteria and Archaea [3,4]. S-layer proteins represent 10–15% of the total protein of the bacterial cell indicating efficient gene expression, S-layer protein synthesis and secretion [3,5]. The S-layer lattice can exhibit oblique (p1, p2), square (p4) or hexagonal (p3, p6) symmetry and is formed by an intrinsic self-assembly process [1,6]. S-layers are normally 5–15 nm thick possessing a smoother outer surface compared with a more structured inner surface [7]. Some organisms have two superimposed S-layers, which are composed of different subunit proteins [8,9]. Each S-layer forms a highly porous structure with pores of an identical size and morphology. The pores comprise up to 70% of the lattice surface area [2].

To date, two types of post-translational modifications of S-layer subunits have been identified. Glycosylated S-layer proteins have been characterized in Gram-positive bacteria and in Archaea [10]. The glycan chains are typically composed of two to six monosaccharides repeated up to 50 units consisting of a wide range of sugars and, in some cases, of non-carbohydrate substituents [10–12]. The sugar residues of S-layer glycoproteins are attached to the protein moiety via *O*-glycosidic or *N*-glycosidic linkages [12]. In addition to glycosylation, post-translational modification of S-layers involving phosphorylation of tyrosine residues has been reported [13].

The S-layer protein subunits are non-covalently linked to each other as well as to the supporting cell wall, and can be disintegrated into monomers by denaturing agents such as urea or guanidine hydrochloride, by metal-chelating agents or by cation substitution [1]. Isolated S-layer subunits can recrystallize into regular arrays on solid supports, liquid-surface interfaces, lipid films and liposomes or in suspension once the disrupting agent used for their isolation has been removed [3,6].

The current knowledge considering the biological functions of S-layers is relatively limited, compared to information that has accumulated on their ultrastructure, biosynthesis, molecular biology and genetics. Many of the functions described to S-layers are still hypothetical and need further experimental data to be revealed. S-layers have been considered to function as protective coats, cell shape determinants, molecule and ion traps, adhesion sites for exoenzymes as well as structures involved in cell adhesion and surface recognition (reviewed in [1,2]). In some pathogenic organisms such as *Campylobacter fetus* [14], *Aeromonas salmonicida* [15] and *Bacteroides forsythus* [16], S-layers have been shown to contribute to virulence. However, a general functional principle for all S-layers has not been determined and will likely not exist, due to the widespread occurrence of S-layer-possessing organisms and their differences in the overall cell surface structure. Due to their highly periodic and regularly arranged porous ultrastructure, S-layers possess great potential for various applications in biotechnology, nanotechnology, nanobiotechnology and biomedical applications [6,17,18].

Lactobacilli are members of the lactic acid bacteria, a phylogenetically diverse group of Gram-positive bacteria characterized by the formation of lactic acid as a sole or main end product of their sugar fermentation [19]. The genus *Lactobacillus* is the largest of the genera included in lactic acid bacteria with over hundred species recognized at present. The genus *Lactobacillus* is very heterogeneous containing species with substantial differences in their phenotypic, biochemical, physiological and genotypic characteristics. Members of the genus *Lactobacillus* are commonly found in nature and they are associated with a number of different habitats rich in carbohydrate or protein such as plants or spoiled food. Moreover, several *Lactobacillus* species are also members of the normal microbiota of human and animal gastrointestinal and genitourinary tracts [19,20]. The food and feed industry utilizes lactobacilli widely in the fermentation of vegetables, silage, sourdough bread, and several dairy and meat products, although some species can be associated with food spoilage [21]. Due to their long history of use in food fermentations and in the food industry as well as lack of pathogenicity, lactobacilli are generally recognized as safe (GRAS) organisms. Several lactobacilli are claimed to be health-benefiting to their host [22] and are therefore currently used as probiotic supplements in several products, intended for either human or animal consumption. Attention has recently also focused on the development of lactobacilli as live antigen delivery vehicles [23].

## 2. Structure and molecular characterization of *Lactobacillus* S-layers

### 2.1. S-layer protein genes of Lactobacillus

Many species of the genus *Lactobacillus* possess an S-layer. S-layer protein encoding genes have been cloned and sequenced from two *Lactobacillus brevis* strains [24,25], one *L. acidophilus* strain [26], one *L. helveticus* strain [27], and one *L. crispatus* strain [28]. Deposited in GeneBank (National Center for Biotechnology Information, Bethesda, MD, USA) are also several S-layer protein encoding gene sequences, which are either unpublished or have not been described in detail in publications. These sequences include five from *L. crispatus* (GenBank, accession numbers AF253043, AF253044,