

TITLE: EFFECTS OF NUTRITIONAL CONDITIONS ON BIOFILM PRODUCTION BY *Corynebacterium* spp

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ABSTRACT:

Some species of the genus *Corynebacterium* are able to form biofilm, causing a major problem to human health. The synthesis of the exopolysaccharide matrix, responsible for the establishment of the biofilm, usually occurs in response to changes in environmental conditions or by changes in the metabolism of the bacterium. In view of the above, the present study aimed to evaluate the effect of different culture medium on *Corynebacterium diphtheriae* and *Corynebacterium ulcerans* biofilm formation. Biofilm formation on negatively charged polystyrene surface was determined quantitatively in 96-well flat-bottomed microtiter plates. Bacterial suspensions were applied to microplate wells containing tryptona soy broth (TSB) or nutrient broth (NB). After incubation, the supernatants were aspirated and the wells were washed three times. The remaining attached bacteria were fixed with methanol and stained with crystal violet. Negative controls contained TSB or NB only. The bound dye was then solubilized with glacial acetic acid and the optical density of the solution was measured by using an enzyme immunosorbent assay reader. All strains were classified into the following categories: non-adherent, weakly, moderately or strongly adherent, based upon the ODs of bacterial films. In this study, both *C. diphtheriae* and *C. ulcerans* strains were able to adhere to and to produce biofilm on hydrophobic plastic surfaces at different intensity levels. *C. diphtheriae* ATCC 27010 (non-toxigenic) strain was classified as moderately producing biofilm on polystyrene with TSB and was weakly adherent with NB. *C. diphtheriae* ATCC 27012 (toxigenic) strain was weakly adherent in the presence of both TSB and NB. However *C. ulcerans* KC 279 strain showed moderate and weak ability to adhere to polystyrene with NB and TSB, respectively. Some environmental signs, including nutritional conditions, are able to modify the microbial gene expression, inducing the synthesis of certain proteins and repressing others, suggesting a partial responsibility in the adherence and production of biofilm. The complete network of regulators of stress responses and the details of their actions remain to be elucidated.

Keywords: *Corynebacterium diphtheriae*, *Corynebacterium ulcerans*, Polystyrene, Environmental Signs

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