CSC PhD Vacancies in Leiden Institute of Biology

Project 1:

Model driven structure-function analysis of *Pseudomonas putida* genes, proteins and metabolic networks in response to solvent stress.

Project description:
We recently sequenced the genome of *P. putida* S12 that is extremely tolerant against a broad range of solvents, such as xylene, toluene and butanol. Under solvent conditions this bacterium shows a high transcriptional response in the first 30 minutes that is required for their defence mechanism. The high oxygen-affinity cytochrome c oxidase is expressed to provide a sufficient proton gradient and the metabolic flux is altered in order to obtain a sufficient redox balance. Comparative genome analysis of *P. putida* strains shows a considerable number of unique genes that are mainly present on the megaplasmid of this bacterium. These genes and clusters may play an important role in adaptation towards solvents in the environment.

In this project, we will use constraint-based modeling to construct a *P. putida* S12 metabolic network based on the available metabolic network of *P. putida* strain KT2440. Analysis of the metabolic network in combination with transcriptional and genomic data will identify candidate novel genes that are involved in implementing solvent tolerance. We will scrutinize structure and function of selected genes and analyze their role during solventogenic conditions. Structure-function analysis of the unique tripartite solvent pump will be an important starting point.

Supervisor: Prof. dr. J.H. de Winde

Selection criteria:
Candidates are skilled in microbial molecular biotechnology and have experience in microbial physiology, molecular biology, -omics technologies, and/or are familiar with bioinformatics, protein structure analysis, metabolic network analysis. Experience in working with *Pseudomonas* is an advantage. Candidates are expected to be flexible with travelling for short research stays in partner laboratories.

Applications:
To apply for this vacancy, please send an email to j.h.de.winde@science.leidenuniv.nl. Please ensure that you upload the following additional documents quoting the project title:

- Curriculum vitae;
- Bachelor's and master's transcripts;
- (Draft of) MSc thesis.

Deadline: *not applicable*

Project 2:

***Pseudomonas putida* as an industrial host for biobased production of value added compounds.**

Project description:
In this project, research focuses on understanding the solvent tolerance and metabolic flexibility of *Pseudomonas putida*, through;

1) extension of a detailed functional annotation of the recently sequenced genome of solvent tolerant P. putida S12;
2) comparative genome analysis of *Pseudomonas* and related genomes;
3) analysis of redistribution of metabolic fluxes and carbon capturing capacity through anaplerotic routes;
4) genetic and metabolic control of redox cofactor regeneration and oxidative stress response under industrial growth conditions.

In this project, metabolic engineering of central metabolism and redox cofactor supply in *P. putida* S12 is applied to enable efficient bioproduction of otherwise relatively toxic added value chemicals. Efficient production of 2,5-furan-dicarboxylic acid (FDCA) may be used as initial starting point.

Supervisor: Prof. dr. J.H. de Winde

Selection criteria:
Candidates are skilled in microbial molecular biotechnology and have experience in microbial physiology, molecular biology, -omics technologies, and/or are familiar with bioinformatics, protein structure analysis, metabolic network analysis. Experience in working with *Pseudomonas* is an advantage. Candidates are expected to be flexible with travelling for short research stays in partner laboratories.

Applications:
To apply for this vacancy, please send an email to j.h.de.winde@science.leidenuniv.nl. Please ensure that you upload the following additional documents quoting the project title:

- Curriculum vitae;
- Bachelor's and master's transcripts;
- (Draft of) MSc thesis.

Deadline: *not applicable*

Project 3:

Microbial cell factories and novel enzymes for selective and efficient recycling of plastics and related polymeric materials.

Project description:
This PhD project will focus on discovery, characterization and implementation of novel plastics and polymer degrading enzymes, including their production and utilization by microorganisms. The robust, solvent tolerant gram-negative bacterium *Pseudomonas putida* will serve as a model cell factory. By screening microbial strain libraries for the degradation of complex synthetic plastics and polymers, novel enzymes will be identified and characterized in detail. Degradation of polymers into the constituent monomeric compounds will be confirmed using various chemical and physical analytical methods, providing insight into the biochemical degradation steps, possible modes of action and efficiency of the enzymes identified. Subsequently, *P. putida* will be engineered for production of novel enzymes without consumption of the monomers to achieve successful monomer retrieval.

Supervisor: Prof. dr. J.H. de Winde

Selection criteria:
Candidates are skilled in microbial molecular biotechnology and have experience in microbial physiology, molecular biology, -omics technologies, and/or are familiar with bioinformatics, protein structure analysis, metabolic network analysis. Experience in working with *Pseudomonas* is an advantage. Candidates are expected to be flexible with travelling for short research stays in partner laboratories.

Applications:
To apply for this vacancy, please send an email to j.h.de.winde@science.leidenuniv.nl. Please ensure that you upload the following additional documents quoting the project title:

- Curriculum vitae;
- Bachelor's and master's transcripts;
- (Draft of) MSc thesis.

Deadline: *not applicable*

Project 4:

Dissecting regulatory mechanisms of transcriptional co-repressors in intracellular receptor-mediated immunity

Project description:

Innate immunity is crucial for both plants and animals to survive from various pathogen attacks, including bacteria, fungi, viruses, etc. One of the main molecular surveillance immune systems in plants is mediated by a large group of intracellular receptors, which recognize effectors and trigger immune responses. One of the key processes during immune activation is transcriptional regulation, including both positive and negative regulation. TOPLESS (TPL) and its close homologs, TOPLESS-related proteins (TPRs) TPR1 and TPR4 were initially reported as transcriptional co-repressors mediating auxin-dependent transcriptional repression during embryogenesis, and loss of function in TPL/TPR1/TPR4 compromises plant resistance against *Pseudomonas syringae*, a bacterial pathogen. Recently, we have found TPL/TPR are required for the resistance mediated by the Toll/interleukin-1 (IL-1) receptor/Resistance protein (TIR) domain-containing nucleotide-binding (NB) leucine-rich repeat (LRR)-type (TIR-NLR) intracellular immune receptors, and this is independent of the well-known plant defense hormone salicylic acid (SA) mediated immune activation. This project has three main objectives: (i) investigate the main target genes of TPL/TPRs during TIR-NLR immune activation; (ii) study how co-repressors TPL/TPRs perceive signals from upstream TIR-NLR immune receptors; (iii) understand how co-repressors TPL/TPRs activate or deactivate their target genes via interacting with histone remodellers, such as the histone deacetylase 19 (HDA19). We will use combinatorial and genome-wide approaches, such as RNA-seq, ATAC-seq and ChIP-seq with statistics and bioinformatics analyses to understand the global effects of the co-repressors in transcriptional regulation during the activation of the intracellular immune receptors.

Supervisor: Dr. Pingtao Ding

Selection criteria: The candidate (1) should have a genuine interest in biology and be enthusiastic to understand fundamental questions, ideally with a strong interest in plant biology, microbiology within the area of host and microbe interactions; (2) must have good understanding and knowledge in basic genetics, molecular biology and biochemistry; (3) must have or about to obtain a master's degree in biology or related disciplines;(4, optional) have experience in the wet lab, esp. working with plants; (5) have experience in basic bioinformatics and statistics analyses; (6) have a strong interest in transcriptional regulation and epigenetics.

Applications:
To apply for this vacancy, please send an email to pingtao.ding@tsl.ac.uk or j.h.de.winde@science.leidenuniv.nl . Please ensure that you upload the following additional documents quoting the project title:

- Curriculum vitae;
- Bachelor's and master's transcripts;
- (Draft of) MSc thesis.

Project 5:

Identification and characterization of novel regulatory components downstream of intracellular receptor-mediated immunity

Project description:

Plant disease resistance involves pattern-triggered immunity (PTI) and effector-triggered immunity (ETI). PTI is induced when cell-surface receptors recognize conserved pathogen molecules. Pathogens overcome PTI by secreting effectors into host cells. These effectors can in turn be recognized by host receptors encoded by R genes. Most R genes encode nucleotide-binding (NB) leucine-rich-repeat (LRR) proteins. They are classed into Toll/Interleukin-1-receptor/Resistance (TIR-NB-LRR/TNL) and coiled-coil (CC-NB-LRR/CNL) R proteins. Most TNLs and some CNLs require protein-folding components, and the lipase-like family proteins EDS1, PAD4 and SAG101, for signaling. Several TNLs appear to act in the nucleus, but some well-characterized CNLs, are associated with the cell membrane and others are predicted to be with chloroplasts. Recently, one CNL and one TNL activation complexes have been resolved by cryogenic electron microscopy (cryo-EM), however, the mechanism by which R proteins activate defense remains elusive and provides opportunities for further research. This project aims to identify novel components shared by both TNLs and CNLs with additional modifications compared to reported forward genetic screens: (i) using multiple NLRs that recognize the same effector to perform EMS-mutagenesis screen for suppressors of ETI, to avoid enrichment of revertant mutations in a single NLR; (ii) using inducible effector expressing lines that combines CNL and TNL to investigate the convergent components; (iii) introducing a mutant that is lack of RNA-directed DNA methylation (RdDM) pathway but not affecting immune responses in the background to reduce transgenerational gene silencing to the transgenic inducible lines. Subsequently, we will further characterize mutants with different pathogenesis assays to validate results, and candidate genes that are responsible for the phenotypes will be mapped using mapping by sequencing (MBS).

Supervisor: Dr. Pingtao Ding

Selection criteria: The candidate (1) should have a genuine interest in biology and enthusiastic to understand the fundamental questions, ideally with a strong interest in plant biology within the area of host and microbe interactions; (2) must have good understanding and knowledge in basic genetics, molecular biology and biochemistry; (3) must have or about to obtain a master's degree in biology or related disciplines;(Optional) (4) already had experiences in the wet lab, esp. working with plants; (5) has a strong interest in genetics and plant innate immunity.

Applications:
To apply for this vacancy, please send an email to pingtao.ding@tsl.ac.uk or j.h.de.winde@science.leidenuniv.nl . Please ensure that you upload the following additional documents quoting the project title:

- Curriculum vitae;
- Bachelor's and master's transcripts;
- (Draft of) MSc thesis.

Project 6:

Functional characterization of transcription factors involved in the adaptation of plant growth to environmental changes

Project description:

Abiotic stresses cause adverse effects on plant growth, development and productivity. Reduction in shoot growth is a typical plant adaptive response to stress. However, the regulatory mechanisms by which plants integrate stress-derived signals into the growth and developmental programs are so far largely unknown. The response to environmental cues in plants is a highly regulated process that requires the coordinated regulation of gene expression. Gene regulation is the task of transcription factors (TFs). Together with their target genes the TFs constitute gene regulatory networks (GRNs) that are central to almost all biological processes.
In this project, we aim to identify and uncover the cellular functions of transcription factors regulating the crosstalk between growth and abiotic stress responses (drought and heat stress) in the model plant *Arabidopsis thaliana*. To this end we have selected TFs of the so-called NAC family that are antagonistically regulated by growth- (gibberellins, and brassinosteroids) and abiotic stress-related hormones (abscisic acid). More specifically, we will analyze the expression of the selected transcription factors using qRT-PCR and by transforming promoter-reporter gene constructs into Arabidopsis. The role of the TFs (for regulating growth and stress responses) will be investigated using knockout mutants (T-DNA insertion lines) and transgenic plants overexpressing the TFs under constitutive, stress-inducible or cell-specific promoters. We also employ cutting-edge genome editing methods (e.g., CRISPR-Cas9, besides others) to alter the plant´s regulatory networks. To identify the *in vivo* target genes of the novel TFs we will perform chromatin-immunoprecipitation coupled to qPCR (ChIP-qPCR) and/or next-generation sequencing (CoIP-seq). We may also consider to study the orthologs of the Arabidopsis genes in important crop species, like e.g. tomato.

Supervisor: Dr. Salma Balazadeh

Selection criteria:

- Superior interest in cutting-edge molecular biology methods
- Strong background and interest in plant molecular biology
- Strong interest in solving societal problems related to climate change and its effect of agriculture and food production

Applications:

To apply for this vacancy, please send an email to s.balazadeh@biology.leidenuniv.nl or j.h.de.winde@science.leidenuniv.nl . Please upload the following additional documents quoting the project title:

- Curriculum vitae;
- Bachelor's and master's transcripts;
- (Draft of) MSc thesis.