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رَضِيْتُ بِاللهِ رَبًّا وَبِالْاِسْلَامِ دِيْنًا وَبِمُحَمَّدٍ نَبِيًّا وَرَسُوْلًا

رَبِّ زِدْنِيْ عِلْمًا وَارْزُقْنِيْ فَهْمًا

"Kami ridho Allah SWT sebagai Tuhanku, Islam sebagai agamaku, dan Nabi Muhammad sebagai Nabi dan Rasul, Ya Allah, tambahkanlah kepadaku ilmu dan berikanlah aku kefahaman"

BIOCONTROL: PLANT GROWTH PROMOTING RHIZOBACTERIA (PGPR)

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PROGRAM STUDI BIOTEKNOLOGI
FAKULTAS SAINS DAN TEKNOLOGI
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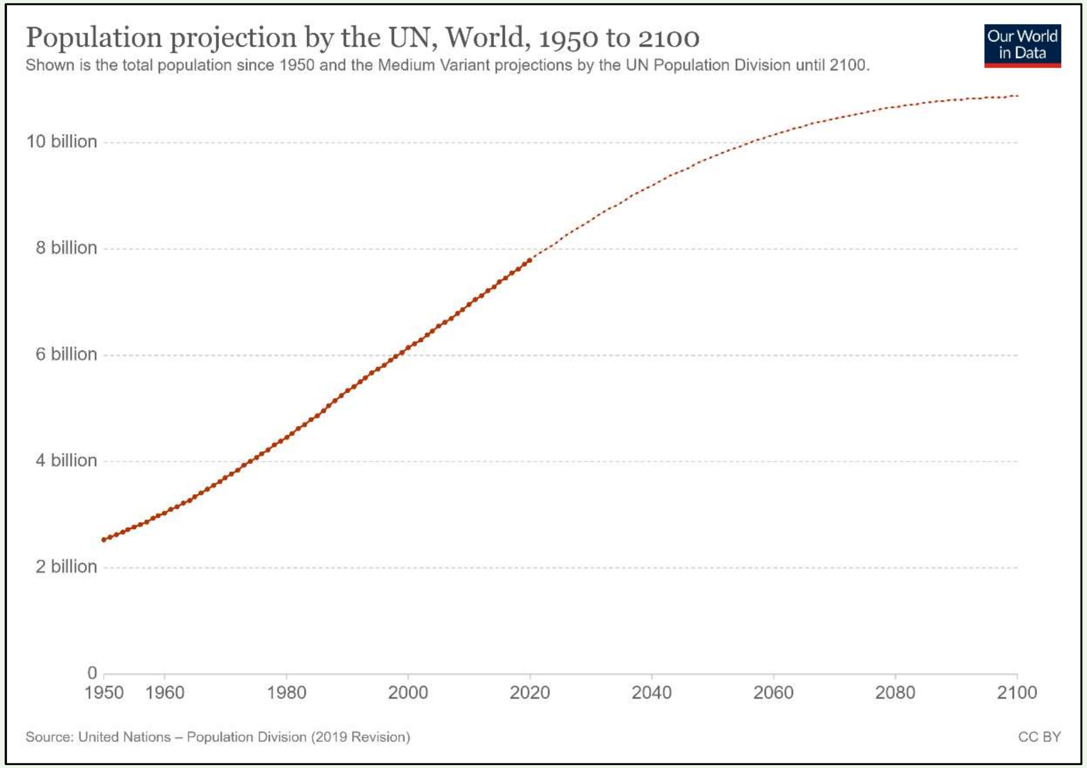
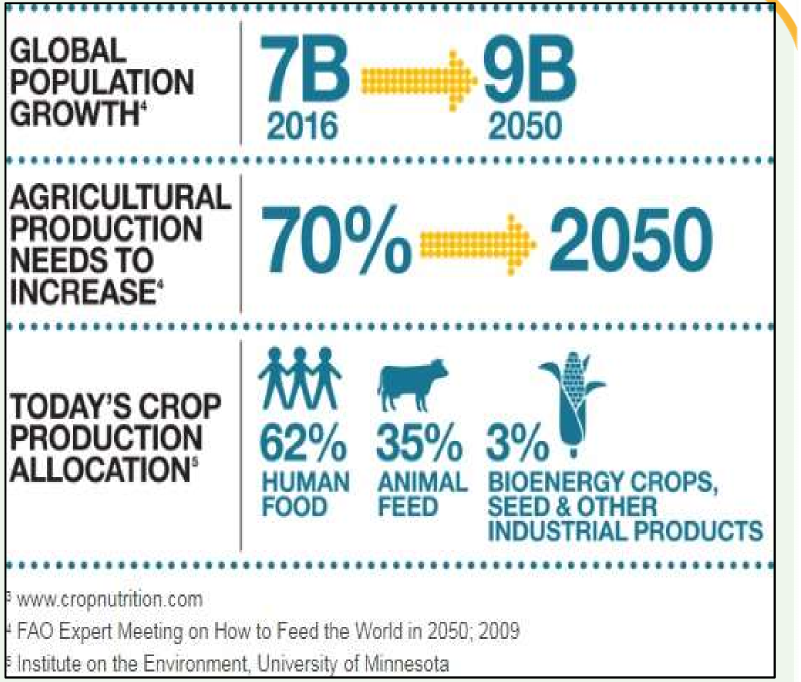
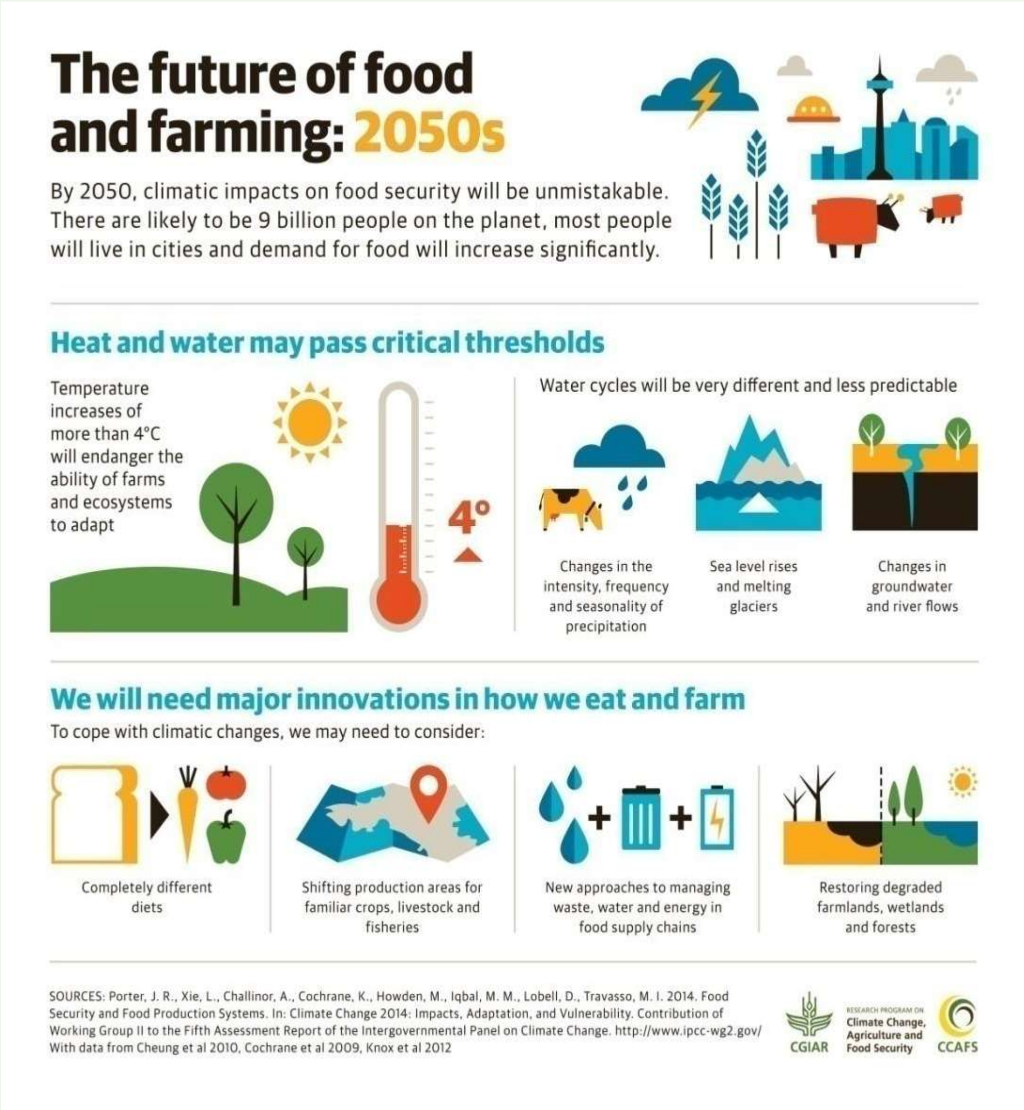
Introduction





Introduction



We will need major innovations in how we eat and farm

PGPR

Introduction

Biological control of plant diseases is the suppression of populations of plant pathogens by living organisms.

- Requires conditions for biocontrol agents:
  1. highly effective against pathogens,
  2. can be multiplied on artificial media
- Application of selected biocontrol agent and mass produced antagonists in high densities is called "**augmentative biological control**".
- Microbial biological control agents protect crops from damage by diseases via different modes of action

What are PGPR

- A group of Soil Born Bacteria (Rhizobacteria)
- Actively colonize plant roots / Rhizosphere
- Enhance plant Growth and Yield
- Directly or Indirectly

- Introduced by JW Kloepper, MN Schroth (1978)

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Introduction

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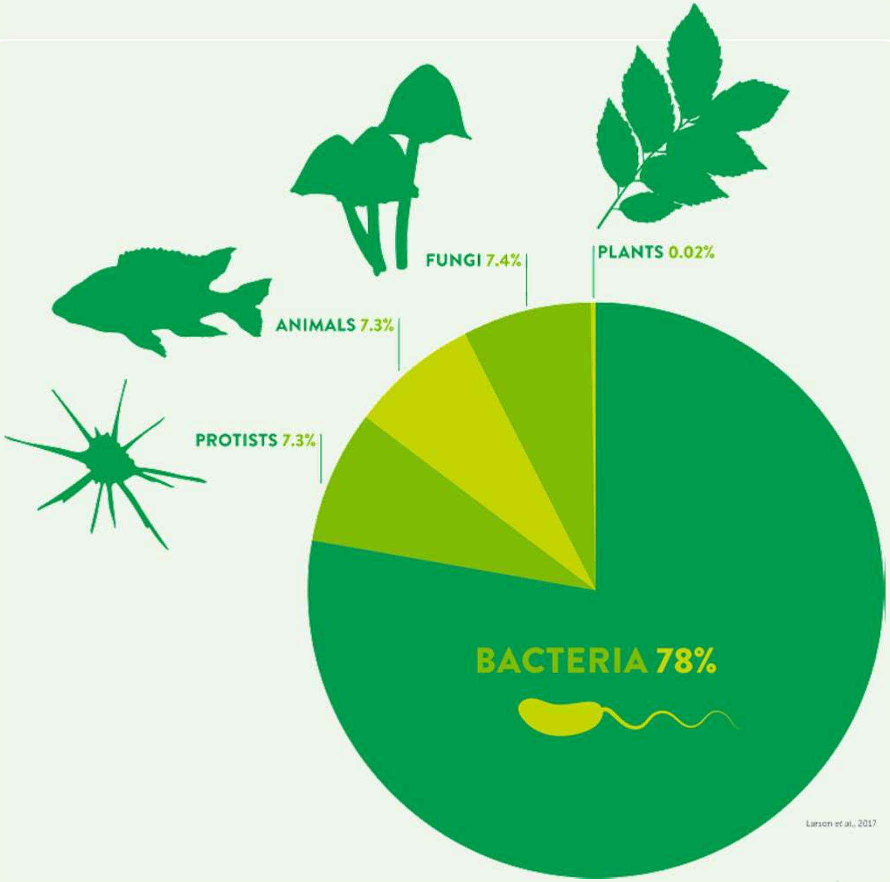
Introduction

Generally, about **2-5%** of **Rhizosphere bacteria** are **PGPR**



Characteristics of PGPR

They must,

1) Able to colonize the root
2) Survive and multiply in microhabitats associated with the root surface, in competition with other microbiota
3) Promote plant growth Or /and
4) Promote plant protection activities

Types of PGPRs

1. Extracellular (ePGPR)

- Existing in/on the
  - Rhizosphere
  - Rhizoplane
  - spaces between cells of the root cortex

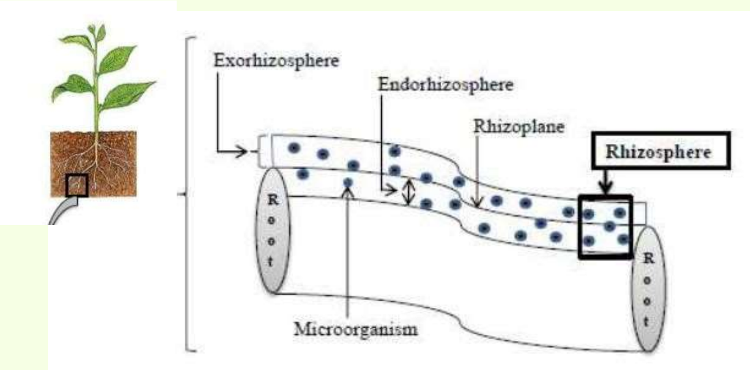


Figure Schematic representation of rhizosphere.

2. Intracellular (iPGPR)

- Exist inside root cells
- Generally in specialized nodular structures.

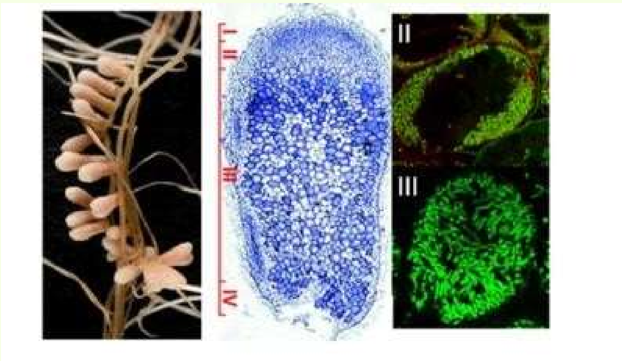


Fig: Structure of nitrogen-fixing root nodules
-Maróti and Kondorosi (2014)

Functional classification of PGPR

a) Direct activity

Nutrient cycling

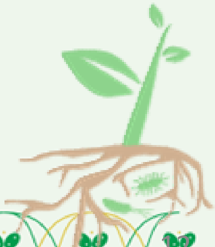
Phyto-stimulation

- MO themselves release growth regulators
- MO act as a sink of plant-released hormones

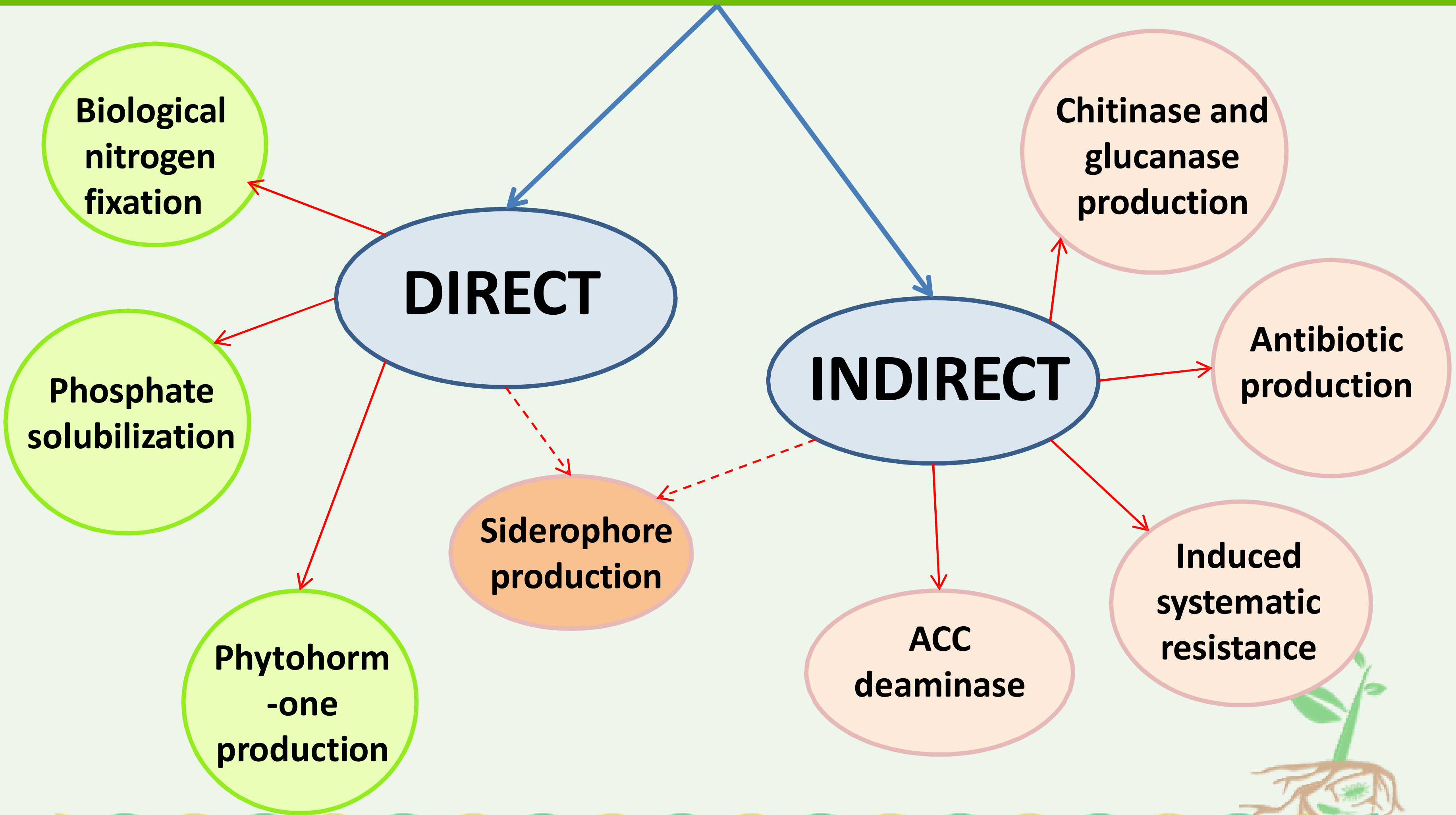
b) Indirect activity

Systemic resistance to Biotic Stress

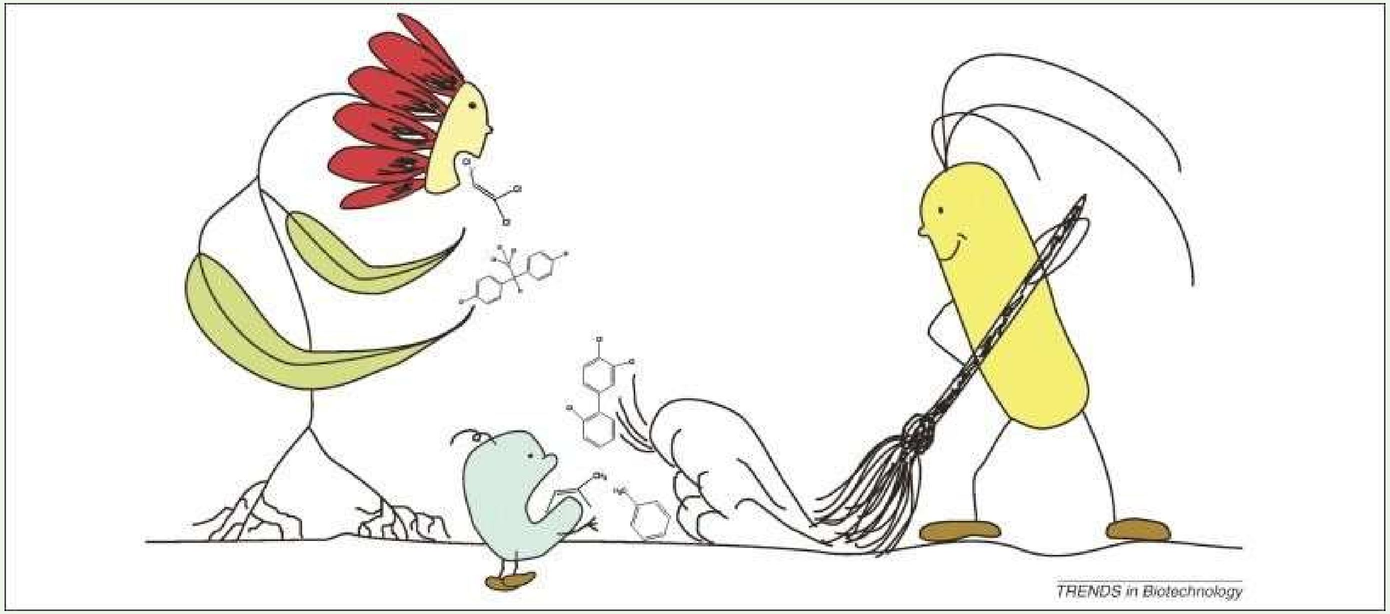
Protection against Abiotic Stress



Mechanisms of PGPR

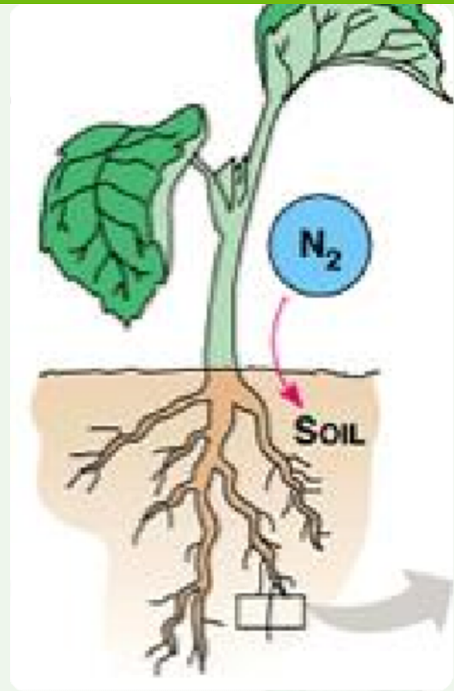


Direct Mechanisms



1. Biological Nitrogen fixation

- Nitrogen is **a vital nutrient** for Plant
- 78% N_2 in the atmosphere
- But **not directly utilizable** for plants



- **BNF fixes ~ 60% of the earth's available Nitrogen**
- **20 - 30 kg per hectare per year**
- **Biofertilizers**

1. Biological Nitrogen fixation…

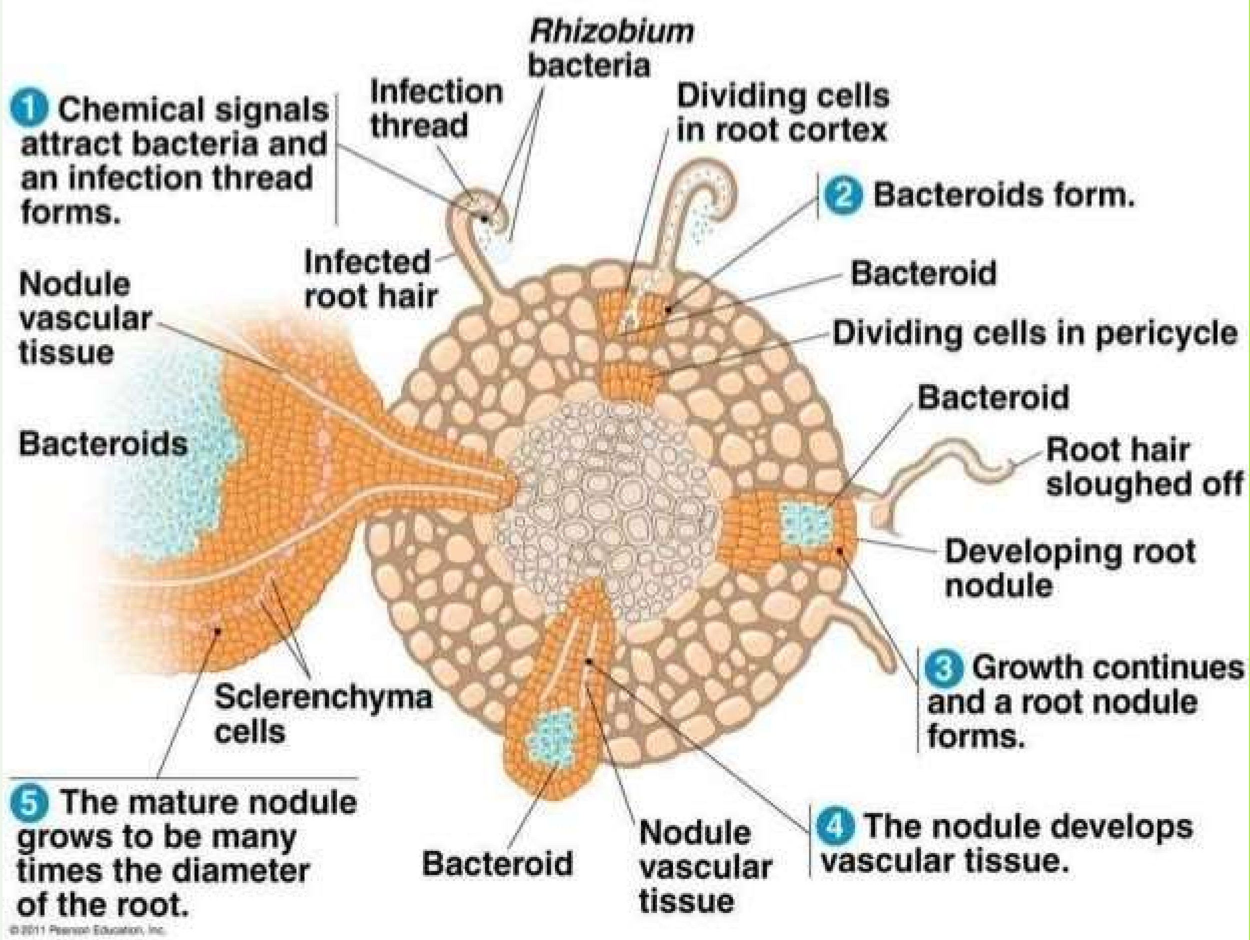
a) Symbiotic

- Specificity
- Infect the roots to produce nodule
    1) With leguminous host plants (e.g., *rhizobia*)
    2) With non-leguminous trees (e.g., *Frankia*)

Fig3: Nodule formation of *Frankia alni*

Sourse:
http://bladmineerders.nl/wordpress/wp-content/uploads/beeld/_752_2.jpg

Nodule formation of *Rhizobium*



1. Biological Nitrogen fixation…

b) Non-Symbiotic

- Free living diazotrophs , associative
- Non-specific / loose symbiosis
- ***Azospirillum*****,** ***Azotobacter****, Burkholderia, Herbaspirillum, Bacillus, and Paenibacillus*

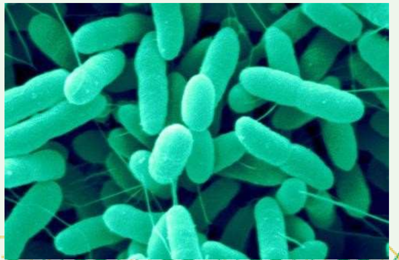
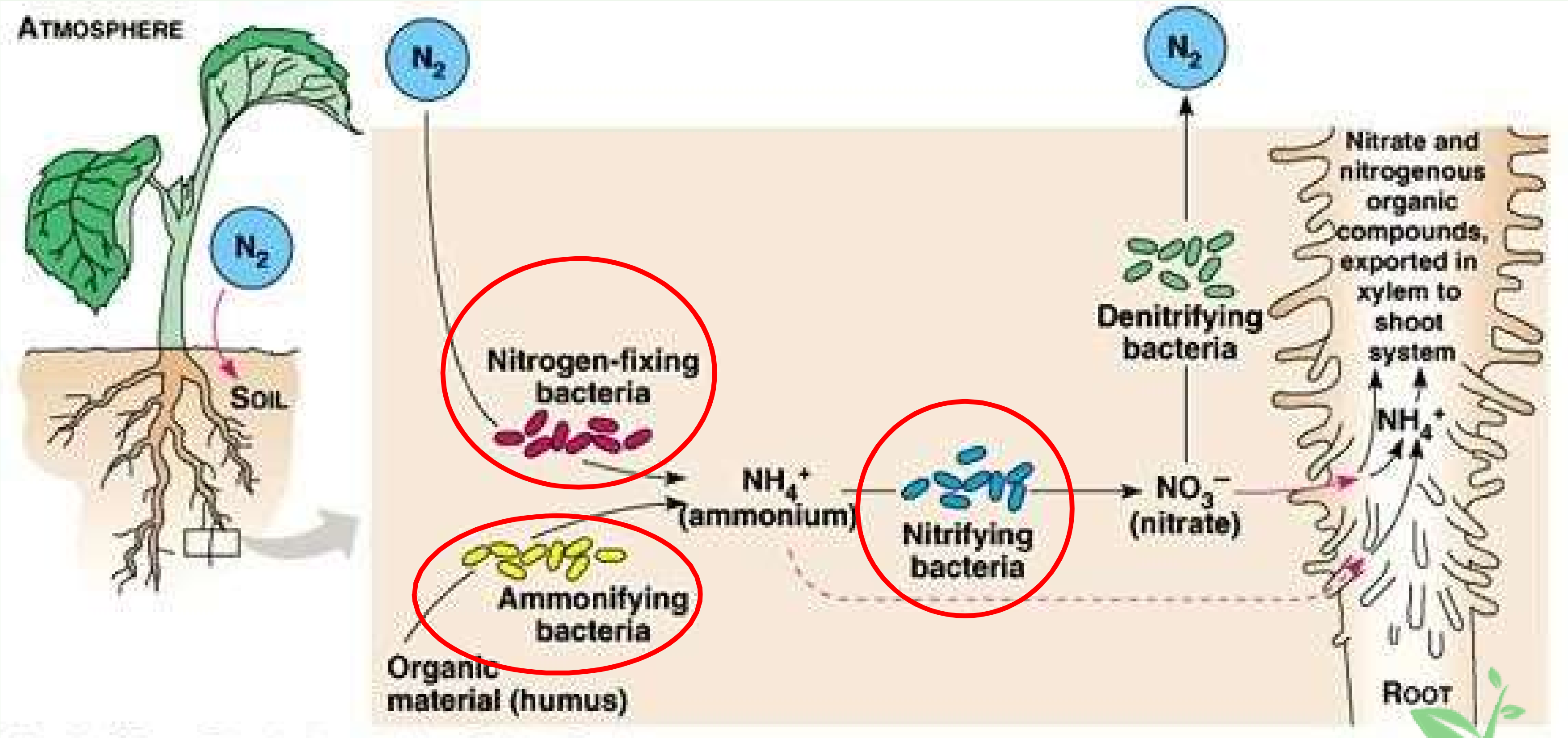


Fig5: ***Azospirillum sp.***

Source:https://www.indiamart.com/proddetail/azospirillum-lipoferum-8534947730.html

Biological Nitrogen fixation



1. Biological Nitrogen fixation…

- **nif gene cluster**

  code for Nitrogenase enzyme
- A key enzyme required
- Eg:
  - *Azotobacter chroococcum*
  - *Azospirillum brasilense*
  - *Paenibacillus azotofixans*
  - *Bacillus spp.*

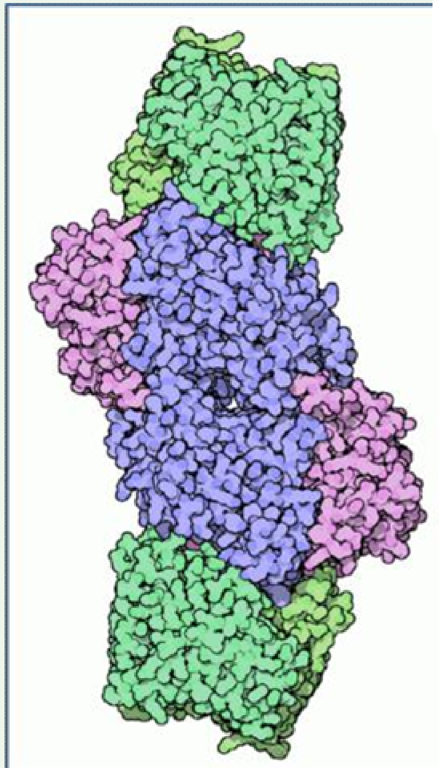
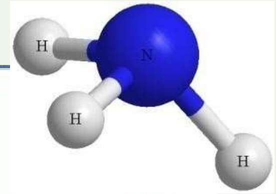
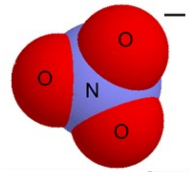


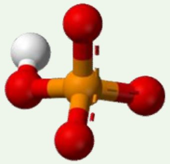
Figure The nitrogenase enzyme with the MoFe protein in blue and purple and the Fe proteins in green [4].

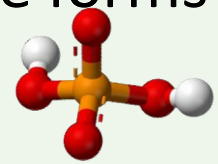




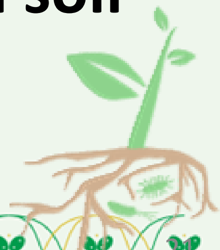
2. Phosphate solubilization

- Limiting nutrient for plants (due to **insoluble forms**)
  - Eg: Tricalcium phosphate, Rock phosphate, Aluminum phosphate, etc.
- Plants are only able to absorb **mono-** and **dibasic phosphate** (soluble forms)
  - ($H_2PO_4^-$)
  - (HPO_4^{2-})





- PGPR mineralize **organic / inorganic phosphorus in soil**
- **Phosphate-solubilizing bacteria**



2. Phosphate solubilization…

Primary Mechanism

1) Organic acid secretion by microbes

- **utilize sugars** from root exudates
- produce **organic acids**
- acetic, lactic, malic, succinic, tartaric, gluconic, 2-ketogluconic, oxalic and citric acids
- act as **good chelators** of divalent Ca2+ cations

2. Phosphate solubilization…

Other Mechanisms

2) Produce extra cellular enzyme

- **Phytase**

  Hexaphosphate salt of inositol (phytate)
- **Phosphatase**

2) Release of Phosphate during substrate dgredation

2. Phosphate solubilization…

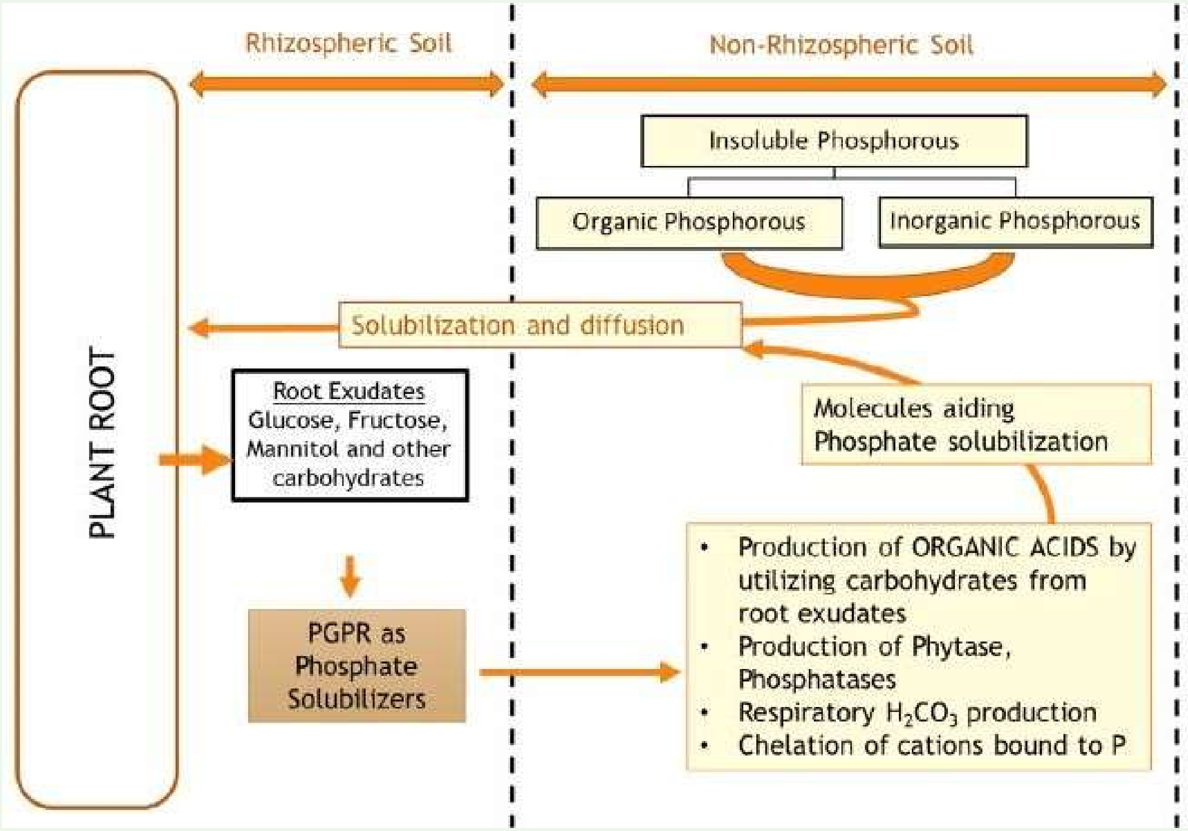
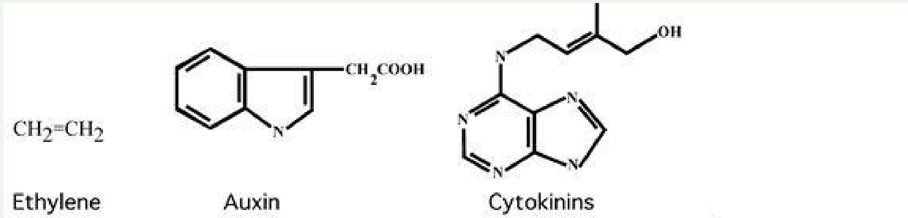


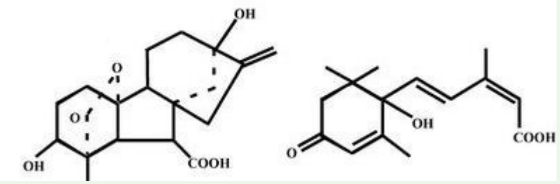
Fig6: **Solubilizationof soil phosphorus by PGPR**.

Goswami et al. (2016)

3. Phytohormone production

- Cause to substantial **increase** in **growth** and **yield** of plants
- Plant responds to any phytohormone in the rhizosphere
  - Auxins
  - Gibberellins
  - Cytokinins

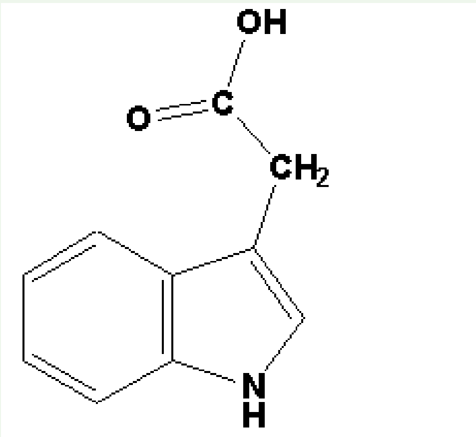




3. Phytohormone production…

1) Indole-3-acetic acid (IAA) *—an auxin*

- cell elongation
- cell division
- tissue differentiation
- aids apical dominance



Indole-3-acetic Acid (Auxin)

- Highly developed roots
- Uptake more nutrients

~ 80% of the bacterial flora in the rhizosphere produce IAA

3. Phytohormone **IAA …**

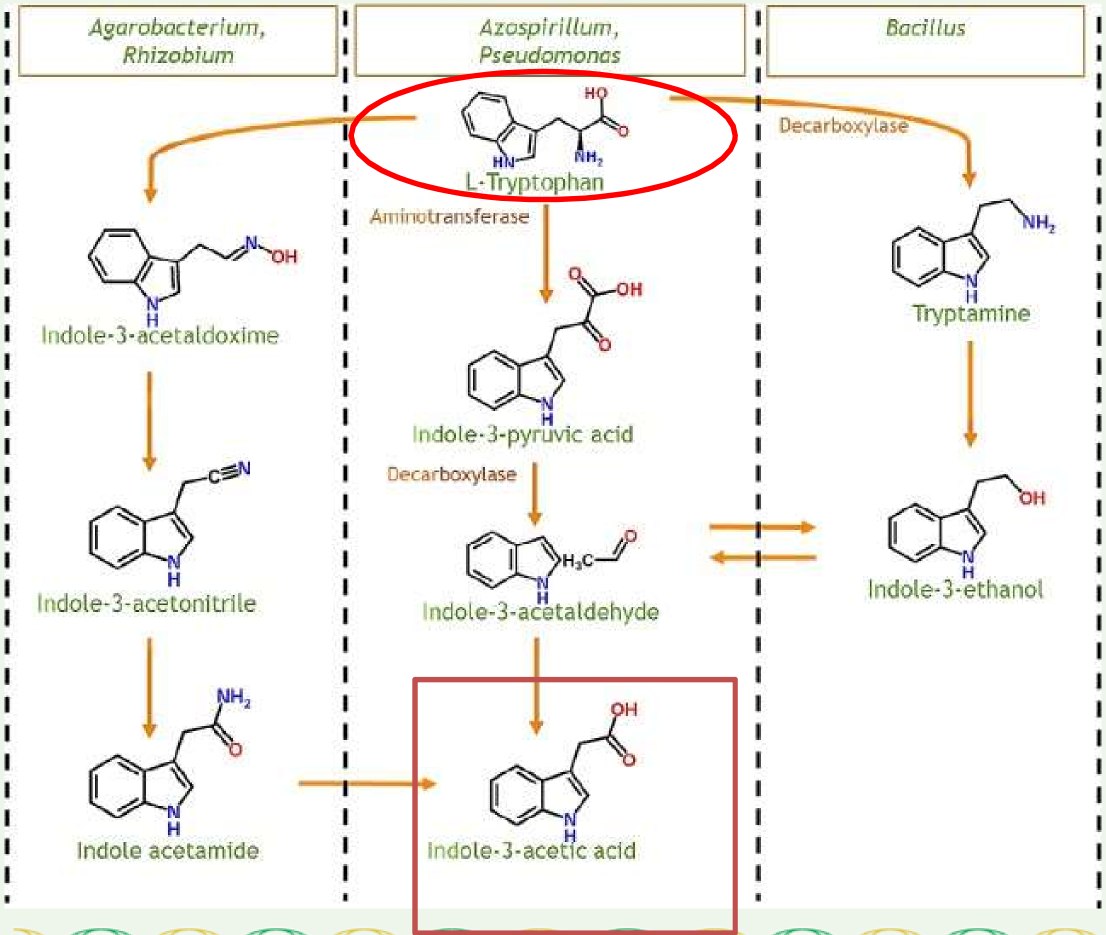


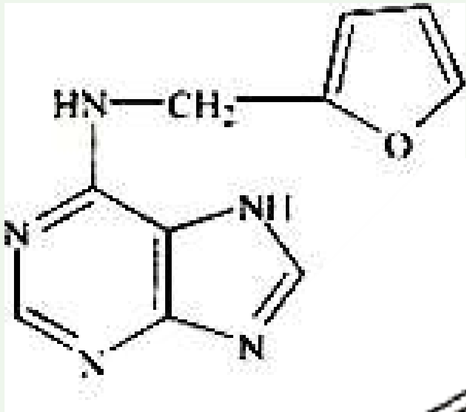
Fig7: Biosynthesis pathways of IAA in PGPR

Ahemad and Mohammad(2011)

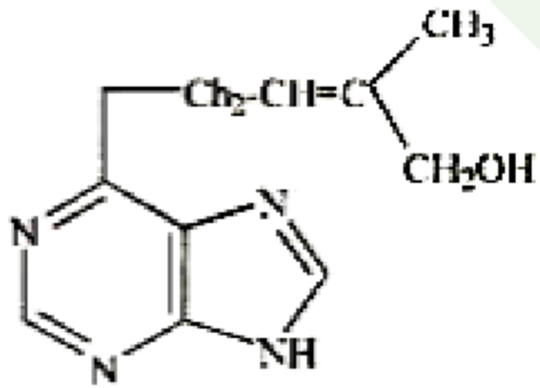
3. Phytohormone production…

2) Cytokinins

- cell division
- root development
- root hair formation
- shoot initiation
- inhibition of root elongation



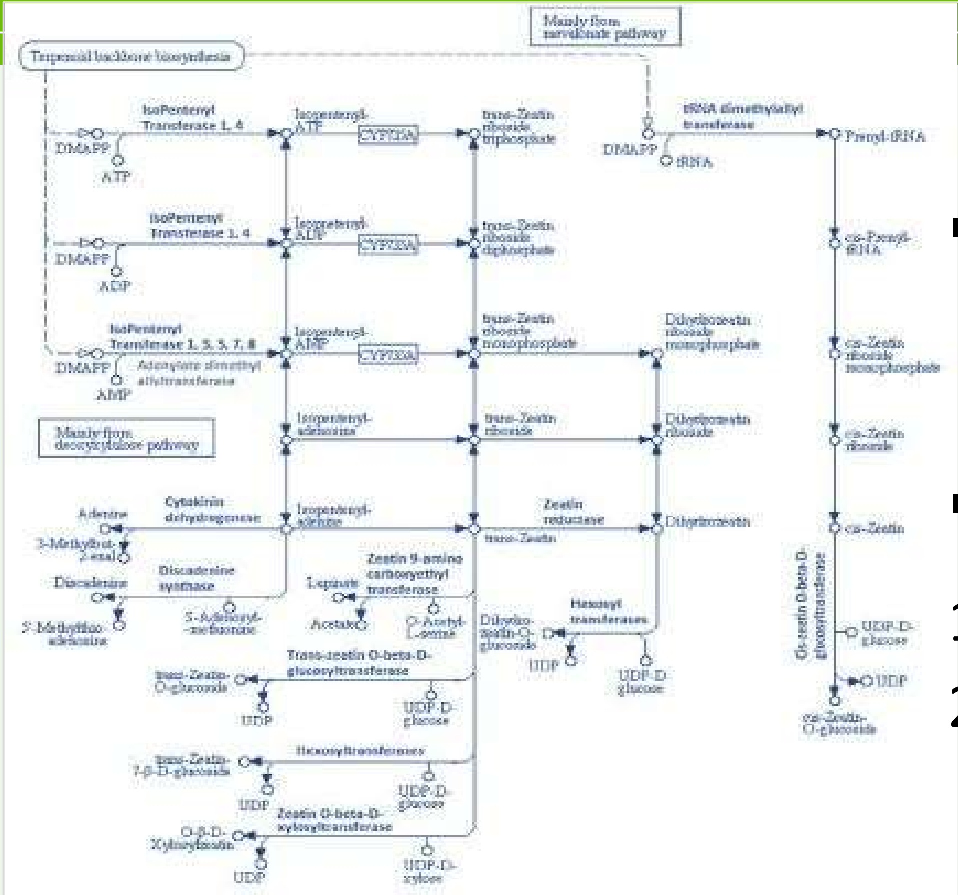
Kinetin



Zeatin

- Influence their physiological and developmental processes
- *Pseudomonas, Azospirillum, and Bacillus*

3. Phytohormone **Cytokinins** …



- Most abundant cytokinins are

  Adenine-type

- Two different pathways

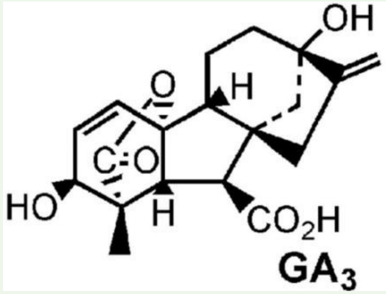
1) the tRNA pathway
2) adenosine monophosphate (AMP) pathway

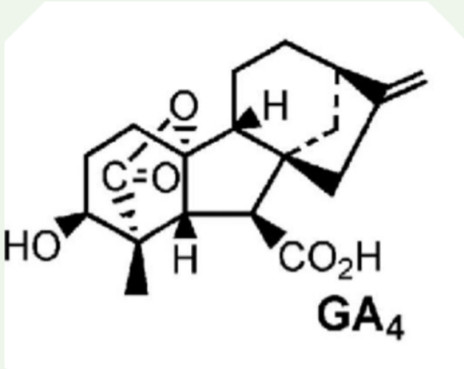
Fig8: Zeatin Biosynthesis pathway studied in *Paenibacillus polymyxa OSY-DF.*
Ahemad and Mohammad(2011)

3. Phytohormone production…

3) Gibberellins (GAs)

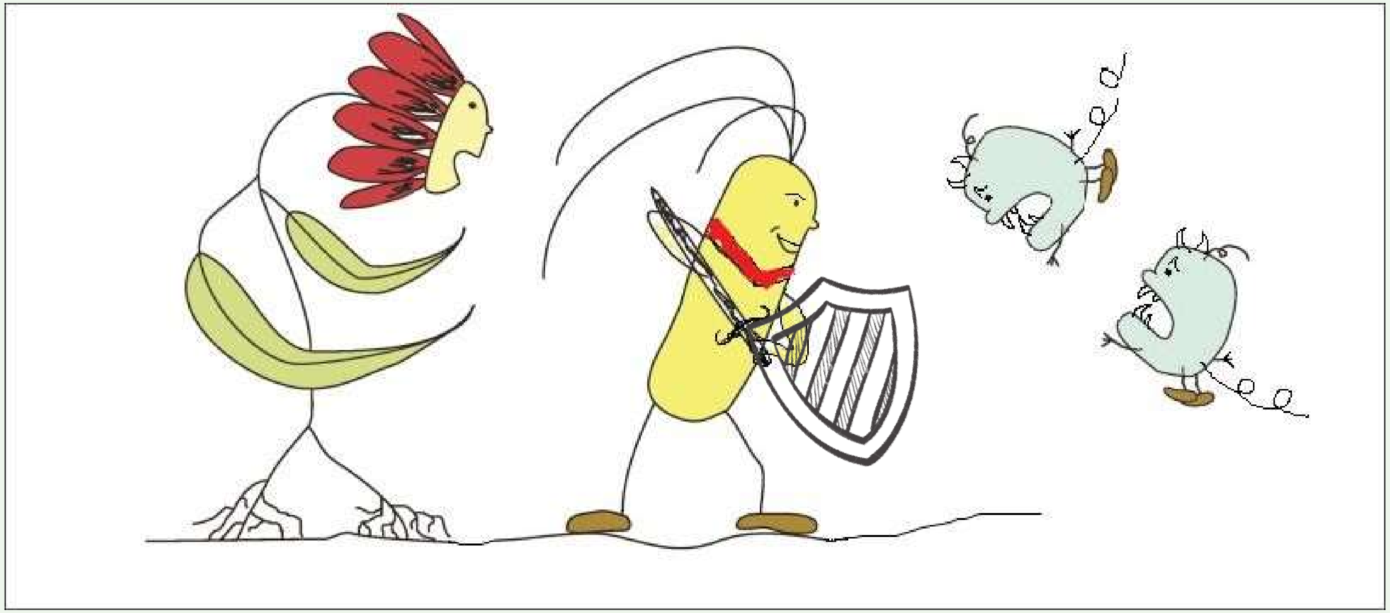
- Seed germination
- Stem elongation
- Flowering & fruit setting





- 136 GA s are known from plants .
- Only 4 from PGPR - GA_1, GA_3, GA_4, and GA_{20}
- **Effect of PGPR producing GAs** on plant **is not exactly known**
- But used in the seed germination
- *B. Pumilus , B. licheniformis*

Indirect Mechanisms



1. Siderophore production

- **Iron** is an essential nutrient
- Quite abundant, but unavailable for plants & MO
- Insoluble oxides and hydroxides inaccessible

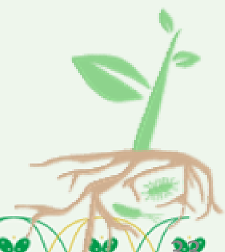
- **Siderophores'** functional groups capable of binding iron in a **reversible way**
- Rhizosphere bacteria release these compounds to increase their **competitive potential**

1. Siderophore production…

- Release **iron-chelating molecules** to the rhizosphere
- Improve iron nutrition - attract iron towards the rhizosphere
- **Inhibit** the growth of other micro-organisms
- Hinder the growth of pathogens by limiting the iron available for the pathogen (Fungi)

PGPR

Pseudomonas fluorescens & *Pseudomonas aeruginosa*



1. Siderophore production…

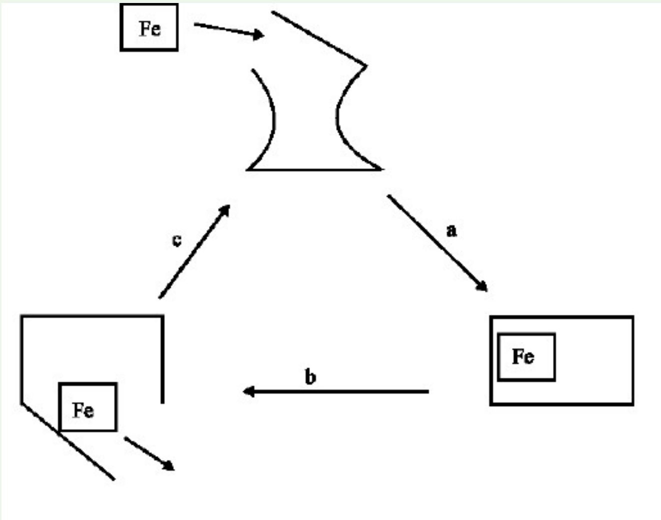


Fig. 6: The usual siderophore iron delivery mechanism. Adopted from Stintzi *et al.* (2000)

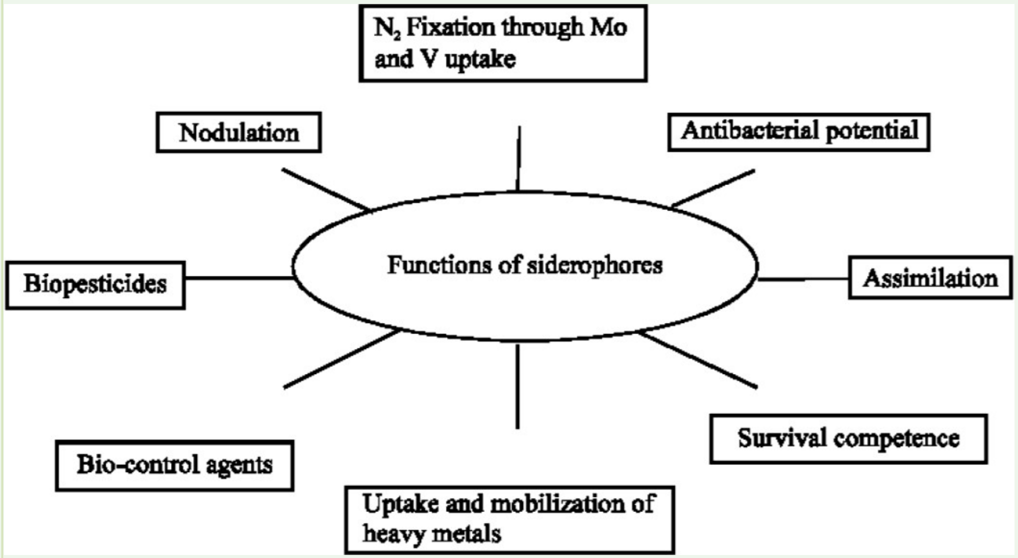
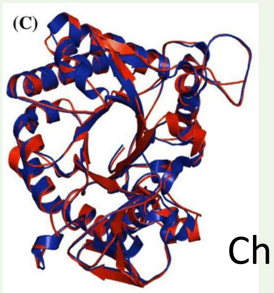


Fig. 8: Impact of microbially secreted siderophores on plant growth

Ahamed and Khan (2014)

2. Chitinase & glucanase production

- Control soil borne pathogens (biocontrol agents)
- Production of **cell wall-degrading enzymes**
    - ß-1,3-glucanase
    - Chitinase
    - Cellulase
    - Protease



Chitinase

- **Direct inhibitory** effect on the hyphal growth of **fungal pathogens**

3. Antibiotic production

- **Microbial antagonists** against plant pathogens
- Alternate to chemical pesticides
- *Bacillus and Pseudomonas* species
- Inhibitory even at low concentrations

Bacillus subtilis 168

antibacterial and antifungal antibiotics

4. Induced systematic resistance

- Protect plant from diseases,
- Via induced systematic resistance **(ISR)**
- **Increase in the level of basal resistance** to several pathogens simultaneously
- *Pseudomonas* strains

- A signal is generated involving **jasmonate** or **ethylene pathway**
- Thus inducing the host plant's defense response.

4. Induced systematic resistance…

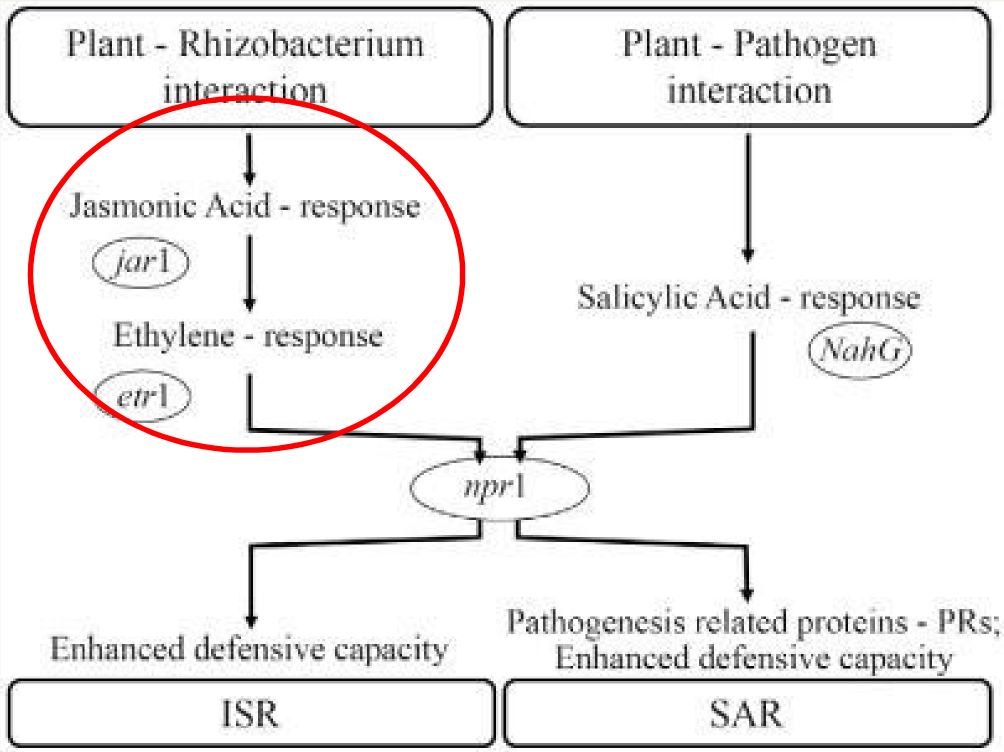
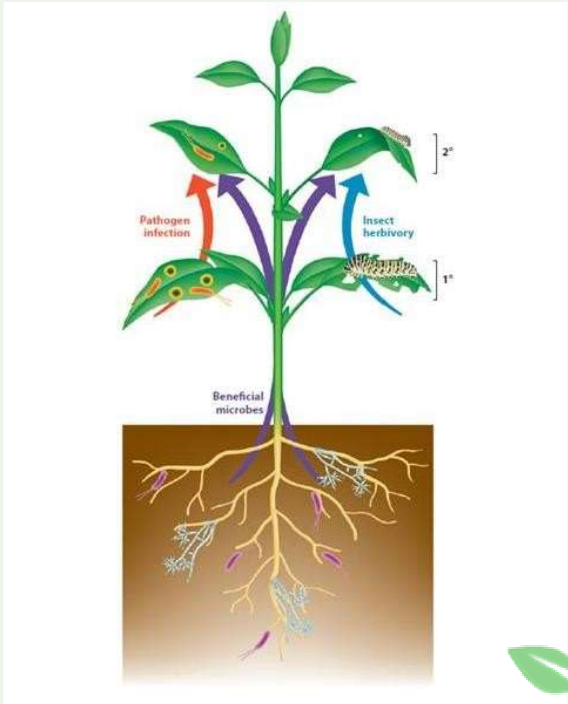


Figure 1 - Signal transduction pathways leading to pathogen-induced systemic acquired resistance (SAR) and rhizobacteria-mediated induced systemic resistance (ISR) in *Arabidopsis thaliana*. *Modified from*: Van Loon *et al.*, 1998.



Pieterse et al. (2014) Annu. Rev. Phytopathol. 52:347-75

5. Plant stress markers Production

- For **environmental stresses** -Temperature, cold, drought, salinity, alkalinity UV, and pathogen infection

Under Higher Salinity,

- Oxidative stress
- Generation of reactive oxygen species **(ROS)**
- **ROS scavenging enzymes** / stress marker enzymes
  - *B. cereus* AR156
  - peroxidase (POX), superoxide dismutase (SOD), catalase (CAT), etc.
- **L-proline**

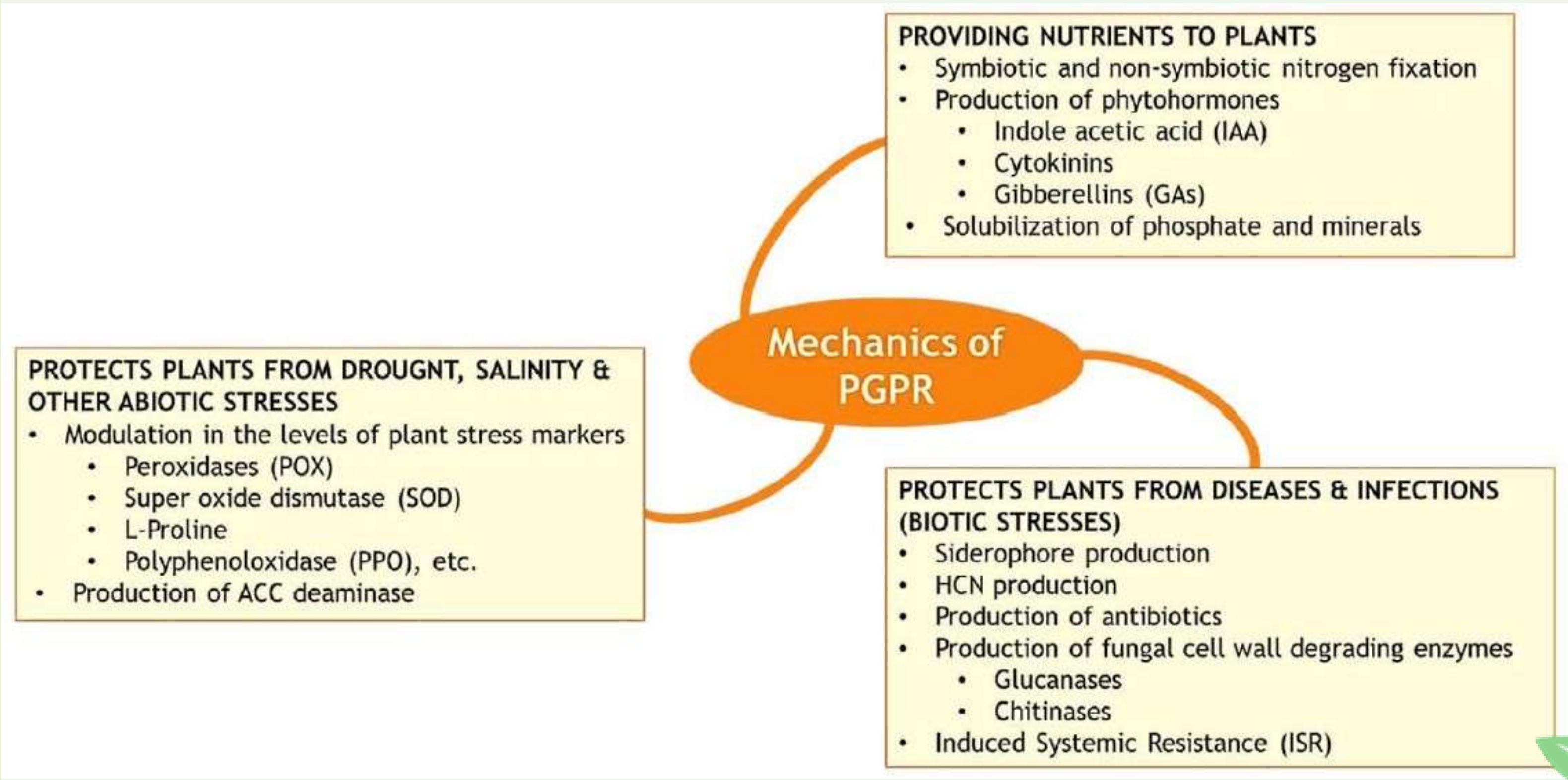
6. Production of ACC deaminase

- 1-aminocyclopropane-1-carboxylic acid (ACC)
- **Precursor of** phytohormone **ethylene**
- Increases dramatically under **abiotic stresses**
- It has **detrimental** effect on plant

ACC deaminase

- Degrades ACC inhibiting – Protect Plants
- *Achromobacter piechaudii* ARV8 - tomato plants
  (Ali, Charles, and Glick (2012)

Summary of the Mechanisms



Ahemad and Mohammad(2011)

Summary of the Mechanisms

Table 1. Mechanisms used by PGPR to promote plant health and growth;

Functions	**Mechanisms**	**References**
Biofertilization	Phosphate solubilization	Yazdani et al. (2009)
	Siderophores production	Vansuyt et al. (2007)
	Exopolysaccharides production	Sandhya et al. (2009)
	Biofixation of atmospheric nitrogen	Weyens et al. (2010)
Phytostimulation	Ethylene production	Glick et al. (2007)
	Cytokinins production	Kang et al. (2009)
	Gibberellins production	Kang et al. (2009)
	Indole Acetic Acid production	Ashrafuzzaman et al. (2009)
Control of pathogens	Antibiotics production	Ongena et al. (2005)
	Lytic enzymes production	Joshi et al. (2012)
	Hydrogen cyanide production	Lanteigne et al. (2012)
	Volatile compounds production	Trivedi et al. (2008)
	Induction of systemic resistance	Doornbos et al. (2012)
	Competition for Iron, nutrient and space	Innerebner et al. (2011)

Noumavo et.al ., (2016)

Commercialization of PGPR

- As
  1) **Biofertilizers**
  2) **Biocontrol agents**

- Several PGPR bacterial strains are commercially available
  - Dry powder products -> G(+)s

  - Suspension of organisms in oil
  - Liquid products -> G(-)s

Commercially available PGPR

Biofertilizers

Bacillus licheniformis SB3086

Azospirillum brasilense Sp245

[http://ztmbpd.iari.res.in/wp-](http://ztmbpd.iari.res.in/wp-content/uploads/2017/09/Azospirillum.png)
content/uploads/2017/09/Azospirillum.png

Biocontrol agents

Pseudomonas aureofaciens

Streptomyces griseoviridis K61

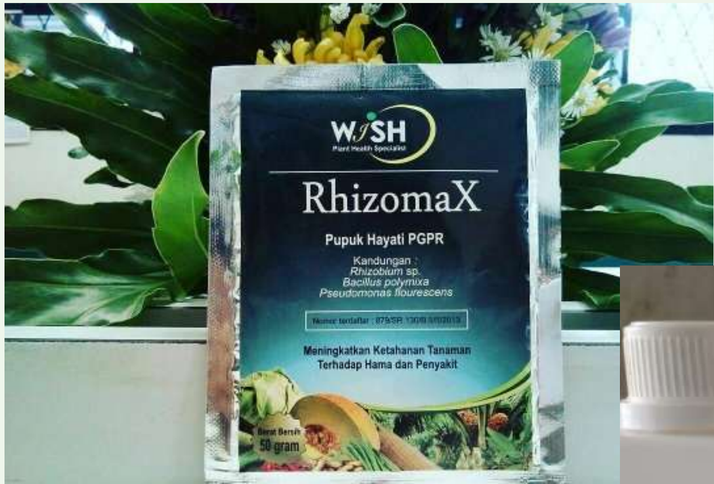
Bacillus licheniformis SB3086

Problem - Crops are grown under a multiplicity of climatic & environmental conditions (farm to farm /within 1 field)

WU1

PGPR in Indonesia

Legin, berisi bakteri Rhizobium sp



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بِسْمِ اللَّهِ الرَّحْمَنِ الرَّحِيمِ

اَللَّهُمَّ أَرِنَا الْحَقَّ حَقًّا وَارْزُقْنَا اتِّبَاعَه ُ وَأَرِنَا الْبَاطِلَ بَاطِلاً وَارْزُقْنَا اجْتِنَابَهُ

Ya Allah Tunjukkanlah kepada kami kebenaran sehinggga kami dapat mengikutinya,
Dan tunjukkanlah kepada kami keburukan sehingga kami dapat menjauhinya.