Microbial adhesion to biomaterial surfaces and subsequent biofilm formation has been observed on nearly all medical devices with severe economic and medical consequences. The significant resistance of biofilms to conventional antibiotic therapies has encouraged the development of new biomaterials and coatings. Biosurfactants represent an interesting approach as they can be used to modify the surface properties conferring it an anti-adhesive and antimicrobial activity, leading to new and effective means of combating colonization by pathogenic microorganisms without the use of synthetic drugs and chemicals. These microbial compounds constitute a diverse group of surface-active molecules occurring in a variety of chemical structures. Biosurfactants from lactic acid bacteria have been used as a strategy to avoid microbial colonization of silicone rubber voice prostheses. Also, they were found to be active against several bacteria and filamentous fungi responsible for diseases and infections in the urinary, vaginal and gastrointestinal tracts, and in the skin. Nevertheless, it is important to stress that the insufficient data on their toxicity for humans, as well as their high costs of large-scale production, have been restraining their commercialization and use in most medical applications. Many biotechnological strategies have been pursued to reduce the biosurfactants production costs including the use of agro-industrial wastes as substrates, optimization of medium and culture conditions, and efficient recovery processes. However, the improvements obtained from these strategies are marginal and to successfully compete with synthetic surfactants, novel microorganisms must be designed. The use of hyper-producer strains allows increasing the production yields and consequently reducing costs. These strains can be screened from the natural environment, or engineered using synthetic biology approaches. Hence, data on the genes involved on the production of biosurfactants is critical for designing organisms with improved features. Once the genes have been indentified and isolated, they can be expressed in other microorganisms, or they can be modified or placed under regulation of strong promoters to increase their expression and so enhance production. This knowledge will also allow the production of novel biosurfactants with specific new properties for different industrial applications. Further advances in genetic engineering of the known biosurfactant molecules could yield potent biosurfactants with altered antimicrobial profiles and decreased toxicity against mammalian cells.