ICGEB International Centre for Genetic Engineering and Biotechnology

ICGEB Research Grants awarded under the 2022 Call for applications

Title: New 4-substituted Pyrazolidine and Isoxazolidine as potential antimicrobial agents
Principal Investigator: Dr. Yousfi Tarek, Biotechnology Research Center (C.R.Bt), Constantine, Algeria
ICGEB Reference No. CRP/DZA22-01*
Abstract: Infectious diseases are currently a significant burden in low- and middle-income countries, although non-communicable diseases are overtaking infectious diseases to become the main problem facing developing countries. The treatment of infectious diseases requires a new generation of antimicrobial agents to deal with the problem of bacterial resistance to currently available antimicrobial drugs. Since 2010, the World Health Organisation has invited scientist partners to strengthen their work in the area of discovering antimicrobial agents that can solve this problem, in a global strategy entitled "Health for 2030" [1b-c]. This project targets the synthesis and characterisation of new chiral compounds of 4-substituted Pyrazolidines and Isoxazolidines, as well as the evaluation, *in silico* and *in vitro*, of their antibacterial and antifungal activities, in order to develop new antimicrobial agents.
***Funded by COMSTECH-ICGEB Scientific Cooperation Programme**

Title: Identifying alternative splicing isoforms from coding genes that can act as long non-coding RNAs
Principal Investigator: Dr. Ezequiel Petrillo, Instituto de Fisiología, Biología Molecular y Celular (IFIBYNE) -CONICET – UBA, Buenos Aires, Argentina
ICGEB Reference No. CRP/ARG22-03
Abstract: Coding genes are transcribed into RNAs that are translated into proteins, making these different from long non-coding RNAs. However, both RNA categories are (mostly) transcribed by the RNA polymerase II and undergo the same processing events: capping, splicing and polyadenylation. Alternative splicing is a mechanism by which different transcripts can be generated from the same gene, potentially expanding the proteome. By adding/changing functions, this mechanism can be of great relevance for sessile organisms like plants, which need to cope with changing environmental conditions to survive. Paradoxically, the most frequent alternative splicing event in plants is intron retention, which, by introducing premature termination codons, generates transcripts that lack coding capacity. Evidence from our lab supports a regulatory role for these non-coding transcripts. We hypothesise that these RNAs may help fine-tune gene expression to adapt to changing conditions, giving alternative splicing new functionality. These could be an unexplored regulatory tool in crop improvement.

Title: Development of a yeast-cell precision medicine platform for uncovering genetic modifiers of Niemann-Pick C disease severity
Principal Investigator: Dr. Andrés Klein, Universidad del Desarrollo, Center for Genetics and Genomics, Santiago, Chile
ICGEB Reference No. CRP/CHL22-02
Abstract: Niemann-Pick type C (NPC) is a rare and incurable multiorgan lysosomal disease. NPC is mainly caused by loss of function of the NPC1 gene. Patients can show huge phenotypic variability, ranging from neonatal death to living for decades, even when sharing identical NPC1 mutations. The biological reasons for such heterogeneities are unknown, but modifier genes are very likely to play a significant role. Discovering them, however, is challenging, because it is difficult to recruit NPC patients with different severities. Yeast possesses an NPC1 functional ortholog and has been used to study NPC. Here, we will model NPC by

chemical inhibition of NPC1 in a panel of already sequenced yeast strains of different genetic backgrounds. We will map modifiers by linkage of the growth curves and vacuolar morphologies, which are good severity parameters. Modifiers will be validated with commercial yeast deletants, overexpression, and in cells derived from NPC patients with different severities.

Title: The critical role and intervention study of Foxm1 in MDS through p21 and Klf4 regulation
Principal Investigator: Dr. Yue Sheng, The Second XIANGYA Hospital of Central South University, Changsha City, Hunan Province, China
ICGEB Reference No. CRP/CHN22-03_EC
Abstract: Myelodysplastic syndrome (MDS) is a highly heterogeneous hematological tumor; since it is caused by complex molecular mechanisms, the treatment of MDS is a major issue among hematologic diseases. This project focus on discovering the molecular mechanisms of MDS and developing new methods of intervention. The applicant found that FOXM1 levels were elevated in MDS patients, indicating that FOXM1 may play a role in MDS development. In order to study FOXM1 functions in MDS, the applicant constructed two mouse models with ectopically expressed FOXM1, and the mice showed MDS-like phenotypes. The applicant will continue to monitor the mice and analyse the phenotypes, examine the molecular mechanisms, and use MDS patient cells to study FOXM1's function and screen potential therapeutic drugs.

Title: Poly-inoculum to drench application as control of rice phytopathogen (*Burkholderia glumae*, *Pyricularia oryzae*, *Gaeumannomyces graminis*) and its effect on the plant and soil microbiome
Principal Investigator: Dr. Nubia Carmenza Moreno Sarmiento, Universidad Nacional de Colombia, Bogotá D.C., Colombia
ICGEB Reference No. CRP/COL22-02
Abstract: Biological control of phytopathogens has become an important alternative to the broad use of chemical pesticides. In our previous work, we have already characterised several strains of phylum Firmicutes (Bacillus, Paenibacillus, Brevibacillus), Actinobacteria (Streptomyces), and even fungi, against Burkholderia or Pyricularia infections *in vitro*. In addition, we have studied some of them at the greenhouse level as bio-controller and promoter microorganisms, acting as a single inoculum. However, poly-inoculum application can boost biological control, but we do not know the scope of the drench application of a poly-inoculum on plant and soil microbial communities. The aim of this research is to identify a poly-inoculum for drench application that can control rice phytopathogens and determine its effects on the plant and soil microbiome. The results obtained will improve the sustainability of agro-ecosystems in the short-, medium- , and long-term, through the decrease in use of chemical pesticides, leading to conservation of soil biodiversity, and soil health.

Title: Study of Inflammatory Bowel Diseases (IBD) in Djibouti : Crohn's Disease (CD) and Ulcerative Colitis (UC)
Principal Investigator: Dr. Ali Merito Ali, Center for Studies and Research of Djibouti (CERD), Djibouti
ICGEB Reference No. CRP/DJI22-01*****
Abstract: Crohn's disease and ulcerative colitis are pathologies linked to an activation of the intestinal immune system against the microbiota in genetically susceptible hosts and under the influence of environmental factors. In this project, we aim to study the pathogenic microorganisms involved in the intestines of Djiboutian patients with chronic inflammatory bowel disease (IBD). Furthermore, other genetic and environmental factors are also known to impact these diseases, independently of the microbiota. Thus, the major genetic analyses carried out in recent years have made it possible to identify predisposing variations for these diseases in more than 150 genes. During this project, we will carry out a molecular study of the CARD15/NOD 2 gene (which increases the risk of developing Crohn's disease) in Djiboutian patients with Crohn's disease, in order to specify the demographic, topographical, phenotypic factors involved, and to examine possible associations between CARD15/NOD2 gene mutations and Crohn's Disease.
***Funded by AICS-Italian Agency for Development Cooperation (BIOTECHNET initiative AID n. 12098)**

Title: Implementing pathogen genomics and nanobiotics to combat antimicrobial resistance
Principal Investigator: Dr. Mohamed Elhadidy Elhadidy, Zewail City of Science and Technology, Giza, Egypt
ICGEB Reference No. CRP/EGY22-03*****
Abstract: The main aim of this project is to implement high throughput sequencing technologies to gain crucial insights into the genetic determinants of antimicrobial resistance (AMR) in Methicillin-resistant Staphylococcus aureus (MRSA). This will allow prediction of the antibacterial capacity of nanoparticles formulated to combat AMR. Through the input sequencing data, genome-wide association studies and machine learning will be implemented to determine genes significantly associated with biofilm formation (which is a major contributor to AMR). Thereafter, the potential ability of several biocompatible nanoparticles loaded with compounds of natural origin will be assessed for antibiofilm activity, both phenotypically and at the molecular level.

A key strength of this project is the translation of the results of fundamental microbial genomics research into drug-delivery systems. The proposed workflow can be tailored for different anti-biofilm systems against a wide range of pathogens, thus combating the increased threat of AMR and contributing to finding solutions for this challenging problem.

***Funded by the Italian Ministry of Foreign Affairs and International Cooperation – Directorate General for Development Cooperation (MAECI–DGCS) under the WESTAR Project (Training Scheme for African women scientists)**

Title: Biomimetic membrane-coated nanoparticles for targeted cardiovascular therapy

Principal Investigator: Dr. Mina Mehanny Habeeb Kaldas, Faculty of Pharmacy, Ain Shams University, Cairo, Egypt

ICGEB Reference No. CRP/EGY22-04_EC*****

Abstract: Cardiovascular diseases are the top causes of deaths worldwide and also in Egypt, where ischemic heart disease and stroke account for most mortalities. Nanotechnology has the potential to be a strong tool to achieve targeted drug delivery and thus minimise undesirable side-effects. We will explore the potential formulation of nanoparticles for the biodegradable and targeted delivery of drugs to combat the urgent problem of cardiovascular diseases. Our project includes: (i) Development of nanoparticles, from biodegradable and safe polymers, and loading them with anticoagulant and vasodilator drugs; (ii) Targeting of prepared nanoparticles using the state-of-the-art cell ghosts membrane coating technique, for the first time in Egypt; (iii) *In vitro* assessment of safety of prepared formulations in cardiac cells; (iv) *In vivo* study of the biomimetic membrane-coated nanoparticles in animal models to verify the safety and efficacy of the prepared formulations. Overall, we will implement novel approaches to endeavor to develop drug-loaded and targeted biomimetic nanoparticles as promising candidates for treatment of cardiovascular diseases.

***Funded by the Italian Ministry of Foreign Affairs and International Cooperation – Directorate General for Development Cooperation (MAECI–DGCS) under the WESTAR Project (Training Scheme for African women scientists)**

Title: Climate change and transmission dynamics of Dengue epidemiology in Ethiopia: A molecular and entomological study

Principal Investigator: Dr. Gizachew Yismaw, Amhara Public Health Institute, Bahir Dar, Ethiopia

ICGEB Reference No. CRP/ETH22-02*****

Abstract: Dengue Hemorrhagic Fever is an infectious disease that causes the death of many individuals, including mothers and children in the countryside of Ethiopia. The main aims of the project are the surveillance and diagnosis of Dengue virus using Enzyme-linked immunosorbent assay (ELISA) and RT-PCR; the assessment of entomological indices of the dengue vector (house index, pupal /index, Breteau index in the study area; determination of the abundance and species diversity of Aedes mosquito in the area; and identification of the seasonal variation and other factors influencing Aedes mosquito abundance as a proxy for epidemics; as well as sequencing and molecular characterisation of Dengue and Chikungunya viruses in Ethiopia. The main benefits of this project will be to demonstrate the magnitude of the problem; moreover, sequencing of the circulating strains will provide information for the design of vaccines, as well as treatment modalities to prevent and control this catastrophic virus in the country. Should this project be awarded it will be published in ICGEB websites.

***Funded by AICS-Italian Agency for Development Cooperation (BIOTECHNET initiative AID n. 12098)**

Title: Overexpression of rate-limiting enzymes, DBTNBT and DBAT, in Taxus cell suspension culture using CRISPR-Cas9 system as a successful strategy for a substantial increase in Taxol

Principal Investigator: Dr. Mohammad Reza Naghavi, University of Tehran, Tehran, Iran

ICGEB Reference No. CRP/IRN22-03*****

Abstract: Taxol is a prominent anti-cancer metabolite approved for control and treatment of a wide range of cancers, including ovarian, lung, breast, and pancreatic cancers, and AIDS-related sarcoma. The bark of the Taxus tree is the natural origin of Taxol, but the Taxol content of bark is extremely low (<0.069%) and the collection of bark for anti-cancer drug development is destructive and threatens wild resources. In our previous studies, we have produced Taxol in a Taxus cell suspension, but now we intend to increase the Taxol content by genetic engineering. Therefore, the aim of this research is to overcome the limitations of Taxol supply by manipulating key genes (DBTNBT) involved in the Taxol biosynthesis pathway in *Taxus baccata* cell culture by using CRISPR-Cas9, a powerful genetic manipulation technology, combined with elicitors for rate-limiting enzymes (DBAT). We believe that the output of our attempt will facilitate the production of sufficient quantities of this valuable anti-cancer compound in bioreactors, at lower cost and with fewer environmental side-effects.

***Funded by COMSTECH-ICGEB Scientific Cooperation Programme**

Title: Insights into the resistome of healthy and mastitis camel milk microbiome

Principal Investigator: Dr. Rita Rahmeh, Kuwait Institute for Scientific Research, Kuwait City, Kuwait
ICGEB Reference No. CRP/KWT22-01*
Abstract: Antimicrobial resistance (AMR) is defined as the resistance of microorganisms to antibiotics. AMR is one of the top ten public health problems worldwide, affecting human, animal, plant, and environmental health, as well as national economies. As a part of the global action plan to tackle the AMR challenge, the goal of this project is to identify antimicrobial resistance genes (ARGs) in camel milk microbiota, using culture-dependent and shotgun sequencing techniques, and to investigate their ability to be spread. This study will provide a comprehensive understanding of the camel milk resistome, as well as the risk of ARG transmission. The findings of this study will be used to develop recommendations for reducing the use of the antibiotic classes to which the majority of camel milk microbiome is resistant, as well as being used in the development of natural antimicrobial peptides that are active against the revealed multi-drug resistant organisms.
***Funded by COMSTECH-ICGEB Scientific Cooperation Programme**

Title: Synthesis and heat transport mechanism of biodiesel from marine macroalgae feedstocks catalysed by activated palm kernel shell waste towards biofuel standard specification
Principal Investigator: Dr. Mardiana Idayu Ahmad, Universiti Sains Malaysia, Pulau Pinang, Malaysia
ICGEB Reference No. CRP/MYS22-01*
Abstract: There is a major need to look into new energy sources as demand grows rapidly. Biodiesel is a viable renewable energy source. With less resource input than animal- and other plant-based sources, marine macroalgae have received attention as a potential third-generation biofuel energy source. However, research on producing bioenergy from marine macroalgae is still in its infancy, as far as commercially viable technical solutions are concerned. Previous investigations of marine macroalgal oil concentrated on using acid or alkaline catalysis of extraction by heating with autoclave, Soxhlet, and microwave. This research will employ a unique technique to extract, synthesise, analyse, and examine the heat-transfer mechanisms of biodiesel derived from marine macroalgae feedstocks. Marine macroalgae oil will be extracted using a supercritical carbon dioxide method, and biodiesel will be synthesised using an activated palm kernel shell waste catalyst, its physico-chemical and heat-transport mechanisms will be characterised. The expectations are optimistic to develop a biodiesel with adapted properties that meet the existing biofuel standard specifications.
***Funded by COMSTECH-ICGEB Scientific Cooperation Programme**

Title: The use of PLGA-Chitosan nanocarriers to improve gentamycin and a novel phytomedicine uptake while targeting *Enterococcus faecalis* biofilm formation in a root canal
Principal Investigator: Dr. Albertina Shatri, University of Namibia, Windhoek, Namibia
ICGEB Reference No. CRP/NAM22-01*
Abstract: Multidrug-resistant and biofilm-forming *Enterococcus faecalis* is the major cause of failure of dental canal treatment reported in 400 million of the African population. Biofilm formation normally reduces the efficacy of antibiotics, resulting in persistent apical periodontitis and antibiotic resistance. Namibia's national developmental goals seek to use the country's natural resources, such as medicinal plants, to improve the access to healthcare for all people. With oral hygiene being a major concern in most African countries, including Namibia, utilising natural and commonly-used medicinal plants to develop complementary and alternative medicines against biofilm-forming resistant pathogens is crucial, as it adds value to natural products while solving national health problems. Moreover, applying cutting-edge nanotechnology to encapsulating antibiotics and phytomedicine, using FDA-approved biodegradable, bio-adhesive, biocompatible polymers enhances their uptake, efficacy, and bioavailability. Hence this study aims to synthesise PLGA-Chitosan-modified nanoparticles to improve the uptake and antibiofilm properties of gentamycin and phytomedicines.
***Funded by the Italian Ministry of Foreign Affairs and International Cooperation – Directorate General for Development Cooperation (MAECI–DGCS) under the WESTAR Project (Training Scheme for African women scientists)**

Title: Optimising root system architecture to improve phosphorus use efficiency in rice
Principal Investigator: Dr. Zaigham Shahzad, Lahore University of Management Sciences, Lahore, Pakistan
ICGEB Reference No. CRP/PAK22-05_EC*
Abstract: Rice is an important source of calories for billions of people across the world. Current rice production strategies rely heavily on phosphorus (P) fertilisation; however, this practice is not sustainable due to the expected exhaustion of P resources and harmful environmental impacts of P fertilisation, such as eutrophication. Therefore, rice varieties with improved efficiency in P-use are becoming a prerequisite to improve yields and reduce environmental impacts. In this regard, plants with topsoil foraging root systems and high capacity to solubilise soil P are suggested to be ideal. A large variation in root growth under P-deficient environments exists between different rice varieties. However, the genetic bases of such variation remain unclear. Here, using a candidate gene approach, coupled with untargeted association genetics, we

aim to uncover genetic mechanisms controlling root adaptation to P deficiency in rice. This research will establish a mechanistic framework for improving P use efficiency in crops.
***Funded by COMSTECH-ICGEB Scientific Cooperation Programme**

Title: Evaluation of efflux pumps responsible for the pyrazinoic acid efflux rate, potentially related to the mechanisms of action and resistance to pyrazinamide in *Mycobacterium tuberculosis*
Principal Investigator: Dr. Patricia Sheen, Universidad Peruana Cayetano Heredia, Lima, Peru
ICGEB Reference No. CRP/PER22-02
Abstract: Pyrazinamide (PZA) is the most important drug against the latent stage of *Mycobacterium tuberculosis*. Despite its importance, its mechanisms of action and resistance are not completely understood. After being hydrolysed, PZA is converted into pyrazinoic acid (POA), which is exported to the extracellular environment by active diffusion, driven by an efflux pump system, which is still unknown.
In our previous studies we found an efflux pump in *M. smegmatis* (MSMEG_0250) that was associated with a decrease in the POA efflux rate after its knock-down by CRISPRi.
In this study we will evaluate the effects of down-regulation and up-regulation of the expression of the MSMEG_0250 homologous gene in *M. tuberculosis,* as well as other efflux pump-encoding gene candidates, on the POA efflux, the susceptibility to PZA, and the bacilli growth rate of *M.tuberculosis*. We wish to decipher the POA efflux mechanism in *M. tuberculosis*.

Title: Genetic alterations and treatment response in patients with diffuse large B-cell lymphoma
Principal Investigator: Dr. Sanda Buruiana, Nicolae Testemitanu State University of Medicine and Pharmacy of the Republic of Moldova, Chisinau, Republic of Moldova
ICGEB Reference No. CRP/MDA22-01
Abstract: Diffuse large B-cell lymphoma is one of the most prevalent hematologic malignancies and is a significant medical issue for the Republic of Moldova and globally. A considerable number of patients do not respond to standard chemotherapy. This is explained, in part, by the marked biological diversity of the disorder. In order to establish an appropriate treatment strategy for every patient, a better understanding of the underlying tumour mechanisms is needed. Many attempts were made to subclassify this disease, but existing approaches still cannot wholly distinguish the full biological spectrum. This study aims to analyse some of the genetic and molecular alterations in patients with diffuse large B-cell lymphoma, and to evaluate the response to standard chemo-immunotherapy, with respect to the presence or absence of these alterations. Assessment of these biological variations is necessary in order to stratify patients into distinct prognostic subgroups and to properly design and apply future targeted treatment.

Title: Double-edged sword of Parkinson's disease: the study of extracellular vesicles as a mediator of pathology spreading and unique source of early diagnostics data
Principal Investigator: Dr. Dominika Frikova, Institute of Neuroimmunology, Bratislava, Slovakia
ICGEB Reference No. CRP/SVK22-04_EC
Abstract: The proposed project represents a necessary step towards understanding the pathological processes underlying Parkinson's disease (PD), the second most common neurodegenerative disease. We aim to fill the gaps in our knowledge of the pathology of this devastating disease, and to provide justification for using extracellular vesicles (EVs) as potential biomarkers, or targeting them as a possible therapeutic venue for the differential diagnosis and treatment of PD. We propose a completely novel strategy to study EVs in PD pathology. We aim to identify the specific signature of PD that is carried by EVs, to cluster the obtained data and perform deep bioinformatic analysis. First, we will generate unique cellular model systems in a uniform genetic background, which will allow us to obtain more consistent data and subsequently proceed to patient studies. Thus, our work will provide new insight into the role of EVs in the pathogenesis and diagnosis of PD, which could lead to better patient management and improve outcomes for PD prognosis.

Title: Development of oncolytic plasmids for cancer immunotherapy
Principal Investigator: Dr. Urska Kamensek, Institute of Oncology Ljubljana, Ljubljana, Slovenia
ICGEB Reference No. CRP/SVN22-02
Abstract: In this project, we propose a new gene electrotransfer (GET)-based cancer treatment, using bacterial toxins to prime and boost the patient's own immune system against the tumour's intrinsic antigens. The main advantage of such an approach is its universality against different cancer types. Further advantages are related to using GET to deliver toxins, instead of using recombinant immunotoxins or viral gene therapy. In GET we have a tool to deliver plasmids encoding the secretory form of toxins directly to tumours, which can then lead to a local paracrine secretion of the produced toxin. Hence, a one-time transfection of only a few cells inside the tumour should be sufficient to cause a therapeutic effect. Furthermore, plasmids encoding toxins are not toxic for the environment and are much safer to produce and use than viral vectors. Toxins are also not mutagenic, unlike most conventional anticancer treatments that kill by damaging DNA.

Title: Improving soybean yield and drought tolerance in South Africa
Principal Investigator: Dr. Olubukola Oluranti Babalola, North-West University, Potchefstroom, South Africa
ICGEB Reference No. CRP/ZAF22-03*
Abstract: The challenges facing soybean production as feedstock for biodiesel production in South Africa include persistent drought, soil degradation, diseases and pest infestation. These constraints have affected the quality of soybean as feedstock, and have caused severe yield losses. As production increases, the incidence of pests also increases, as is evident in the number of root knot and lesion nematodes reported to be associated with soybean in South Africa. Drought is also a major threat to soybean production in South Africa, which is being exacerbated by climate change. The study aim is to identify and characterise plant growth-promoting organisms from the rhizosphere soils of two genotypes of soybean for their growth promotion and drought tolerance activities, using molecular and metabolomics techniques.

***Funded by the Italian Ministry of Foreign Affairs and International Cooperation – Directorate General for Development Cooperation (MAECI–DGCS) under the WESTAR Project (Training Scheme for African women scientists)**

Title: Investigating the EML1-microtubule cytoskeleton Interaction in heterotopia
Principal Investigator: Dr. Nurhan Ozlu, Koç University, İstanbul, Turkey
ICGEB Reference No. CRP/TUR22-03
Abstract: The adult cerebral cortex is a complex structure. Any mistake during cerebral cortex development may lead to cortical malformations that result in multiple rare disorders with no available treatments. Cortical malformations that are characterised by abnormal neuronal position within the cortex are called heterotopia. Patients with heterotopia typically demonstrate recurrent epileptic seizures. Mutations in the EML1 gene, which codes a microtubule-associated protein from the EMAP family, cause severe heterotopia in both mice and humans. The proposed research aims to characterise heterotopia-causing mutations in the EML1 gene and investigate how the microtubule cytoskeleton network is deregulated during cortical malformations. The outcome of this project will contribute to the understanding the activities of the pathological form of EML1 in humans, and will reveal molecular details of cortical malfunction within the cortex. It will also shed new light onto EMAP family proteins and their interactions with the microtubule cytoskeleton.

Title: The role of trypanosome RNA binding protein TcUBP1 in post-transcriptional regulation
Principal Investigator: Dr. Pablo Smircich, Instituto de Investigaciones Biológicas Clemente Estable, Montevideo, Uruguay
ICGEB Reference No. CRP/URY22-03
Abstract: *Trypanosoma cruzi* is the parasite causing American trypanosomiasis or Chagas disease, a neglected disease that affects more than 8 million people, leading to 10,000 annual deaths in poor areas of Latin America. Current pharmacological treatments are toxic and frequently ineffective. Understanding how the parasite regulates its genome expression can lead to filling in gaps of knowledge, thereby facilitating the development of more effective and less toxic compounds to treat Chagas disease. *T. cruzi* mainly controls gene expression at the post-transcriptional level, highlighting the relevance of RNA binding proteins (RBPs) in the definition of the transcriptome and proteome profiles. This proposal will focus on the study and characterisation of TcUBP1, an RBP that our previous results highlighted as a key player in the parasite's biology. The proposal aims to understand the role of TcUBP1 in post-transcriptional regulation, describing its molecular partners and its effects on the shaping of the parasite gene expression profiles.