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Besides being part of human commensal flora, *S. epidermidis* has the ability to colonize and form biofilms in artificial implants. Due to the particular characteristics of biofilms, conventional methods used to disrupt and lyse biofilms from Gram positive bacteria may include association between mechanical, enzymatic and chemical methods. Nevertheless, proteomic characterization is highly dependent of the extraction procedure. In order to characterize proteome from *S. epidermidis* biofilms grown in glucose excess, we used mechanical lysis (glass beads) associated with two distinct lysis solution with different charge characteristic detergents, namely, SDS (an ionic detergent) or CHAPS (a zwitterionic detergent). Protein extracted was separated by SDS-PAGE and identified by LC-MS/MS. SDS lysis buffer combined with glass-beads showed the highest number of identified proteins (332 proteins). With zwitterionic detergent extraction, most the identified proteins presented a lower GRAVY value (grand average of hydropathy) and a protein molecular weight under 30 KDa. In overall, this work evidence that SDS lysis buffer is the optimal protocol to proteome analysis of *S. epidermidis* biofilms. This work was funded by Fundação para a Ciência e a Tecnologia (FCT) and COMPETE grants PTDC/BIA-MIC/113450/2009, FCOMP-01-0124-FEDER-014309. Thanks are due to Fundação para a Ciência e a Tecnologia, European Union, QREN, FEDER and COMPETE for funding the QOPNA research unit (project PEst-C/UI0062/2011) and the Portuguese National Mass Spectrometry Network (RNEM), also supported by funds from FCT. VC was funded by FCT fellowship SFRH/BD/78235/2011.