

# World Biotechnology Congress

July 16-17, 2018 Berlin, Germany



## Posters



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## Druggability analysis and classification of bacterial histidine kinase

**Mohammad Al Sorkhy**

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Anti-Microbial Resistance (AMR) is becoming a real threat to humanity with a resistant strains emerging very frequently. The arising of antibiotics resistance is mainly attributed to the misuse of these agents, in addition to the decline in the development of new drugs by the pharmaceutical industry. The development of new novel antibiotics is an emerging necessity to counteract this serious phenomenon. Bacterial Histidine Kinases (HKs) are one of the most promising targets for novel antibacterial drugs. They are part of the bacterial Two-Component Systems (TCSs), the main signal transduction pathways in bacteria, regulating various processes including virulence, secretion systems and antibiotic resistance. Since mammalian histidine kinases signal transduction pathway is different than the prokaryotic one, the inhibition of those pathways could be a potential target for a novel antimicrobial agent. In this study, the druggability of histidine kinase will be assessed for several bacterial species. In this work, different histidine kinases of varies bacterial origins were assed. First we performed some extensive data mining in the Protein Data Bank (PDB) to obtain a large library of crystal structures of histidine kinases, each one of the proteins were then prepared using the Molecular Operating Environment software (MOE). Druggability assessment of the prepared histidine kinases was then carried out using SiteMap module of the Schrödinger molecular modeling suite.

### Biography

Mohammad Al sorkhy is an assistant professor of cell and computational biology, Dr Al sorkhy started his career as a cell biologist with interest in protein interactions. Then he started to shift towards computational biology to answer his major question about protein interactions.

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# World Biotechnology Congress

July 16-17, 2018 Berlin, Germany

## Phenotypic and proteomic evaluation of EMS-induced mutants of *Nigella sativa*

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*Nigella sativa* L. (Black cumin) is a small herbaceous plant, belonging to family Ranunculaceae. Seeds of this plant have huge medicinal properties. However, the low seed yield is not fulfilling the demand for pharmaceutical purposes. Therefore, an attempt has been made in this study to develop mutant lines of *Nigella sativa* by using EMS. It has been found that 0.1% EMS resulted in the mutant line which is taller with pro-fused branching with high yield compared to wild type plant. Interestingly 1.0% EMS treatment produced dwarf mutant with few branches and low yield. To investigate the contrasting character of both the mutants, proteome analysis of leaves was carried out through 2D-gel electrophoresis. Analysis of proteome of wild type and mutant lines showed that 32 proteins were differentially expressed. These differentially expressed proteins were sequenced through MALDI-TOF and identified by using MASCOT software. The function and location of these proteins were analyzed by using Uniprot software. These proteins were categorized on the basis of their functions and correlated with the phenotypic characters of mutant lines. Validation of differentially expressed proteins was carried out through studying expression pattern of respective gene through quantitative real time PCR. It has been found that expression of 26 proteins was due to mutation in the mutant lines.

### Biography

Ambreen Asif is attending the University of Aberdeen, Scotland, United Kingdom for an Advance Research in Genetic Mapping after being awarded the prestigious Newton-Bhabha Research Fellowship. She is pursuing PhD in AMU with specialization in Genetics and Cytogenetics. She has earlier been awarded the INSPIRE Research Fellowship of Department of Science & Technology (DST), Government of India. She has also been conferred with Anjalina Khan Award and Regularity Award by the Women's College in AMU.

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# World Biotechnology Congress

July 16-17, 2018 Berlin, Germany

## Effect of spray drying, high pressure and UV radiation in the quality of human milk

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Many studies have proved that human milk is the ideal food to feed children during the first six months of life, complementing their feeding with other more complex foods up to two years. But there is the problem of those children whose mothers cannot feed them for some reason beyond their control. For this, it has begun the transformation of mother's milk in powder treated with High Pressures (HP) and UV radiation; with the purpose of supplying human milk Banks in Mexico. It is essential to know if this change allows human milk to conserve its nutritional properties, it is also important to know if the shelf life of the product allows it to compete in quality with the big companies that make formulations of cow's milk powder for babies. The results obtained over the course of 10 weeks have shown that there is no significant loss in the protein and carbohydrates content of the spray-dried human milk powder relative to fresh human milk ( $p > 0.05$ ). Microbiological analyzes performed after high pressure treatment and spray drying did not show growth in standard count, dextrose and Macconkey agar, except in MRS agar  $\leq 12$  UFC; treated with UV radiation there were growth in standard count, potato dextrose, and MRS agar. The results allow to continue with the transformation of the human milk powder and know which treatment is the best for its conservation.

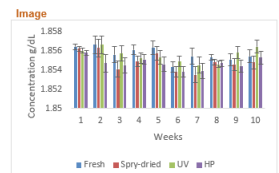


Figure 1. Comparison of concentration of proteins between fresh human milk and different treatments applied during ten week.

### Biography

Ariana Rodríguez Arreola is pursuing PhD from the Department of Biotechnological Process in University of Guadalajara, México. Her research area of interest includes food biotechnology, microbiology, food processing and preservation.

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# World Biotechnology Congress

July 16-17, 2018 Berlin, Germany

## Screening of crop seedlings to salinity stress tolerance: The case of *Pisum sativum* var. *abyssinicum* A. Braun in Ethiopia

**Berhane Gebreslassie Gebreegziabher and Berhanu Abraha Tsegay**  
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**D**ekoko (*Pisum sativum* var. *abyssinicum*) is one of the most important food legumes grown in south Tigray and north Wollo, northern Ethiopia. It is one among the most important food legumes in terms of price and protein content. It grows alone and mixed with many cereal crops growing in north Ethiopia. This study was conducted with the objective of selecting tolerant and relatively high yielding *P. sativum* var. *abyssinicum* collections under different salt (NaCl) concentrations at laboratory conditions. The seeds of the six collections were obtained from four districts; two regional states of north Ethiopia with different altitudinal ranges 1868 m a.s.l. being the lowest and 2457 m a.s.l. the highest. The six on farm vigorously growing local collections, three from Ofla (T-001/08OF, T-002/08OF and, T-003/08OF), one from Sirinka (T-025/08Sr), one from Emba-Alaje (TA-026/15E/A), and one from Endamohoni (T-023/15MW) were studied for salt stress tolerances in controlled condition by priming in four salt treatment levels (5 dS/m, 7 dS/m, 9 dS/m, and 15 dS/m). Distilled water (0 dS/m) was used as control. 50 surface sterilized seeds per petri dish were sown for the four salt treatments and the control. Collections T-001/08 from Ofla and T-023/08 from Endamohoni showed good growth performance at 5 dS/m. However, T-025/08Sr from Sirinka and TA-026/15E/A from Emba-Alaje responded positively up to 7dS/m. At higher salinity level (9 dS/m) growth features decreased with increasing salinity stress. But, T-023/15MW, T-001/08OF, T-025/08Sr followed by TA-026/15E/A from lower to the higher resistances, respectively, could withstand lower (5 dS/m) to medium (7 dS/m) concentrations of salinity as compared to the other collections.

### Biography

Berhane Gebreslassie has his expertise in plant physiology and particularly of the legume crops physiology in improving the agronomic performances and yield increment in this climatic change scenario era. His experience both in Academic and research works on saline areas of the tropics and sub-tropics creates new pathways for improving legume crops production and productivity. He started his study on salt priming particularly of sodium chloride (NaCl) at different concentration for optimization of salt stress tolerance that trigger rapid biochemical and metabolic activities to tolerate the stress thereby increasing their growth starting from germination of *Lathyrus sativus* L. and *Pisum sativum* var. *abyssinicum*. This approach is responsive to all stakeholders and has a different way of focusing on crops challenged by many factors in the nowadays climate changed world. Thus, take measures that enable them to tolerate and produce good yields requires a scientific research for better solution and recommendation.

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# World Biotechnology Congress

July 16-17, 2018 Berlin, Germany

## Accepted Abstracts



# World Biotechnology Congress

July 16-17, 2018 Berlin, Germany

## Identification of cis-regulatory elements of butyrophilin gene of the mammary gland

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Butyrophilin subfamily 1 member A1 is a highly expressed gene in mammary gland of all mammals during lactation. It is found to be the major integral protein in the milk fat globule membrane. Its interactions with other membrane elements and soluble protein of the mammary epithelial cells regulate the secretion of milk fat. In order to reveal any shared cis-acting elements, the human butyrophilin subfamily 1 member A1 5' genomic sequence was analyzed and compared with four mammals comprising of a rodent and three primates, viz., *Mus musculus*, *Macaca mulata*, and three other mammary specific human milk genes using publicly available bioinformatics tools. Prior to a multiple sequence analysis, low complexity DNA sequences were masked using CENSOR. The multiple sequence analysis revealed nine highly conserved regions of similarities in the 5' butyrophilin genes across species. Consensus putative transcription factor binding sites were identified using MatInspector and compared with SiteGA results. They were subsequently examined for the expression in the mammary gland as well as for their occurrence in the previously identified region of homology. Finally, CCCTC binding factor (CTCF) and nuclear receptor subfamily 2 at a similar distance from the transcription start site in the 5' butyrophilin gene across the species. However, the exact interaction of these transcription factors with the butyrophilin gene is not known, which needs further investigation.

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# World Biotechnology Congress

July 16-17, 2018 Berlin, Germany

## Generation of bacterial strains of production, with a growth-coupled focus for its application in synthetic biology

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The present project presents a combination of methodologies that manages to turn around the design-construction-test cycle of bacterial strains of metabolic engineering production. We started with an *in silico* design generated by the genomic scale model of last generation *Escherichia coli* (ME-iOL1554). From this, the strains were generated using molecular biology tools. The strains generated were characterized in a simple experimental system but with strict micro aerobic conditions and underwent a process of adaptive evolution in the same experimental system, managing to generate strains with fermentative pathways interrupted but that manage to grow under strict micro aerobic conditions. The strains generated produced L-alanine (although not in titles close to that predicted by the metabolic model at genomic scale), the exo metabolomics analyzes of one of the strains show that it is igniting latent fermentation pathways not previously described. This is why this work constitutes a conceptual advance for several reasons: (1) Test the use of computer models as a design tool, a combination of systems biology and synthetic biology is achieved. Both sciences of great importance and relevance today, (2) the concept of growth-coupled (growth-coupled), a fundamental quality in a production strain, was experimentally validated, (3) a combination of methodologies was implemented: Computational design, molecular biology, fermentations, adaptive evolution and exo-metabolomics by H1-NMR and (4) an advance was achieved in the generation of L-alanine producing strains, however the most important result of the project was the use of computational models as a design tool and the discovery of latent fermentation pathways (ethylene glycol and methanol) in *Escherichia coli*, which could reinforce what has been said and proposed by other researchers. At the moment, there are two strains whose characteristics make them candidates for strains "Chasis".

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# World Biotechnology Congress

July 16-17, 2018 Berlin, Germany

## Improved shoot regeneration, salinity tolerance and reduced fungal susceptibility in transgenic tobacco constitutively expressing PR-10a gene

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Abiotic stresses such as drought and salinity exerts adverse effects on plant growth and development. To combat these stresses plants are having unique mechanism and it is largely regulated by plant hormones, which in turn, orchestrate the different biochemical and molecular pathways to manoeuvre stress tolerance. The PR-10 protein family is reported to be involved in defense regulation, stress response and plant growth and development. The JcPR-10a overexpression resulted in increased number of shoot buds in tobacco (*Nicotiana tabacum*), which could be due to high cytokinin to auxin ratio in the transgenics. The docking analysis shows the binding of three BAP molecules at the active sites of JcPR-10a protein. JcPR-10a transgenics showed enhanced salt tolerance, as evident by increased germination rate, shoot and root length, relative water content, proline, soluble sugar and amino acid content under salinity. Interestingly, the transgenics also showed enhanced endogenous cytokinin level as compared to WT, which, further increased with salinity. Exposure of gradual salinity resulted in increased stomatal conductance, water use efficiency, photosynthesis rate and reduced transpiration rate. Furthermore, the transgenics also showed enhanced resistance against *Macrophomina* fungus. Thus, JcPR-10a might be working in co-ordination with cytokinin signaling in mitigating the stress induced damage by regulating different stress signaling pathways, leading to enhanced stress tolerance.

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# World Biotechnology Congress

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## Evaluation of the efficacy of recombinant pollen and fruit allergens by ELISA and immune-blotting

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**Introduction & Aim:** Allergenic extracts from different sources extensively use for the diagnosis and treatment of allergic disorders. However, the potency and stability of commercial natural extract is vary and in some cases dramatically decrease by time mostly due to inherent enzyme activities producing recombinant allergens is proposed as an alternative method which can provide enough allergens and improves stability and potency of allergens. Among different methods for expressing the allergenic proteins, the *E. coli* system is the first-choice for the initial screening of as it can be easily manipulated, cultured inexpensively and grows rapidly. The aim of this study was to evaluate the potency of some recombinant allergens with natural extract by ELISA and immune-blotting methods.

**Method:** Recombinant major allergens of melon, grape, *Chenopodium album* and *Salsola kali* pollen were expressed in *E. coli*, lyophilized and purified by gel extraction. Total protein content was measured by Bradford method and after adjustment of the concentration with commercial natural extract; the potency of extracts was assessed by ELISA and immune-blotting methods using patients' sera.

**Results:** The potency of pollen recombinant allergens was comparable with natural extracts but for grape and melon, the potency was better than natural extracts. With increasing the concentration of major allergens in extracts, the potency of extracts increased accordingly.

**Conclusion:** Recombinant allergens particularly for fruits which are unstable are the suitable alternative sources and can use in *in vitro* and *in vivo* diagnostic methods for allergy evaluation.

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# World Biotechnology Congress

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## Biochemical and molecular characterization of some *Papaver* species from Iran, a valuable potency for producing of Morphinan alkaloids

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There are 150 species in the genus of *Papaver* in the world which 28 of them are natives of Iran and five of them are endemic distributed in most part of Iran. Phytochemical evaluation of some *Papaver* species such as *Papaver bracteatum*, *Papaver somniferum* and *Papaver orientale* from Iran revealed valuable metabolites such as noscapine, morphine; codeine; thebaine and papaverine that mainly are used in medicine and pharmacy. The accumulation of morphinan alkaloids was different among organs of each species. However, we found inverse relations among various metabolites which suggest a competition among biosynthetic pathways for intermediates or organs for accumulation of final products. In addition, we found common morphinan biosynthesis pathway in different *Papaver* species as what reported in *Papaver somniferum* using quantitative PCR. Relative gene expression of important genes (*TYDC*, *BBE*, *SAT*, *COR*, *T6ODM*, and *CODM*) in morphinan biosynthesis pathway was highly varied depend on growth, developmental stages and alkaloid content of the species. Our results also indicated that the elicitation of *Papaver* cell culture by hormonal and nano elicitors can be a good system for enhancement the production of the alkaloids. In addition, our findings suggested that inducing the hairy roots in *Papaver* species can be used as a good source for producing commercial alkaloids.

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# World Biotechnology Congress

July 16-17, 2018 Berlin, Germany

## Development of chestnut (*Castanea sativa Mill.*) micro-propagation through zygotic embryogenesis

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To establish an effective protocol for plant regeneration through zygotic embryogenesis effects of explant, culture media and plant growth regulators on chestnut (*Castanea sativa Mill.*) regeneration were investigated. Explant (zygotic embryo), two different media Murashige and Skoog medium (MS) and Woody Plant Medium (WPM), different plant growth regulators (6-benzylaminopurine (BAP), Indole-3-Butyric Acid (IBA) with different concentration (0.1, 0.2 and 3.0 mgL<sup>-1</sup>) and phenol inhibitors (activated charcoal, citric acid, Polyvinylpyrrolidone (PVP)) for shoot and root induction were chosen. The culture of chestnut showed the better initiation and multiplication rates in WPM medium. 0.1 mgL<sup>-1</sup> BAP in combination with activated charcoal was the best growth regulator for shoot elongation. WPM supplemented with 0.1 mgL<sup>-1</sup> BAP and citric acid was the best shoot proliferation. Rooting of *in vitro* plantlets was achieved on WPM medium supplemented with 3.0 mgL<sup>-1</sup> IBA showing 60% intensity and was the most efficient in terms of secondary root production. For acclimatization rooted plants were transferred to plastic pots filled with mixture of sphagnum peat:perlite at the ratio 2:1. This protocol provides a basis for future studies on protection of rare and endangered plant species using biotechnological approaches, thus preserving diversity of the forest ecosystems.

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