

TITLE: FORMATION OF BIOFILM OF *ALICYCLOBACILLUS* SPP. ON STAINLESS STEEL SURFACES ISOLATED FROM THE ORANGE JUICE INDUSTRY

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ABSTRACT:

Alicyclobacillus spp. are microorganisms related to the deterioration of acidic products, such as drinks and citrus juices. The objective of the present study was to evaluate the capacity of different isolates and a reference strain of *Alicyclobacillus* spp. (vegetative cells and spores) to form biofilms on stainless steel surfaces. In independent experiments, the reference strain of *Alicyclobacillus acidiphilus* 0247^T and two isolates of *Alicyclobacillus* sp. from different stages of the industrial process, such as: concentrated juice - following pasteurization (CCT 7272) and concentrated juice (CCT 6280), were evaluated. Orange juice reconstituted previously contaminated with 10⁴ CFU/ml of the microorganism was used for microbial growth in the experiments. The stainless steel coupon was submerged in a microtube containing 1 ml of orange juice. The culture conditions were: time (24, 72 and 120 hours) and contact temperature for *A. acidiphilus* 0247^T (25 and 45 °C), CCT 7272 (30 and 45 °C) and CCT 6280 (45 and 60 °C). Subsequently, the coupons were removed from the orange juice and transferred to microtubes containing 1.0 ml of saline solution 0.85% where they were submerged for 1 minute at rest for the removal of the planktonic cells. Then, each vial was submerged in 1.0 ml of 0.85% saline solution and subjected to ultrasound at 25 kHz for 5 minutes to remove sessile vegetative growth. After that, in-depth plating was performed with BAT agar, and incubation was carried out at 45°C for 24 h. For the spore count, the same coupons were subjected to heat shock at 80°C for 10 minutes, followed by plating with BAT agar and incubation at 45°C for 24 h. The 0247^T strain did not form biofilm at any of the times or temperature conditions evaluated. *Alicyclobacillus* sp. CCT 7272 formed biofilm in the form of vegetative cells at 30 °C (4.61 log CFU/cm²) from 72 h of contact onwards. However, at 45 °C there was biofilm formation at 24 h (5.27 log CFU/cm²). The CCT 6280 isolate, meanwhile, formed biofilm in vegetative cell form at 45 °C at 120 h of contact (5.58 log CFU/cm²). However, biofilm formed at 60 °C from 24 h of contact onwards. Furthermore, the CCT 7272 isolate sporulated over time, with counts between 4 and 5 log CFU/cm². The CCT 6280 isolate formed biofilm in spore form only at a temperature of 60 °C with counts between 3 and 4 log CFU/cm². The results demonstrate the varied behavior of *Alicyclobacillus* spp. when forming biofilm on a stainless steel surface.

Keywords: *Alicyclobacillus* spp., stainless steel, orange juice, biofilm, spores

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