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ICGEB Research Grants Programme

RESEARCH GRANTS COMPLETED in 2021

CRP - ICGEB RESEARCH GRANTS COMPLETED IN 2021

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BANGLADESH

Title: Elucidation of taxol biosynthetic pathway in endophytic fungus *Grammothele lineata* -SDLCO-2015-S1

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Abstract: Initial screening had confirmed the production of taxol by *Grammothele lineata* -SDLCO-2015-S1, an endofungus isolated from jute. Subsequent investigation for taxol production could not be reproduced and biosynthetic pathway could not be elucidated. While searching for taxol the strain was found to harbor biosynthetic gene cluster for another anticancer drug, epothilone. Both the genome and RNA-Seq data showed the presence of epothilone gene clusters in *G. lineata*. TLC, LC-MS/MS were used to isolate and characterise epothilone. Purified sample showed signal at ($M+H^+$) = 508.1 Da, identical to standard epothilone B. Product ions were found with the m/z values at 321.2 Da, 421.2 Da; atypical MS2 fragments for standard Epothilone B. Investigation of *G. lineata* genome using bioinformatic tools for industrially important enzymes and secondary metabolites allowed identification of 28 gene clusters for secondary metabolites and a diverse range of CAZymes, including numerous lignocellulolytic enzymes. Transcriptome and secretome analyses of *G. lineata* were performed to understand the lignocellulosic enzyme production capacity, nature of the enzymes, and their possible use for biomass hydrolysis. A study of untargeted metabolomics led to the identification of artemisinin, a widely known antimalarial compound.

Objectives:

(i) *In silico* prediction of Taxol biosynthetic pathway in *Grammothele lineata* through genome analysis. (Initially this was not enumerated as an objective of the proposal. However, since the genome sequence was available it was thought pertinent to try and elucidate the taxol biosynthetic pathway in the endofungus as this would be highly relevant to the project);
(ii) Optimisation of the growth conditions and other parameters for taxol production and quantification;
(iii) Identification of the optimum combination of elicitors (media optimisation). List of reported elicitors expected to increase taxol production includes methyl jasmonate (MJ), vanadyl sulfate (VS), salicylic acid (SA), phenylalanine, benzoic acid, indole acetic acid (IAA) and cobalt chloride;
(iv) Quantification of the amount of taxol produced for each combination of elicitors after 15 to 21 days;
(v) Isolation of total RNA from control and optimised elicitors at 4 different time intervals starting from 15 to 21 days;
(vi) RNA -sequencing analysis using Illumina Hi-seq platform;
(vii) Isolation of total protein from control media and optimised elicitor media at 4 different time intervals;
(viii) Proteomics analysis using LC-ESI-MS/MS iTRAQ labeling;
(ix) Annotation of proteo-transcriptomics data and identification of the overlapping elicited pathway.

Results Obtained:

G. lineata is under the Basidiomycota phylum and fungi under the phylum of Basidiomycota are known to produce a variety of cytotoxic compounds (Rosa et al., 2009). Thus, *G. lineata* with the ability to produce epothilone was not found to be surprising. The epothilones are a class of potential cancer drugs. Like taxanes, they prevent cancer cells from dividing by interfering with tubulin, but in early trials epothilones have been shown to have better efficacy and milder adverse effects than taxanes. BLASTx has also been carried out for the RNA-Seq data generated in this project primarily for identifying taxol biosynthetic pathway. For identifying the epothilone gene cluster the *G. lineata* transcripts were matched for homology with epothilone gene clusters identified from the genome analysis. Each of these clusters showed 100% similarity with two of the genes from the RNA-Seq data. In order to establish epothilone production capacity of the fungus, purification of the compound was carried out using thin layer chromatography along with standard Epothilone B. Later, MS-MS identified the molecular weight of epothilone B. Mass-spectra were acquired in positive ionisation mode with auto MS2 fragmentation. Both the standard epothilone B and HPLC collected peak of 40^{th} minute produced a molecular mass of 508.1 Da ($M+H^+$). They also produced the typical MS2 fragments at m/z 420.0 Da and 320.2 Da in the product ion mode. This confirmed the presence of epothilone in *G. lineata* extract. Further confirmation will be made through NMR once enough of the compound is purified.

To understand the chemodiversity of *G. lineata*, we studied the untargeted metabolome of the strain at three different time points. One of the objectives for this was to *identify taxol or its intermediates in the metabolome data. Unfortunately, we failed to find such metabolites.* However, a detailed comparison between the transcriptomic and the secretome data was made to understand the

fungus's lignocellulosic enzyme production capacity, nature of the enzymes, and their possible use in plant biomass hydrolysis. This led to the identification of artemisinin in the untargeted extracellular metabolome. This well characterised antimalarial compound was initially isolated from the plant, Artemisia annua. We also investigated whether monoisotopic mass of the intermediate metabolites of the artemisinin biosynthetic pathway can be predicted from the metabolome data. 10 intermediate compounds were predicted as they were found to match with the metabolites of the artemisinin biosynthesis pathway. *G. lineata* genome was also searched and annotated to identify the genes involved in this pathway. As fungi and plants are distantly related, only three genes could be identified in the *G. lineata* genome with a maximum of 51% identity.

As a basidiomycete and an endophyte to a host plant (i.e., jute) with massive lignocellulosic content, the presence of numerous carbohydrate-active enzymes was expected in *Grammothele lineata*. Considering these, cellulases produced by *Grammothele lineata* were studied extensively in search of an efficient cellulase system applicable in biofuel production. *In silico* analysis of the genome of *G. lineata*, identified a total of 485 genes which were assigned to CAZyme families, and the number of glycoside hydrolases (~53.97%) present was found to be significantly higher than the other families. Secretory proteins were analysed using LC-MS/MS to identify cellulases in cellulase-inducing conditions. A total of 378 proteins were identified from the secretome with high confidence. Among them, 198 proteins were related to metabolism, and 145 proteins were enzymes. dbCAN, a web resource for automated carbohydrate-active enzyme annotation, analysis suggested that a good number of enzymes and associated proteins are secreted, which is the key component for pretreated as well as untreated biomass hydrolysis. More than 70% of secreted total proteins were suggested to be directly related to biomass hydrolysis by Label-Free Quantification (LFQ) using the Protein Discoverer. The richness of CAZymes as well as terpene synthases identified in this endophytic fungus suggests that it is a great candidate to pursue for development into biofuel development platform.

Results Unforeseen in the Original Project:

Initial studies in our lab had revealed that *G. lineata* has the potential to produce a diterpenic polyoxygenated pseudoalkaloid-paclitaxel (taxol), in culture conditions. Therefore, initially the focus of this project was to elucidate the taxol producing pathway functional in *G. lineata.* However, in subsequent studies we failed to isolate taxol from this endofungus. Also, an analysis of *G. lineata* genome failed to pinpoint the genes involved in the biosynthesis of the compound. Even an analysis of the metabolomic data failed to identify taxol or its metabolites. There are reports that the molecular mechanisms required to produce such phytochemicals is likely to be acquired from the plant host. After several generations of growth in axenic conditions, endophytes usually undergo attenuation and thus the endophytes may lose the capacity to produce the compound. Even genome analysis of these endophytes often fails to reveal the complete biosynthetic pathway of the compound.

Publications:

Ehsan, T., Reza, R.N., Das, A., Ahmed, O., Baten, A.K.M.A., Ferdous, A.S., Islam, M.R., Khan, H. Genome and secretome analysis of jute endophyte *Grammothele lineata* strain SDL-CO-2015-1: Insights into its lignocellulolytic structure and secondary metabolite profile. 2020. Genomics, **112(4)**, 2794-2803

KENYA

Title: Deciphering resistance and virulence in sorghum-*Striga* interaction

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Abstract: Sorghum is a major food stable in sub-Saharan Africa (SSA) but its yields are constrained by the parasitic plant Striga that attaches to roots of cereal crops causing severe stunting and loss of yield. Both sorghum and Striga co-evolved in Eastern Africa with no serious threat by the parasite. However, transformation of wild sorghum into elite cultivars through domestication has led to a gradual decline in sorghum resistance, and a corresponding increase in Striga virulence. In this ICGEB-CRP project, we sought to expand the genetic basis of cultivated sorghum to cope with evolving Striga virulence in order to build durable and broad-spectrum resistance. Our hypothesis hinged on: (i) our previous findings that revealed wild sorghum accessions are important reservoirs of Striga resistance genes and (ii) emerging literature that Striga is able to evade host immunity using effector molecules. The project achieved following outputs: (i) development of tissue culture protocols for the obligate parasitic plant Striga hermonthica; (ii) elucidation of gene networks for sorghum-Striga interactions; (iii) establishment of a platform for genetic transformation of sorghum for Striga resistance; (iii) identification of new Striga resistant sorghum adapted to Africa agro-ecologies; (iv) leveraging new partnerships for establishment of a sorghum genome-editing platform.

Objectives:

In this application, we sought to expand the genetic basis of cultivated sorghum to cope with evolving Striga virulence in order to build durable and broad-spectrum resistance. We wanted to achieve this by developing resistance in sorghum against Striga based on interactions between effectors, virulence genes from Striga and resistance genes from sorghum. Our work was to provide critical information on the resistance mechanism of sorghum to Striga and how that resistance can be maintained. Outputs from this project are directly applicable in SSA and enable farmers to grow Striga resistant sorghum in the short-term.

We were guided by two specific objectives:

(i) To determine the role of Pathogenesis-Related Proteins in the sorghum-Striga interaction,

(ii) To determine the role of effectors in the sorghum-Striga interaction.

Results Obtained:

To determine the underlying genetic course of this resistance, we used RNA sequencing to determine genes, in wild sorghum, that are differentially expressed at early and late Striga infection and compared their profile to those of susceptible cultivated sorghum. In addition, we compared the profile of differentially expressed genes between Striga infecting wild and cultivated sorghum. We found that more genes were differentially expressed in wild sorghum upon Striga infection and that more Striga genes were differentially expressed when the infection was on wild sorghum. These findings suggest that wild sorghum has more genetic diversity to cope with Striga and that more genes in Striga are required to overcome resistance from the host. RNA sequencing data further led us identify a set of candidate Striga resistance genes from wild sorghum as well as a rapporteur of molecules (effectors) that may be aiding Striga to overcome the innate immunity of its hosts.

In summary, we found the following genes as important regulators of resistance in sorghum:

(i) dehydration-responsive element-binding (DREB) protein/C-repeat binding factors (CBFs), which belong to APETALA2 (AP2) family transcription factors. In our analysis, DREB was downregulated in susceptible but upregulated in resistant varieties.

(ii) Universal Stress Protein A. This gene was also upregulated in resistant varieties relative to susceptible varieties and has been demonstrated to confer resistance against a broad range of pathogens.

(iii) Cytokinin-Activated Transcription Factor ARR2. This gene confers plant immunity via the Non expressor of pathogenesis related protein 1 (NPR1)-dependent salicylic acid signaling in Arabidopsis.

(iv) Leucin rich repeat receptor like genes. Two LRR-like were also significantly differentially expressed. LRR point to programmed cell death resistance. On the virulence component of Striga-sorghum interaction. The most prominent genes were the Pectin methylesterase inhibitors (PMEI) that work to inhibit Pectin methylesterases (PMEs) that catalyse the demethylesterification of the homogalacturonan domains of pectin in the plant cell wall.

To further elucidate the intricate host-parasite interactions between Striga and sorghum, we sought to carry out functional validation of resistance and virulence genes. To achieve this goal, we developed efficient protocols for Striga *in vitro* culture in order to achieve optimal growth and proliferation of Striga plants. This is a necessary step for all subsequent *in vitro* culture based experiments that require aseptic Striga tissue. In addition, we required a protocol that will lead to production of a large amount of Striga somatic embryos that can be efficiently regenerated. For many tissue culture protocols, role of growth regulators is critical for somatic embryogenesis and regeneration. We therefore varied concentrations of the auxin Naphthaleneacetic acid (NAA) and cytokinin 6-Benzylaminopurine (BAP). These experiments led us to establish further protocols for Striga somatic embryogenesis, regeneration and organogenesis. To analyze the Striga resistance component in sorghum, we developed a sorghum transformation platform using candidate resistance genes.

Results Unforeseen in the Original Project:

To increase the efficiency of candidate gene validation we are now taking a genome editing approach. This has been made possible through establishment of a strategic partnership with Corteva Agriscience, leaders in genome editing protocols, and Pennsylvania State University. This partnership allowed us to get proof-of-concept that genome editing can be used as a Striga-sorghum functional genomics tool and also as a strategy for introducing resistance against Striga. This proof was obtained following on previous knowledge that Striga germination occurs in response to stimulation by the plant hormone strigolactone and that Striga resistance in sorghum occurs due to a natural mutation in the loci called *LOW GERMINATION STIMULANT1* (*LGS1*) that prevent them from producing the germination stimulant. CRISPR/Cas9 was used to precisely introduce mutations in a farmer popular sorghum variety called Marcia in the *LGS1* loci. These Striga resistant gene-edited material have been developed and will be evaluated at Kenyatta University.

In addition to genome editing we have also embarked on a screening a large population of sorghum for Striga resistance based on mutation on the LGS1 loci. To identify more targets for CRISPR we have taken a Genome wide association studies (GWAS) approach using Genotyping-by-sequencing (GBS) based Single Nucleotide Polymorphism (SNPs). This work led us to identify new sorghum varieties harboring pre-attachment resistance against Striga. We were also able to able to determine new genetic loci underpinning the resistance.

Publications:

Waweru, D., Kuria, E., Bradley, J., Scholes, J., Runo, S. Tissue culture protocols for the obligate parasitic plant *Striga hermonthica* and implications for host-parasite co-cultivation. 2019. Plant Cell Tiss. Organ Cult. **138,** 247–256

Bellis, E.S., Kelly, E.A., Lorts, C.M., Gao, H., DeLeo, D.L., Rouhan, G., Budden, A., Bhaskara, G.B., Hu, Z., Muscarella, R., Timko, M.P., Nebie, B., Runo, S.M., Chilcoat, N.D., Juenger, T.E., Morris, G.P., dePamphilis, C.W., Lasky, J.R. Genomics of sorghum local adaptation to a parasitic plant. 2020. Proc. Natl. Acad. Sci. USA, **117 (8),** 4243-4251

Mallu, T.S., Mutinda, S., Githiri, S.M., Odeny, D., Runo, S. New pre-attachment Striga resistant sorghum adapted to African Agro-ecologies. 2021. Pest Management Science (in press)

MALAYSIA

Title: D-amino acids as potential modulators of age-related functional decline in natural and accelerated aging

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Abstract: D-amino acids (AA), which is a mirror-image of L-AA, have been associated with age-associated diseases including Alzheimer's, chronic kidney disease, Parkinson's disease and frailty; raising speculation that D-AAs may contribute to the aging process. D-AAs are abundantly found in bacterial cell wall structure and intestinal microbial products and the translocation of bacterial products from intestine to the circulation has been shown to occur in many age-associated diseases and in HIV disease, a disease model for accelerated aging. The breakdown of D-AAs by D-amino acid oxidase (DAO) enzyme produces hydrogen peroxide, which may contribute to increased oxidative stress in the tissue microenvironment, leading to eventual organ damage/decline. We found that D-aa measured in plasma was significantly correlated with age in individuals with and without HIV disease and significantly associated with surrogate markers of liver and kidney disease. Additionally, D-aa were correlated with markers of inflammation suggesting their potential role as a biomarker of inflammaging. *In vitro* mechanistic studies in HepG2 cell lines, confirm that the breakdown of D-aa by DAO generates hydrogen peroxide, albeit variably and leads to the activation of NF-kB and the subsequent release of inflammatory cytokines in cell supernatant. Our findings collectively suggest the potential role of D-aa as a previously unrecognised biological entity associated with the process of inflammaging in humans.

Objectives:

(i) To explore the accumulation of D-AAs and its association with geriatric conditions and immunological aging in HIV-infected and uninfected individuals;
(ii) To elucidate the mechanistic pathways affected following exposure to D-AAs through cell-based assays.

Results Obtained:

The concentration of 4 common D-AAs, namely D-Ser, D-proline (D-Pro), D-alanine (D-Ala) and D-asparagine (D-Asn), have been quantified using three dimensional-HPLC in plasma samples of 60 HIV-infected and 59 HIV-uninfected controls. The markers of immune activation and inflammation namely, sCD14, IL-6, TNF-α, IL-1B, sCD163 and hsCRP as well as markers of intestinal permeability I-FABP, LBP and zonulin have been measured in all samples using platforms of ELISA and ELLA. Activity of IFN-gamma-induced indolamine-2,3-deoxygenase, as represented by ratio of kynurenine and tryptophan concentrations (K/T ratio) in the plasma, has also been quantified by LC-MS/MS. Cellular markers of T-cell activation (CD38+, HLA-DR+) and senescence (CD57+, CD28-) have also been quantified by flow cytometry in cryopreserved peripheral blood mononuclear cells.

In our findings we have found that the concentrations of D-Asn, D-Ser, D-Ala and D-Pro increased as both HIV-infected participants and HIV-uninfected controls were older in age. The concentration of D-AAs was also significantly associated with clinical markers of kidney, liver organ function and with the composite Veterans Aging Cohort mortality risk scores in both the HIV-infected and uninfected controls. In addition, specific D-AAs were associated with inflammation markers (TNF-α, K/T ratio and sCD14), suggesting the potential role of D-AAs as putative markers of inflammaging. Furthermore, a higher concentration of D-AAs was observed in HIV-infected individuals presenting with various geriatric conditions (cognitive impairments, urinary incontinence, functional impairment, polypharmacy and polypathology). However, these differences were no longer significant when adjusted for age implying that exposure to D-AAs in individuals with functional aging phenotypes were modulated by age. We did not find a consistent correlation between D-AAs in plasma with direct markers of increased gut permeability and damage in HIV-infected participants to suggest that the source of D-AAs in plasma is a result of microbial products translocating into the systemic circulation. In short, D-AAs may likely play a role in driving multiple organ dysfunction and immune activation in HIV-infected and uninfected controls but are unlikely to be driven by microbial translocation.

To understand the effect of D-AAs on cells, we investigated the activity of DAO following exposure to D-aa to HepG2 cells by measuring the production of H_2O_2. D-AAs is metabolised by DAO and generates hydrogen peroxide (H_2O_2), but variable kinetics were detected with the two D-aa tested *in vitro*. There was an increased in H_2O_2 production for D-Ala, but reduced production following D-Ser treatment. We further determined the levels of DAO gene and protein expression, to understand the difference in H_2O_2 production for different treatment. We found that DAO gene expression was upregulated for D-Ala treatment but downregulated when the cells were treated with D-Ser. These results were consistent with the levels of DAO protein expression measured by Western blot following D-Ala and D-Ser treatment. We next explored if the production of H_2O_2 following D-aa exposure

modulates NF-kB protein expression. We analysed activated NF-kB protein expression following D-AAs treatment using Western blot and found that activated NF-kB protein levels in HepG2 were decreased initially in low concentrations of Daa but increased significantly in high D-aa concentrations after treatment with both D-Ser and D-Ala. We also analysed the cell culture supernatants for inflammatory cytokine secretion. The concentrations for TNF-α and IL-8 increased for both D-Ala and D-Ser treatment. However, IL-6 was not detectable in the supernatants. Taken together, these results suggest that the breakdown of D-Ser and D-Ala by DAO is associated with the production of hydrogen peroxide leads to NF-kB activation and cytokine release, consistent with that observed in plasma samples of aging individuals.

Results Unforeseen in the Original Project:

Different D-AA may affect cellular response differently as we found in our experiments that the regulations of DAO gene and protein expression, as well as the hydrogen peroxide production, by D-Ser and D-Ala were different. However, both amino acids were found to be metabolised by DAO enzyme as found in previous studies and led to the production of H_2O_2. The mechanistic pathway behind this difference is unclear and a thorough investigation on the cellular pathway involved is needed. Additionally, we anticipated that the levels of D-aa will be higher in plasma concentrations of people living with HIV, a disease model for accelerated aging as a result of increased microbial translocation in this group. However, HIV status was found to not be independently associated with concentration of circulating D-aa and that markers of microbial translocation was not associated with D-aa levels. D-aa could still be associated with changes in metabolites produced gut microbiome, as aspect which was not explored in our study and worth investigating in future work.

RUSSIA

Title: Role of zinc-driven interaction of amyloid-beta with neuronal proteins in Alzheimer's disease
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Abstract: Endogenous amyloid-beta (Aβ) is normally a monomeric component of biological fluids, however, it forms soluble neurotoxic oligomers in Alzheimer's disease, and also accumulates as insoluble polymeric aggregates (amyloid plaques) in the brain tissues. Earlier, we determined that zinc-dependent oligomers of certain isoforms of Aβ are capable of triggering pathological aggregation of endogenous Aβ, as well as causing neuronal death. There is evidence that formation of zinc-dependent oligomers of Aβ is dependent on its rigidly structured segment 11-14. This site provides two coordination bonds for one zinc ion and through symmetric coordination of this ion a second Aβ molecule forms one of the two zinc-dependent interfaces in each molecule of Aβ in oligomers. By means of the 11-14 site Aβ is able to form zinc-bound interfaces not only in Aβ homo-oligomers but also in zinc-induced Aβ interactions with other endogenous proteins in which there are sites similar in structure to the site 11 -14 of Aβ. The project has addressed the role of zinc-driven interaction of Aβ with neuronal proteins in Alzheimer's disease.
Objectives:
(i) Determination of potential sites of zinc-dependent interaction between isoforms of Aβ and neuronal proteins associated with the progression of Alzheimer's disease;
(ii) Determination of the structural and functional properties of zinc-linked intermolecular complexes formed by Aβ isoforms and partner proteins and/or their fragments;
(iii) Testing on animal AD models (*C. elegans*) of possible therapeutic effect of specific inhibition of zinc-dependent interactions of Aβ with partner proteins.
Results Obtained:
(i) 3D models of the Aβ(1-42) and isoAsp7- Aβ(1-42) structure with predetermined structure of site 11-14 (bearing a zinc ion chelated by Glu11 and His14) were used as ligands for molecular docking. As target proteins interacting with above Aβ isoforms we have probed Na,K-ATPase, α4β2 nicotinic acetylcholine receptor (α4β2-nAChR), and sheep prion (PrP^C). Computer docking of ligands and target proteins was done using four servers (Gramm-x, ClusPro, SwarmDock, Zdock). Scoring, analysis and ranking of protein-protein docking models was performed using QASDOM Server (http://qasdom.eimb.ru/Qasmod.html). Unlike the PrP^C and α4β2-nAChR, for the Na, K-ATPase there were no suitable sites for zinc-induced interactions with Aβ. The simulations performed show that, the interaction interfaces remain similar for Aβ and isoAsp7- Aβ. Residues His64 and His72 from octarepeat sequence of flexible N-terminal fragment of ovine PrP^C are the most like ligands for zinc coordination on the interface with Aβ and isoAsp7- Aβ. Complexes of Aβ and isoAsp7-Aβ with C-terminal fragment of ovine PrP^C are mediated by PrP^C residues His143 and Asp147. Complexes of Aβ and isoAsp7-Aβ with α4β2-nAChR are mediated by His38 and Asp41 from HAEE tetrapeptide site of α4 subunit of the receptor.
(ii) Along with the post-translational modifications of Aβ, hereditary mutations were found in certain patients, some of which lie in the region of the metal-binding domain 1-16. Such mutations affect the toxicity and rate of Aβ oligomerisation in the presence of zinc ions, causing the development of early onset AD. Among them, the Aβ peptide with the "Taiwanese" mutation (D7H-Aβ) has the greatest aggregation ability (Istrate, Kozin et al. 2016). We have investigated the molecular mechanism of zinc-induced oligomerisation of the metal-binding domain of D7H-Aβ(1-16). The critical role of 7 and 13 histidine residues in enhancing the aggregation properties of the peptide D7H-Aβ(1-16) is established by using turbidity measurements, isothermal titration calorimetry and mass-spectrometry. It is shown that the primary binding site of the zinc ion in the D7H-Aβ(1-16) peptide is the 11EVHH14 site, and the E11 and H14 residues of the interacting peptides form a symmetrical zinc-bound intermolecular dimer interface. Then, in this dimer, the E3/H6 and H7/H13 pairs form two more interfaces, through which the formation of D7H-Aβ(1-16) oligomers and aggregates in the presence of zinc ions occurs. Thus, the D7H mutation leads to the emergence of a new mechanism of the zinc-dependent oligomerisation of the metal-binding domain of Aβ(1-16): the role of E11/H14 as primary zinc-bound interface is preserved (as for the zinc-dependent dimer Aβ(1-16)), but the association constant for this site is increased by an order of magnitude, and instead of the H6/H13 interface found for Aβ(1-16) zinc-bound oligomers, two interfaces appear - E3/H6 and H7/H13. This mechanism explains why Aβ(1-16) with the "Taiwanese" mutation, D7H-Aβ(1-16), is the most prone to zinc-induced aggregation in comparison with other Aβ(1-16) isoforms and confirms that 11EVHH14 is a universal target for drugs aimed to suppress zinc-dependent oligomerisation of various Aβ isoforms.
(iii) We have analysed changes in the lifespan of transgenic nematodes *C. elegans* CL2120 (dvis14 [(pCL12) unc-54 :: beta 1-42 + (pCL26) mtl-2 :: GFP]) and *C. elegans* CL2122 (dvis15 [(pPD30.38) unc-54 (vector) + (pCL26) mtl-2 :: GFP]) (obtained from Caenorhaditis Genetics Center) by adding to the culture medium various molecular agents (Aβ, isoD7-Aβ, zinc ions), which are present in amyloid plaques of patients with AD, as well as the tetrapeptide Acetyl-HAEE-NH_2, which is known to be a specific inhibitor of zinc-dependent Aβ oligomerisation and cerebral amyloidogenesis in vivo (Tsvetkov, Cheglakov et al. 2015). It was shown that the simultaneous addition of zinc ions and isoD7-Aβ leads to a significant decrease in the average lifespan of nematodes, which indicates a sharp increase in the integral toxicity of endogenous Aβ aggregates, which are formed, presumably, by a zinc-induced mechanism under the action of seeding centers with the participation of isoD7-Aβ. At the same time, the

presence of the tetrapeptide Acetyl-HAEE-NH2 in the nutrient medium completely neutralises the negative effects of the combination of zinc ions and isoD7-Aβ on the lifespan of nematodes. The results obtained indicate the fundamental role of non-covalent complexes between the zinc ion and isoD7-Aβ as a trigger for pathological aggregation of endogenous Aβ molecules. Also, using the tetrapeptide Acetyl-HAEE-NH_2 as an example, it was confirmed that molecular agents that suppress the ability of isoD7-Aβ to participate in zinc-dependent oligomerisation can act as potential drugs that stop the development of cerebral almyloidogenesis in Alzheimer's disease.

Results Unforeseen in the Original Project:

On the basis of our findings on zinc-induced oligomerisation of the metal-binding domain of various Aβ species, we hypothesise that upon phosphorylation of Ser8, isoD7-Aβ loses its ability to form zinc-bound oligomeric seeds. We have found that: (i) *in vitro* isoD7-Aβ with phosphorylated Ser8 (isoD7-pS8-Aβ) is less prone to spontaneous and zinc-induced aggregation in comparison with isoD7-Aβ and intact Aβ as shown by thioflavin T fluorimetry and dynamic light scattering data, and (ii) intravenous injections of isoD7-pS8-Aβ significantly slow down the progression of institutional β-amyloidosis in AβPP/PS1 transgenic mice as shown by the reduction of the congophilic amyloid plaques' number in the hippocampus. The results support the role of the zinc-mediated oligomerisation of Aβ species in the modulation of cerebral β-amyloidosis and demonstrate that isoD7-pS8-Aβ can serve as a potential molecular tool to block the aggregation of endogenous Aβ in AD.

Publications:

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TURKEY

Title: Functional and molecular characterization of novel mitotic exit inhibitors
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Abstract: Mitotic exit, the transition from mitosis to G1 phase, is a critical step in the life of a cell and it has to be well regulated. In order for cells to exit mitosis, cyclin-dependent kinase (Cdk) activity has to be down regulated after sister chromatid separation and chromosome segregation. Cdk inactivation relies upon the interplay of mitotic cyclin degradation, Cdk inhibitors and reversal of the Cdk1-Cyclin B driven phosphorylation events that promoted the mitotic state in the first place. A single conserved phosphatase, Cdc14, drives mitotic exit in *Saccharomyces cerevisiae.* In this study we identified the bud cortex protein Bud14 as a novel mitotic exit inhibitor in budding yeast. We further showed that Bud14 together with protein phosphatase 1, Glc7, is a part of the Spindle Position Checkpoint, which halts mitotic exit in response to the failure of spindle positioning along the cell polarity axis. We anticipate that similar mechanisms apply in higher eukaryotes.
Results Obtained:
Among 29 genes identified in a genetic screen that is designed to find mitotic exit inhibitors, one gene, Bud14, was found to be a part of Spindle Position Checkpoint, which halts mitotic exit in response to the failure of spindle positioning along the budding yeast polarity (SPOC) axis. We showed that Bud14 works together with the protein phosphatase 1, Glc7, to promote activity of the SPOC downstream effector Bfa1-Bub2 through dephosphorylation of Bfa1.
Results Unforeseen in the Original Project:
Interestingly, only one gene among many was found to be involved in a known checkpoint. It will be interesting to uncover the roles of remaining genes.
Publications:
Kocakaplan, D., Karaburk, H., Kirdok, I., Erkan, S.N., Dilege, C., Caydasi, A.K. Protein Phosphatase 1 in association with Bud14 inhibits mitotic exit in *Saccharomyces cerevisiae*. 2020. bioRxiv 273946

VIET NAM

Title: Development a simple, low-cost, high sensitivity fluorescent biosensor for hydrogen peroxide (H_2O_2), glucose and cholesterol sensing based on ZnO nanorods decorated metal nanoparticles

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Abstract: In chemical, biological, clinical, environmental applications the detection of hydrogen peroxide (H_2O_2) is of vital important. It is due to the fact that H_2O_2 can be harmful to biological systems and appears to be involved in the neuropathology of central nervous system diseases. For example, the H_2O_2 level in urine is considered as a potential biomarker for oxidation stress in patients with a malignancy. Recently, diabetes is one of the leading causes of death and disability around the world. When the glucose concentration in human blood is not regulated, life threatening diseases, such as diabetic mellitus and acute diabetes, develop. Cholesterol and its fatty acid esters, on the other hand, are one of the main constituents for the human beings as they are the components of nerve and brain cells; they are precursors of other biological materials, such as bile acid and steroid hormones. High serum cholesterol level is often related to various clinical disorders, such as heart disease, coronary artery disease, arteriosclerosis, hypertension, cerebral thrombosis, etc. In recent years, a new kind of H_2O_2, glucose and cholesterol sensing methodology called fluorescent based methodology has been attracted a lot of attention. Comparing to the conventional electrochemical methods, the fluorescent-based method is a non-destructive testing and do not require the implementation of electrodes. These are the outstanding advantageous of the fluorescence-based methods in designing biosensor devices. Herein, the change in fluorescence of luminescent materials such as nanoparticles or a semiconductor can be used as an indicator of the presence and the concentration of target substances such as H_2O_2, glucose or cholesterol.

In this project, we aim to develop a simple, low-cost, high sensitive fluorescent biosensor for hydrogen peroxide (H_2O_2), glucose and cholesterol sensing. The fluorescent biosensor is based on ZnO nanorods (NRS) decorated with metal nanoparticles (or can be called as nano-heterostructure of ZnO NRs/metal nanoparticles). The working principle of the biosensor is based on the detection of H_2O_2/glucose/cholesterol immobilised directly on ZnO NRs/metal nanoparticles through the photoluminescent quenching of ZnO NRs decorated with metal nanoparticles. The presence of the H_2O_2/glucose/cholesterol will give rise to the corresponding change of photoluminescent signals, which in turn can be used for the detection of glucose and cholesterol concentration in the solution. Due to the presence of metal nanoparticles, the sensitivity and the limit of detection of the sensor will be significantly enhanced compared to the case of bare ZnO nanostructures based fluorescent biosensor.

Objectives:

In this project, we aim to develop a simple, low-cost, high sensitive fluorescent biosensor for hydrogen peroxide (H_2O_2), glucose and cholesterol sensing. Based on this main objective, we clarified into three objectives as follow:

(i) To succeed in synthesising ZnO NRs decorated metal nanoparticles with high density, high vertical alignment, and high crystallinity;

(ii) To succeed in investigating the fluorescent behaviours of the as-synthesised ZnO NRs decorated metal nanoparticles treated with target substances such as H_2O_2, glucose or cholesterol. Investigate the sensitivity, the limit of detection, the selectivity and the response time of the sensor;

(iii) To succeed in clarify the accuracy of the proposed fluorescent sensor when it is treated with real blood sample;

(iv) Upgrading the current free beam Photoluminescence setup for further applications.

Results Obtained:

In this study ZnO NRs/Au NPs were successfully synthesised and implemented for non-enzymatic fluorescent glucose sensing. It was shown that due to the adhesion of Au NPs, the sensitivity was significantly increased in compared with that of the sensor based ZnO NRs. The sensor exhibits a working range of 0.01 mM – 12 mM with a remarkable sensitivity, for example it of (22 ± 2) % mM-1 for glucose sensing, and of (29 ± 2) % mM-1 for H_2O_2 sensing in the range of less than 2 mM. The sensor's sensitivity is at least 7 times higher than other fluorescent sensors. This ultra-high sensitivity enables its application in non-invasive, highly sensitive glucose devices, which can monitor glucose levels in low glucose concentration fluids such as tears, sweat, and saliva. Additionally, good selectivity, high response time are other advantages of the proposed sensor. The significant enhancement in sensitivity is due to surface plasmon resonance effect, while the good selectivity toward other interfering species is probably based on energy level matching of ZnO NRs decorated metal nanoparticles. Most importantly, when treated with human blood serum, the sensor exhibits a high accuracy, which is compatible with that of clinical devices. This ensures the potential application of ZnO NRs decorated metal nanoparticles for a single use, high sensitivity, high selectivity, non-invasive glucose sensing in clinical measurements.

Results Unforeseen in the Original Project:

The upgraded fluorescent setup can also be used for lasing emission investigation from biological microspheres.

Publications:

Mai, H.H., Janssens, E. Au nanoparticle–decorated ZnO nanorods as fluorescent non-enzymatic glucose sensor. 2020. Microchim. Acta **187**, 577

Mai, H.H., Nguyen, T.T., Gian,g K.M., Do, X.T., Nguyen, T.T., Hoang, H.C., Ta, V.D. Soft Matter. 2020. (in press)