

**Title:** *In silico* metatranscriptomic approach for tracking biofilm-related effectors in dairies

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**Abstract:**

Microbial biofilms are communities that depend on complex interactions elicited in cascade signalling to be able to persist in harsh environments. The regulation of gene expression related to flagellum, production of exopolysaccharides and release of extracellular DNA are key features for biofilm formation and maintenance. In this study, to evaluate the expression of effectors linked to biofilm formation, all metatranscriptomics data of dairy foods available on the public database JGI GOLD were downloaded, except for bioprojects from lab-scale fermentations and from dairy products prepared with fungal starter cultures. In total, there were deposited milk samples from Holland (n=8), and cheese samples from Finland (n=6), Italy (n=27) and France (n=12). The sequencing files from all the 53 samples were quality-filtered to remove reads with low-quality base calls and adapter/barcodes. To search for biofilm-related effectors, downloads were done from the UniprotKB database for diverse protein sequences considered important for biofilm formation: (i) QS and QQ (autoinducer, LuxS, AHL, AHL-acylases and AHL-lactonases and cyclic-diguanosine-GMP); (ii) biofilm-associated proteins - BAP; (iii) flagellum; (iv) adhesins; (v) two-component systems (histidine kinases – HK and response regulators - RR). A custom database was built based on 1,154,446 de-replicated sequences which were indexed in DIAMOND for sequence alignment. The results revealed reads assigned to genes involved with flagellar functions, adhesion, QS (AHL, HKs, RR and LuxS) and QQ (AHL-acylase) for milk and cheese samples. However, reads from BAP were found only in the dataset from milk samples. There were significant associations (Pearson correlation;  $p < 0.05$ ) among flagellum, RRs, Hks, adhesins and LuxS. On the other hand, no correlations were observed for BAP, AHL/acylase, adhesins, LuxS, flagellum and response regulator. There were also no correlations between AHL-adhesins or AHL-acylase-HKs versus AHL-acylase-adhesins. Sequences for effectors related to interspecific and intraspecific QS mechanisms (LuxS, HKs and RRs) were also detected. In counterpart, the absence of correlation observed for BAP and other important effectors might indicate a polysaccharide-independent mechanism of biofilm formation. In conclusion, the presence of many biofilm-related effectors in metatranscriptomics datasets evidenced regulatory mechanisms in action for bacterial persistence in dairy plants, which might contribute to design strategies for biofilm control.

**Key-words:** Biofilms, quorum sensing, microbial interactions, dairy.

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