Molecular aspects of interactions between plant-growth promoting rhizobia and legume host plants

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World population and consumption of nitrogenous fertilizer

![World population projection of the United Nations](image1)

Population (billions)

- **Asia**: The population of Asia shows a significant increase over the years, reflecting the growth in the world population.
- **Africa**: The population of Africa is also rising, contributing to the overall increase in global population.
- **N/S America**: The population of Central and South America has grown, but at a slower rate compared to Asia and Africa.
- **Europe**: The population of Europe has been stable but has shown a slight decline in recent years.
- **Oceania**: The population of Oceania is the smallest among the regions shown, but it has also been growing.

**World population**

- **Population projection of the United Nations**

![World consumption of synthetic nitrogenous fertilizer](image2)

Kg of fertilizer (billions)

- **World consumption of synthetic nitrogenous fertilizer**

- **FAOSTAT 2006**
Effects of rhizobial inoculation

*Vigna unguiculata*

Control  
*Sinorhizobium* sp. NGR234
Root nodules induced by the broad-host range *Sinorhizobium* sp. NGR234

*Kennedia rubicunda*

*Leucaena leucocephala*

*Erythrina crista-galli*

*Acacia pendula*

*Albizia lebbeck*

*Glycine max*
Biological and industrial nitrogen fixation

**Legumes**
- Symbiotic $N_2$ fixation
- $N_2$ uptake
- Organic $N$
- $N_2 \rightarrow NH_4^+$

**Non-legumes**
- Industrial $N_2$ Fixation
- $N_2 \rightarrow NH_4^+$
- Nitrogen fertilizer
- $NH_3$ Volatilization
- Root uptake
- $NO_3^-$ Nitrification
- $NH_4^+$ Fertilizer runoff
- Denitrification
- Nitrate in groundwater
- Leaching
Infection of root hairs and nodule formation

A – Attachment of bacteria
B – Root hair deformation
C – Infection thread
D – Nodule primordium
E – Infected cell of a nodule

Root hair

Infection thread

Cell division branching

Root cortex

Bacteroids

Infected cell

Bacteroids

Infected cell
Research work in our laboratory

1. The Nod factor hydrolase of *Medicago truncatula*, an enzyme that specifically inactivates Nod factors.

2. Rhizobial type 3 effectors such as NopM (an E3 ubiquitin ligase) and NopL (a MAP kinase substrate) of *Rhizobium* sp. NGR234.

3. Chimeric LysM domain receptors that mediate Nod factor-induced defense signaling in *Arabidopsis thaliana* and chitin-induced nodulation signaling in *Lotus japonicus*.
1. MtNFH1, the Nod factor hydrolase of *Medicago truncatula*, cleaves and inactivates Nod factors of *Sinorhizobium meliloti*
Cleavage specificity of Nod factor cleaving enzymes

Known plant chitinases

<table>
<thead>
<tr>
<th>Nod factors</th>
<th>Stability</th>
<th>Chitinase</th>
</tr>
</thead>
<tbody>
<tr>
<td>IV(Ac,S)</td>
<td>+++</td>
<td>none</td>
</tr>
<tr>
<td>IV(S)</td>
<td>++</td>
<td>class III</td>
</tr>
<tr>
<td>IV(Ac)</td>
<td>++</td>
<td>class III</td>
</tr>
<tr>
<td>V(Ac,S)</td>
<td>++</td>
<td>class III</td>
</tr>
<tr>
<td>V(S)</td>
<td>+</td>
<td>class I, III, V</td>
</tr>
<tr>
<td>V(Ac)</td>
<td>+</td>
<td>class I, III, V</td>
</tr>
<tr>
<td>IV</td>
<td>+</td>
<td>class I, III,</td>
</tr>
<tr>
<td>V</td>
<td>-</td>
<td>all</td>
</tr>
</tbody>
</table>

MtNFH1: Nod factor hydrolase of *Medicago truncatula*

<table>
<thead>
<tr>
<th>Nod factors</th>
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<tbody>
<tr>
<td>IV(Ac,S)</td>
</tr>
<tr>
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<tr>
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<tr>
<td>V(Ac)</td>
</tr>
<tr>
<td>IV</td>
</tr>
<tr>
<td>V</td>
</tr>
</tbody>
</table>

The Nod factor hydrolase MtNFH1 lacks chitinase activity
Homology modeling: Prediction of a fatty acid groove in MtNFH1

**MtNFH1** + NodSm-V(C16:2, S)  
*M. truncatula*  

**NtChiV** + (GlcNAc)$_5$  
*N. tabacum* (tobacco)  

**AtChiC** + (GlcNAc)$_5$  
*A. thaliana*  

Tian *et al.* 2013 Plant Physiol. 163, 1179-1190
2. The Rhizobial type 3 protein secretion system

Translocation of toxin-like effector proteins into plant cells
Chimeric proteins [here NopM(1-58)-AvrBs1(Δ58) as an example] consisting of a secretion signal sequence from a rhizobial T3 effector candidate and AvrBs1(Δ58) are expressed in the X. campestris mutant strains 8004ΔavrBs1 and 8004ΔhrpF. Once infiltrated into ECW-10R leaves, T3SS-dependent translocation of the fusion protein expressed in strain 8004ΔavrBs1 results in AvrBs1-induced HR. Such an HR does not occur when the fusion protein is expressed in strain 8004ΔhrpF.
The type 3 effector NopM is an E3 ubiquitin ligase

NopM possesses E3 ligase activity

NopM is secreted by the type 3 secretion system

Mutant analysis: NopM activity is required for optimal nodulation of *Lablab purpureus*

**Used inoculum strains:**

1. NGR234 (parent strain)
2. NGRΔnopM (knockout mutant)
3. NGRnopMC338A (inactive NopM)
4. NGRΔnopM pFAJ-nopM
5. NGRnopMC338A pFAJ-nopM

The type 3 effector NopL is a MAP kinase substrate

Serine-proline residues in NopL required for MAP kinase interaction are also important for symbiotic NopL activity

Mutant analysis with NGR234 derivatives and Tendergreen beans

Suppression of defense proteins in transformed tobacco cells

Conclusion: NopL interferes with MAP kinase signaling by mimicking a MAP kinase substrate in the nucleus.

Poster PP-O6
3. Chimeric LysM receptor kinases

- **NFR1**: Nod factor receptor of *Lotus japonicus*
- **NFR5**: Nod factor co-receptor of *Lotus japonicus*
- **AtCERK1**: Chitin oligosaccharide receptor of *Arabidopsis*

Perception of Nod factors → Nodulation
Perception of chitin from pathogens → plant defense

Expression in a *cerk1* mutant of *Arabidopsis thaliana*
Induced defense gene expression in the *Arabidopsis cerk1-2* mutant co-expressing *NFR1-CERK1* and *NFR5-CERK1* in response to Nod factors (NFs)

NFs = Nod factors of *Rhizobium* sp. NGR234

Non-legumes can be modified to initiate defense reactions in response to Nod factors instead of chitin.

Wang *et al.* 2014 Plant J., 78, 56-69
*Fusarium oxysporum* infection of the *Arabidopsis* *cerk1-2* mutant expressing indicated chimeric receptors after pretreatment with Nod factors.

**Analysis of fungal infection**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Degree of Infection (%)</th>
</tr>
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<tbody>
<tr>
<td>Col-0</td>
<td>10</td>
</tr>
<tr>
<td>NFR1-CERK1 + NFR5-CERK1</td>
<td>90</td>
</tr>
<tr>
<td>NFR1-CERK1</td>
<td>80</td>
</tr>
<tr>
<td>NFR5-CERK1</td>
<td>80</td>
</tr>
<tr>
<td><em>cerk1-2</em> mutant</td>
<td>20</td>
</tr>
<tr>
<td>Col-0 (wild-type)</td>
<td>10</td>
</tr>
</tbody>
</table>

Wang et al. 2014
Plant J., 78, 56-69
Conclusion

Non-legumes can be modified to initiate defense reactions in response to Nod factors. This opens the possibility of using rhizobia or Nod factors as biocontrol agents.
Thank you!

**Visiting scientist:** Dr. Christian Wagner

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- MtNFH1: Jie Cai, Lan-Yue Zhang, Ye Tian, Wei Liu
- NopL: Ying-Ying Ge, Qi-Wang Xiang, Ling Zhang
- NopM: Da-Wei Xin, Sha Liao, Chang-Chao Xu, Di Zhang
- Effector translocation: Min Chen, Qi-Wang Xiang
- LysM receptors: Wei Wang

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- The Chinese University of Hong Kong, Hongkong, China

**Financial support:**
- National Basic Research Program of China (973 program) and
- National Natural Science Foundation of China