TITLE: Down-regulation of EF-Tu impairs host cell interaction and virulence by *Paracoccidioides brasiliensis.*

AUTHORS: MARCOS, C.M.[1]; OLIVEIRA, H.C.[1]; ASSATO, P.A.[1]; DA SILVA, J.F.[1]; SCORZONI, L.[2]; DE PAULA E SILVA, A.C.[1]; SINGULANI, J.L.[1]; SANTOS, C.T.[1]; SILVA, R.A.M.[1]; ALMEIDA, R.[1]; ANDRADE, R.A.[3]; TAMAYO, D.P.[4]; LOPEZ, A.M.[4]; HERNÁNDEZ-RUIZ, O.[4,5]; MCEWEN, J.G.[4,6]; FUSCO-ALMEIDA, A.M.[1]; MENDES-GIANNINI, M.J[1].

INSTITUTION: 1. FACULDADE DE CIÊNCIAS FARMACÊUTICAS DE ARARAQUARA, Unesp - UNIVERSIDADE ESTADUAL PAULISTA, ARARAQUARA-SP, BRAZIL; 2. INSTITUTO DE CIÊNCIA E TECNOLOGIA, Unesp – SÃO JOSÉ DOS CAMPOS-SP, BRAZIL; 3. FACULDADE DE ODONTOLOGIA DE ARARAQUARA, Unesp – ARARAQUARA, BRAZIL; 4. UNIDAD DE BIOLOGÍA CELULAR Y MOLECULAR, CORPORACIÓN PARA INVESTIGACIONES BIOLÓGICAS (CIB) - MEDELLÍN, COLOMBIA; 5. ESCUELA DE MICROBIOLOGÍA. UNIVERSIDAD DE ANTIOQUIA, MEDELLÍN, COLOMBIA; 6. FACULTAD DE MEDICINA, UNIVERSIDAD DE ANTIOQUIA, MEDELLÍN, COLOMBIA.

ABSTRACT:

Members of the genus *Paracoccidioide*s are dimorphic fungi responsible for causing a deep systemic mycosis in humans geographically restricted to subtropical areas of Latin America; however the molecular mechanisms employed by *Paracoccidioides* spp. to cause disease remain poorly understood. *Paracoccidioides* spp. contain genes that encode proteins involved in the adhesion and invasion required for the pathogenicity of these fungi and therefore to mediate virulence. We previously identified the EF-Tu protein of *P. brasiliensis* (PbEF-Tu) through mass spectrometry and suggest its participation in the pathogenicity of this fungus by several assays. EF-Tu is a protein responsible for critical steps in protein synthesis, but in several pathogenic microbes is associated with the cell surface where it then interacts with host participating in adhesion, invasion and evasion of host immune response. To confirm the function of this protein in *P. brasiliensis*, PbEF-Tu was silenced by antisense-RNA (aRNA) technology, a well-establish methodology for this fungus, with a 55% reduced gene expression of PbEF-Tu (PbEF-Tu aRNA). Silencing of PbEF-Tu gave no apparent phenotype differences with normal growth and morphology of yeast-cells. Nonetheless,PbEF-Tu aRNA strain exhibits reduced levels of interaction with pneumocytes and additionally lower virulence in the alternative invertebrate,*Galleria mellonella,* and murine infection models, emphasizing the importance of PbEF-Tu for full virulence of *P. brasiliensis*. Therefore, the potential for application of this protein as target for antifungal agents against paracoccidioidomycosis should be applied.

KEYWORDS: *Paracoccidioides brasiliensis*, elongation factor Tu, virulence, gene knockdown

DEVELOPMENT AGENCY: Fundação de Amparo à Pesquisa do Estado de São Paulo (Fapesp) 2016/17048-4 and Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) AUXPE - 88881.141306/2017-01.