TITLE: DESIGN AND ANALYSIS OF PRIMERS *In silico* OF *Pseudomonas aeruginosa* OF REGION 16S OF rRNA TO POLYMERASE CHAIN REACTION

AUTHORS: SANTOS, D.C.P.[1]; MUNIZ, H.V.L. [1]; SILVA NETO, A.F. [1]; SALES, L.A.T. [1]; BORGES, P.D.B. [1]; MENDES, T.L. [1]; FRANÇA, H.L. [1]; ABREU, V.F. [1]; NUNES, M.A.S. [1]; FIRMO, W.C.A. [1]

INSTITUTION: 1. UNIVERSIDADE DO CEUMA, SÃO LUÍS, MA (RUA JOSUÉ MONTELLO, 1, RENASCENÇA II, CEP 65075-120, SÃO LUÍS - MA, BRAZIL)

ABSTRACT:

Pseudomonas aeruginosa is a bacterium being a Gram-negative bacillus, and can be found in several environments, mainly in soil and water, being also associated with plants and animals, causing opportunistic infections in humans. The pathogen can easily adhere to a variety of materials and is highly resistant to antibiotics, evidencing reports of nosocomial infections, especially in patients admitted to an intensive care unit, so that infections caused by this agent are difficult to control. The platform of the *National Center for Biotechnology Information* was used to obtain the specific sequence of the *P. aeruginosa* gene from the 16S region of the rRNA, then 15 primers were designed by the BLAST platform, the thermodynamic characteristics analyzed using the *Integrated DNA Technologies* platform with the OligoAnalyzer tool, finally, the primers were submitted to *in silico* PCR amplification. Nevertheless, it was necessary to establish general patterns of the primers for a good refining with the best characteristics for a good reaction; melting temperature (C°) between 50 and 60, with differences not exceeding 5°C, amount of cytosine and guanine between 40 and 60%, number of nucleotides between 18 and 25 base pairs and the size of the DNA fragment between 150 and 250 base pairs and, in dimers relations, *hairpin dimer* with melting values below 50ºC, *self-dimer* with values higher than -9 kcal/mole[1] and *hetero dimer* using the same criterion of the previous item. From the design, analysis and amplification in the *in silico* PCR, three pairs of primers amplified pairs of bases for *P. aeruginosa*, demonstrating specificity for the species, however, there was an amplification for different strains (B136-33 / DK2 / LES431 / LESB58 / M18 / MTB-1 / NC1 / PA1 / PA7 / RP73 / SCV20265 / UCBPP-PA14), being the best primer with size of 20 base pairs, CG quantity at 50%, *melting* temperature of 52.5°C and the *dimers*, *hairpin dimer* at 35°C, *self-dimer* with a value of -6.3 kcal/mole and *hetero dimer* yielding -4.38 kcal/mole. Thus, in theory the primer becomes efficient for base-pair amplification for *P. aeruginosa*, but more studies are needed using molecular techniques to ensure specificity for the strains.

Keywords: Polymerase Chain Reaction, Primers, *Pseudomonas aeruginosa*

Development agencies: Universidade CEUMA