

## ORT\_31 - Evaluation of *Pseudomonas aeruginosa* biofilm isolated in a pharmaceutical industry by Scanning Electron Microscopy

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**Introduction:** *Pseudomonas aeruginosa* is an opportunist human pathogen capable of forming biofilm in different surfaces. The biofilm formation in the stages regarding the manufacture of biological products, must be evaluated and investigated.

**Objectives:** This study aimed to evaluate the biofilm formed by *P. aeruginosa* strains isolated in a pharmaceutical industry by scanning electron microscopy (SEM).

**Methodology:** Twenty *P. aeruginosa* strains identified by polyphasic characterization 16S rRNA sequencing, VITEK®2 and MALDI-TOF MS were tested using SEM on stainlesssteel surfaces. The strains were transferred to 15 ml of brain hearth-infusion broth (BHI) and incubated at 37°C/24h with shaking (150 rpm). Each well of a sterile 6-well polystyrene plate containing a 2.5 cm<sup>2</sup> diameter stainless-steel disc was filled with 4.0 ml of bacterial suspension. The plate was incubated at 37°C/48h. Then, the wells were washed two times with 2.0 mL of phosphate buffer saline (PBS). The biofilm was fixed for 1 h with 2.5% glutaraldehyde in 0.1 M cacodylate buffer. After fixation, the biofilm was washed three times in PBS for 5 min, post-fixed for 15 min in 1% osmium tetroxide (Os4) and washed again three times in PBS for 5 min. Next, the samples were dehydrated in an ascending series of ethanol (7.5, 15, 30, 50, 70, 90 and 100% ethanol) for 15 min each step, critical point dried with CO<sub>2</sub> using a Critical Point Dryer machine, sputtercoated with a 15-nm thick layer of gold and examined in a Jeol JSM 6390 scanning electron microscope.

**Results:** All strains cultivated in stainless-steel surfaces was able to produce biofilm. Nine strains (45.0%) produced biofilm with scattering cells; eight strains (40.0%) produced homogeneous biofilm; and three strains (15.0%) produced biofilms heterogeneously forming cellular aggregates.

**Conclusion:** The isolation of biofilm-forming *P. aeruginosa* during the production steps should be investigated to identify the root cause and subsequently the adoption of corrective/preventive actions for elimination of this pathogen.

**Keywords:** *Pseudomonas aeruginosa*, Scanning electron microscopy, biofilm