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D.31- Structural and biophysical characterization of Trypanosoma cruzi proline dehydrogenase - TcPRODH

LINO, M. E. S. D.1, SCULACCIO, S. A.1, SILBER, A. M.2, THIEMANN, O. H.1
1Department of Physics and Interdisciplinary Science, University of São Paulo (SP, Brazil), 2Parasitology Department, University of São Paulo (SP, Brazil)

Proline dehydrogenase is part of the proline metabolic route, central in glutamate synthesis, that participates in the mitochondrial electron transport chain, essential to the parasite life cycle. Proline and glutamate promote cell differentiation from epimastigotes to metaciclic trypomastigotes and to intracellular trypomastigotes. Therefore, TcPRODH is recognized as a target to inhibit proline synthesis. Characterize structurally and biophysicially the recombinant TcPRODH enzyme through circular dicroism (CD), dynamic light scattering (DLS), 3D structural prediction using AlphaFold and determine the atomic structure X-ray diffraction of TcPRODH single crystals. This effort will contribute to the search of specific inhibitors to proline methabolism, with therapeutic potential against T. cruzi infections. The heterologous recombinant TcPRODH gene was cloned in Codon Plus strain of Escherichia coli in the vector pET.TRX.LIC, expressed and purified using metal-affinity chromatography and size exclusion chromatography. CD spectra have been obtained in a Jasco J-815 Spectrometer (single temperature of 4°C, from 270 to 200 nm in 0,1 mg/mL), DLS in a Malvern Zetasizer UV. The 3D structural prediction was obtained using AlphaFold system (DeepMind) of the dimeric TcPRODH with 5 degrees of relaxations. Molecular docking was conducted by AutoDock Vina (active site used 6, -1, -10, 525 Å2 and 26,3 Å). The recombinant TcPRODH was obtained with approximately 95% purity and in a monodisperse conformation of a monomeric protein, coherent with the DLS data. Screening for crystallization conditions is underway. Initial conditions have been achieved previously. Initial compound screening revealed a promising candidates as ligand, the 3,5-dichloropyridyl-aminomethylenebisphosphonic acid. TcPRODH is a monomeric enzyme and 3,5-dichloropyridyl-aminomethylenebisphosphonic acid can act as a potent inhibitor in the proline methabolism. Future work is been performed to obtain the experimental atomic structure of TcPRODH and further explore potential inhibitors. Keywords: Proline Dehydrogenase, Trypanosoma cruzi, Drug-discovery

D.32- Structural studies on cyanide degrading nitrilases

Santiago Justo Arevalo1, Valeria Valleriestra Felice2, David Pabon Rodriguez3,1, Sofia Romani Moreyra3, Roberto Pineda Chavarria3, Andrea Balan2, Shaker Chuck Farah1
1Bioquimica, Instituto de Química e 2Microbiologia, Instituto de Ciencias Biomedicas, Universidade de Sao Paulo (SP, Brazil), 3Ciencias Biologicas, Facultad de Ciencias Biologicas, Universidad Ricardo Palma (Lima, Peru)

Cyanide is widely used in industries due to its high affinity for metals, a characteristic that is also responsible for its toxicity. Thus, industries have to reduce the cyanide concentration from wastewater before its final disposal. Physical, chemical and biological methods have been developed to achieve this goal, but knowledge about structure of enzymes involved in cyanide degradation is still scarce. Furthermore, since hydrogen cyanide is volatile at neutral pH, decontamination methods that operate at elevated pHs are preferable. We have cloned, expressed, and purified a cyanide dihydratase (CynDPER-URP-08) from Bacillus safensis PER-URP-08, a strain isolated from mine wastewater in Peru selected for its ability to grow in the presence of elevated levels of HCN and a cyanide hydratase from Gloeocercospora sorghi (CynHG.sorghi). Here, we report the 2.75 and 3.0 angstroms resolution cryo-electron microscopy structure of CynDPER-URP-08 and CynHG.sorghi at pH 8.0. Under these conditions, these enzymes form helical filaments consisting of 14 to 18 subunits in length. The polymers can be considered polymers of dimers forming a left-handed helix. Alternatively, they can be considered intertwined antiparallel double-helices stabilized by crisscrossed beta sheets in the inner dimeric interface. The knowledge of the structure, could help us understand the molecular basis of its pH-dependent activity that could lead to its optimization for eventual application in the decontamination of industrial wastes containing cyanide. The objectives of this work were to verify the degradation potential of bacteria isolated from diesel and biodiesel and analysis the antagonistic properties of bacteria against filamentous fungi. Bacillus cereus, B. licheniformis, B. amyloliquefaciens, B. kochii, B. subtilis and B. thuringiensis were isolated from diesel oil and Solibacillus sp. and Exiguobacterium acetylicum, from diesel. The analysis of the degrading activity of organic substances was carried out by verifying the degradation halo in specific media. It was also investigated the antibiotic susceptibility profile and antagonistic activity of bacteria against the fungi Curvularia lunata, Fusarium sp. and Drechslera sp. B. cereus, B. amyloliquefaciens and B. kochii showed higher amylolytic activity. All bacteria isolated from diesel blends showed cellulolytic activity, except Solibacillus sp. and E. acetylicum. For the analysis of lipase activity, was found that B. kochii and B. thuringiensis had a high capacity for olive oil degradation. In Tween-80, within 48 h, only B. amyloliquefaciens showed activity. After 5 days incubation, degradation halos were observed for the other isolates, being more pronounced for B. licheniformis, B. kochii, B. subtilis, and B. thuringiensis and E. acetylicum. Proteolytic activity was more pronounced for B. subtilis, B. thuringiensis and E. acetylicum. All isolates showed sensibility to tested antibiotics, except for oxacillin. All Bacillus spp. inhibited the growth of Fusarium sp., C. lunata and Dreschslera sp. The bacteria isolated from diesel, E. acetylicum and Solibacillus sp. did not present antagonistic action. These results reinforce the biotechnological potential for degradation of organic substances, low microbial resistance profile and potential for use in controlling phytopathogens, of bacteria, mainly of the Bacillus genus, isolated from biodiesel blends. Keywords: Cryo-EM, Biorremediation, Enzymes