

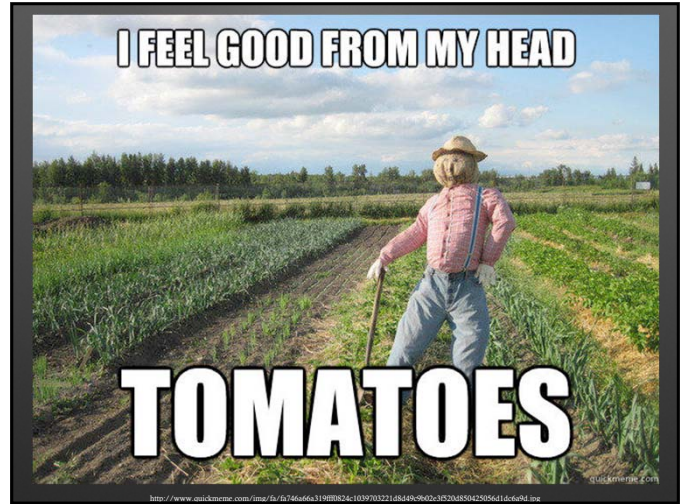
Living Soils

Steve Becker
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IPPS
Presented at the 10th Annual Meeting of the International Plant Pathogen Society
Western Region of North America, October 17-20, 2017, Williams, Oregon, USA

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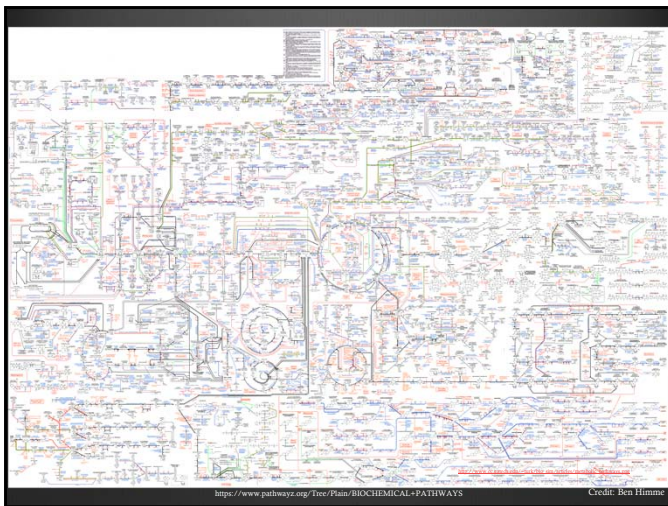
www.tainio.com



I FEEL GOOD FROM MY HEAD

TOMATOES

<http://www.quickmeme.com/img/5a/5a746a6631988824c1039703221d8449e9602c3520a850425056d1d06f9d.png>

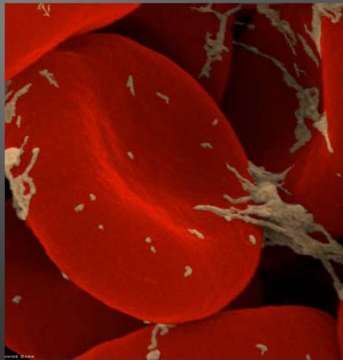


<https://www.pathwayz.org/Tree/Plain/BIOCHEMICAL+PATHWAYS>

Credit: Ben Himme

Life is AMAZING!

- Inside of YOU!
- ~2.4 million new RBC/second!
- 250 million hemoglobin each
- 600,000,000,000,000 hemoglobin produced-Trill
- 344,400,000,000,000,000 amino acids-Quad
- 1,033,200,000,000,000,000 bases read-Quint
- Life is AMAZING!



<http://www.boredpanda.com/looking-at-the-world-through-a-microscope-pics/>

Surfaces Scratched:

- Living Soils
- Small Friends
- Exudate Energy
- PGPR Functions
- Examples



<http://www.humansociety-feeding.com/wp-content/uploads/2017/05/keberg.png>

Pretend your soil is a person...



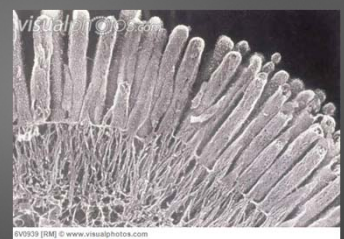
gardeningjones.com/blog

Pretty easy for me...



Plant Gut?

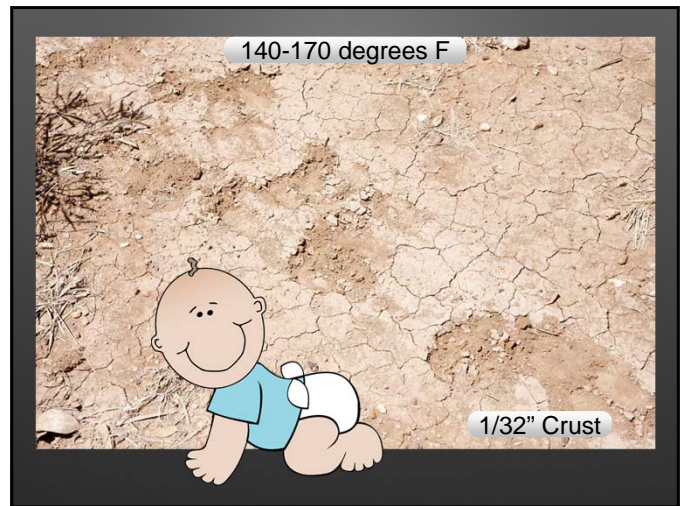
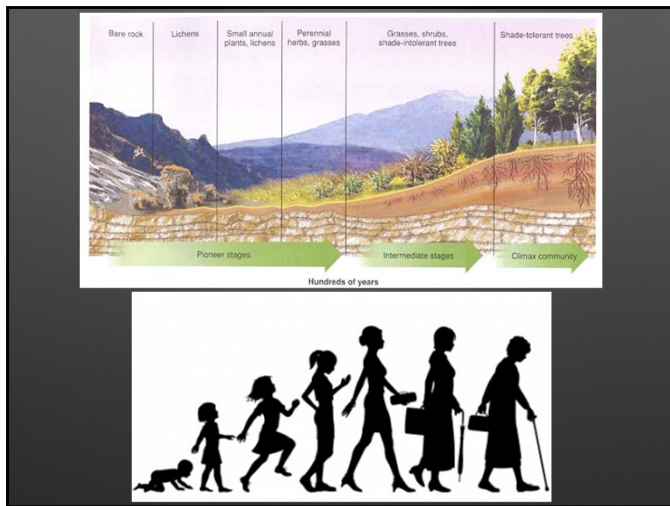
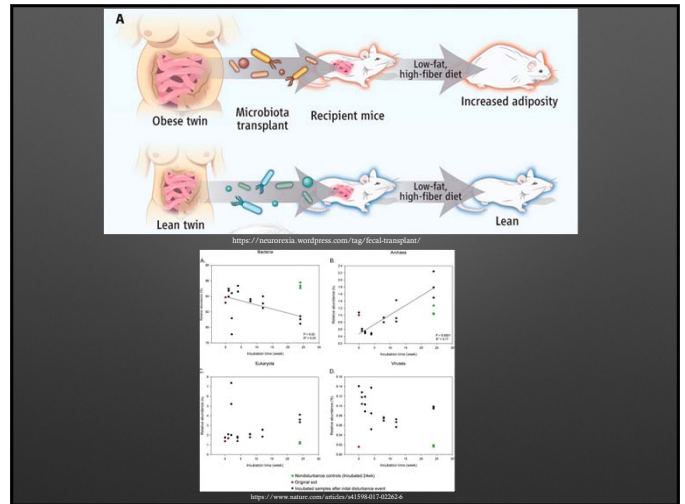
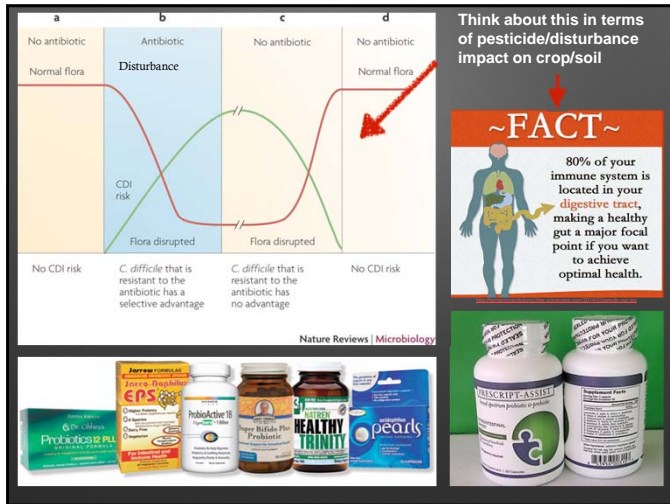
- Berendsen et al. (2012) highlights similarities in importance of microbes between human and plant
- "[The] complex plant-associated microbial community, also referred to as the second genome of the plant is crucial for plant health."
- "In humans, the effects of intestinal microbial communities on health are becoming increasingly apparent. Similar functions can be ascribed to microbial communities in the human gut and plant rhizosphere."



EV9539 [RM] © www.visualphotos.com



Berendsen et al., 2012; R.L. Berendsen, C.M. Pieterse, P.A. Bakker. The rhizosphere microbiome and plant health. Trends Plant Sci., 17 (2012), pp. 478-486



Healthy. Happy. Simple. first foods

6 Months	7 Months	8 Months	9 Months	10 Months	11 Months	12 Months
— Rice Cereal	— Oatmeal (infant cereal)	— Yogurt	— Onions	— Spinach	— Fish	— Cow's Milk
— Avocado	— Carrots	— Wheat B	— Garlic	— Blueberries	— Tomatoes	— Strawberries
— Banana	— Asparagus	— Wheat Germ*	— Beans	— Cherries	— Stronger cheeses	— Raspberries
— Sweet potato	— Green Beans	— Flax	— Light cheeses	— Sweet Pepper	— Corn/cornmeal	— Blackberries
— Butternut Squash	— Peas	— Okra	— Basil	— Mushroom	— Egg Yolk (consult physician)	— Honey
— Pumpkin	— Summer Squash	— Kale	— Parsley	— Cottage Cheese	— Smooth Nut butter (not peanut)	— Hand-cooked egg white
— Peach	— White Potatoes	— Broccoli	— Oregano	— Cream Cheese	— Oranges & Juice	— Grapefruit
— Prune		— Watermelon	— Cinnamon	— Light Cheddar		
— Apple		— Turkey	— Rosemary	— Gouda		
— Pear		— Cranberry	— Dill	— Cantaloupe/ Melons		
— Mango		— Chicken	— Chives	— Pork (lean)		
— Plum			— Nutmeg			

Fruits may only be served raw after 8 months. Bananas and avocados do not need to be cooked.

Vegetables should be cooked until baby has mastered chewing and the risk of choking diminishes.

Consult your pediatrician before offering yogurt.

Consider trying whole grain pasta, breads, and cereals made from introduced grains. Watch for choking.

All cheeses at this age must be made of pasteurized milk.

Always watch for signs of an allergic reaction: facial swelling, vomiting, diarrhea, rash or problem breathing.

Citrus and other acidic foods may cause rash and digestive upset.

This schedule should be reviewed with your pediatrician.

For more information & updates, visit healthyhappysimple.blogspot.com/



Who are we feeding?

Organism	Pounds of liveweight/acre
• Bacteria	1000
• Actinomycetes	1000
• Molds	2000
• Algae	100
• Protozoa	200
• Nematodes	50
• Insects	100
• Worms	1000
• Roots	2000

In one square meter of soil...

Organisms decrease in size and increase in number

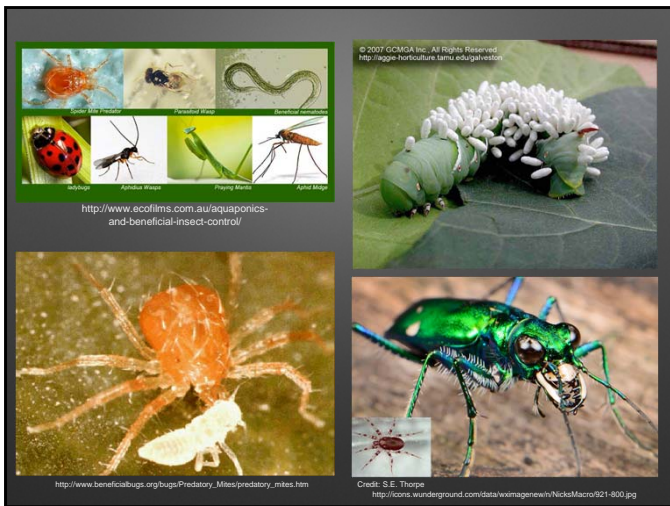
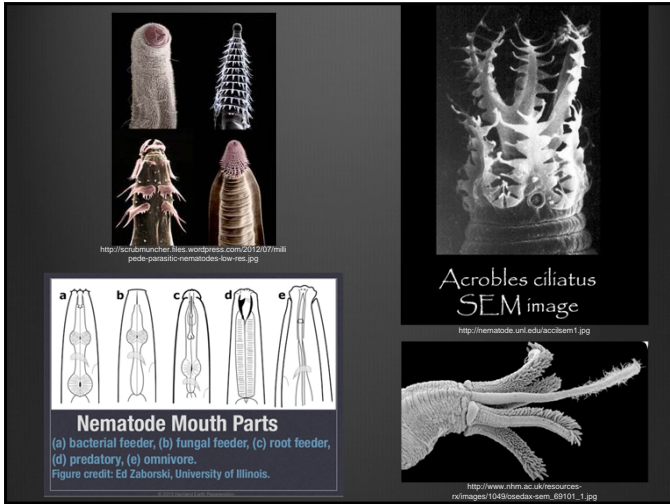
*1 Million (1,000,000) layer

Bollen, Walter B. 1959. Microorganisms and Soil Fertility. Oregon State College. Oregon State Monographs, Studies in Bacteriology, Number 1, 22 p.

Who Knew?

Credit: Val Behan-Pelletier

1 sq. ft., top 2 inches of litter





Bacteria

- Very Small!
- So small that by themselves, they can not have an impact on larger organisms
- But anyone who has been sick or had an infections knows that this is not the whole story

Scaled 2000 times on 8.5 x 11 sheet

<http://www.ctgclean.com/downloads/HowBigisAMicron.jpg>

<https://mckag.files.wordpress.com/2011/05/090527170308-large.jpg>

http://upload.wikimedia.org/wikipedia/en/b/b2/Broomstick_Bunny.jpg

Micro Machines

Size of Bacteria

Bacillus cells on the tip of a pin.

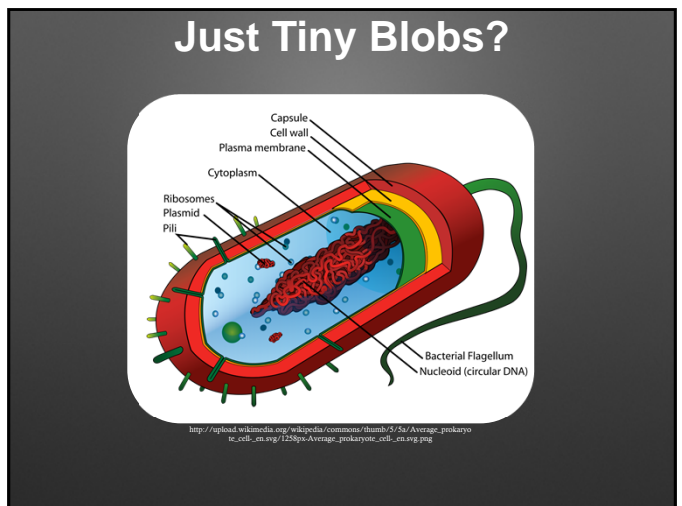
100 um http://19thpsalm.org/Ch108/index_00144499aSize.gif 10 um

-Based on a quick calculation, a 1 square inch by 1/8th inch colony may have over 5 trillion individuals!

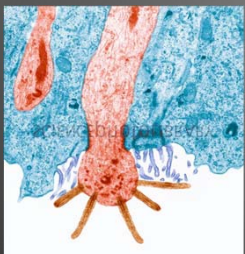
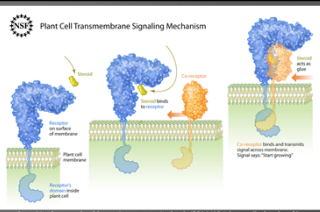
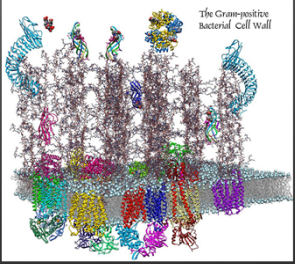
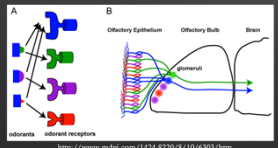
-Earth has 7 billion people

-700x population of people in 1" of bacteria!

Scale



Senses?

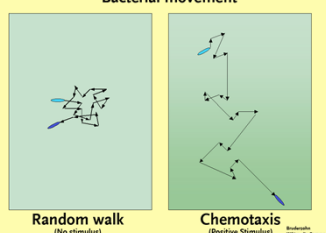

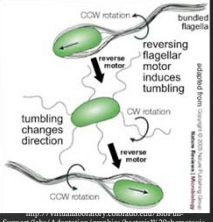





Plant Cell Transmembrane Signaling Mechanism

The Gram-positive Bacterial Cell Wall

olfactory receptors

Bacterial movement

Random walk (No stimulus)

Chemotaxis (Positive Stimulus)

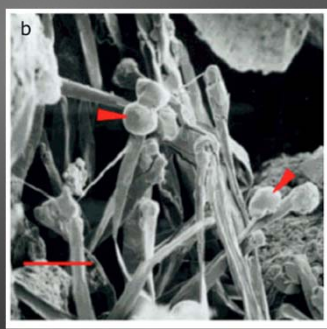
OOH!
PIECE OF CANDY.

reversing flagellar motor induces tumbling

tumbling changes direction

Follow the food

- Huge quantities of carbon are sent to the roots as exudates
- Research shows close to 25% of photosynthetic sugar is sent to the root
 - Some research shows 75% during certain plant stages
 - Young seedlings typically exude about 30-40% of their fixed carbon as root exudates (Whipps, 1990)
- That is a LOT of energy
 - If 25%, some crops "dump" over 20,000 lbs of exudate/acre/year



b) Red arrows point to droplets of root exudates released from the tips of root hairs on the surface of broom corn (Sorghum sp.).

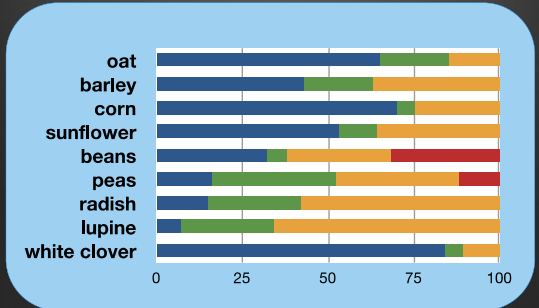
Hutsch B, Augustin J, Merbach W. (2002) Plant rhizodeposition – an important source for carbon turnover in soils. *Journal of Plant Nutrition and Soil Science*, 165, 4, 399-407

Carvalhais LC, Davies PJ, Fidosoyewu D, Hainzeel MR, Borriss R, von Weren N. 2011. Root exudates of sugars, amino acids, and organic acids by maize as affected by nitrogen, phosphorus, potassium, and iron deficiency. *Journal of Plant Nutrition and Soil Science* 174: 3-11

Whipps J.M. (1990) Carbon economy. In: *The Rhizosphere* (ed. J.M. Lynch), pp. 59-97. John Wiley & Sons Ltd, Essex, UK.

2012 Nature Education McGully, M. The rhizosphere: the key functional unit in plant/microbe interactions in the field: implications for the understanding of allelopathic effects. Division of Plant Industry, Forth World Congress on Allelopathy, The Regional Institute Ltd.

The percentage of N in the roots as nitrate (blue), amino acids (green), amides (yellow) and ureides (red). These compounds leak from the roots as exudates and are part of the plant's signature to create a unique rhizosphere.



Plant	Nitrate (%)	Amino acids (%)	Amides (%)	Ureides (%)
oat	~45	~15	~25	~15
barley	~45	~15	~25	~15
corn	~45	~15	~25	~15
sunflower	~45	~15	~25	~15
beans	~35	~15	~25	~25
peas	~35	~15	~25	~25
radish	~45	~15	~25	~15
lupine	~45	~15	~25	~15
white clover	~45	~15	~25	~15

Credit: Dr. Jill Clapperton

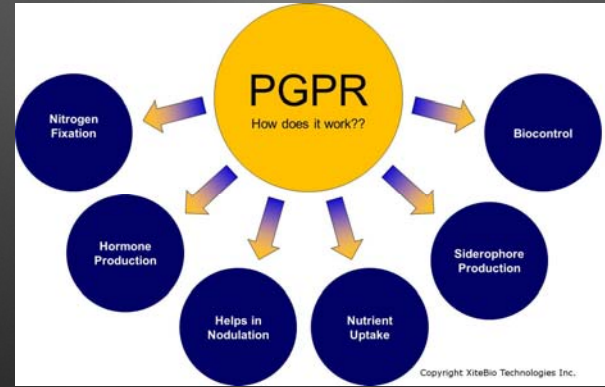
Why so much Energy?

- That is a lot of energy being released through the roots
- Why would a plant do that?
- ROI?



Credit: Dr. Richard Box Wirelessly lit Fluorescent field

Because Plants Need Help



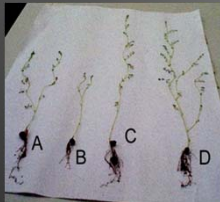
Copyright Xitellio Technologies Inc.
Klopper, J.W., Schroth, M.N. 1978. Plant growth-promoting rhizobacteria in radish. pp 879-882. Proc. 4th Int'l. Conf. Plant Pathogenic Bact. Gilbert-Clary, Tours, France

Phosphorus

- *Pseudomonas putida*
- *P. fluorescens*
- *Bacillus megaterium*
- *Glomus*



P. fluorescens phosphate solubilization

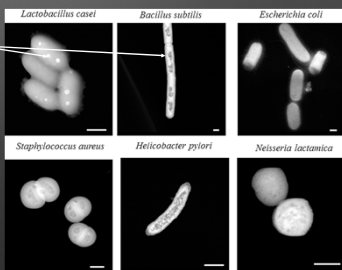


Pseudomonas

A = Garden soil with zinc phosphate B = Garden soil only C = CMG860 alone without zinc phosphate D = CMG860 with zinc phosphate

Alkhar, M.S. and Z.A. Siddiqui. 2009. Effects of phosphate solubilizing rhizobacteria and phosphate on the growth, nodulation, yield and root: root diameter complex of chickpea under field condition. African J. Biotechnol., 8: 3489-3495

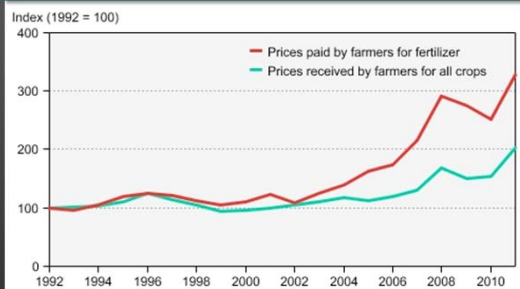
Noori M.S., Saad H.M. (2012) Potential Plant Growth-Promoting Activity of *Pseudomonas* sp. Isolated from Paddy Soil in Malaysia as Biocontrol Agent. J. Plant Pathol. Microb. 3: 205. doi:10.4172/2291-7471.1000192



http://c431376.776.c22.rackcdn.com/24445/1c6mb-02-00063-2/1c6mb_02-00063-2003.jpg

Nitrogen

Prices paid by farmers for fertilizer and prices received for all crops, 1992-2011



Source: USDA Economic Research Service using data from USDA-NASS, and Bureau of Labor Statistics.

With these trends, business as usual can not work!

Free-living N Fixers

- Dr. Arden Andersen
 - "University personnel tell farmers that they cannot generate much nitrogen bacteria activity without legumes. However, research in 1942 revealed that "root-nodule bacteria of lucerne grew equally under lucerne and under cotton."
- Azotobacter
- Azospirillum
- Many photosynthetic
- Rhodospirillum, Rhodobacter, Rhodospira, etc...
- **Don't forget your vitamins!**
- Mg, Fe, Mo, Ni, Co, V




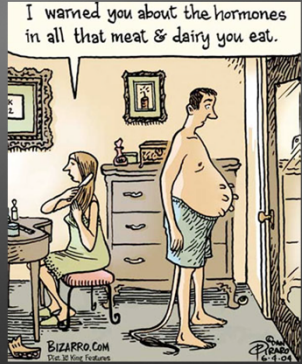
Photo: Marangola Hungria

<http://www.agribio.com.br/noticias/NoticiaDetalhe.aspx?codigoNoticia=34613>
 Dobereiner, J. et al. How to isolate and identify diazotrophs of non-leguminous plants. Brasília: EMBRAPA-SPI. Itajai, RJ: EMBRAPA-CNPAB, 1996.
<http://www.scribd.com/doc/17804307/Arden-Andersen-1942>

Biofertilization accounts for approximately 65% of the nitrogen supply to crops worldwide (Bloembergen et al., 2001).

Hormones

- Hormones, at their core, are chemical messengers.
- Extremely small quantities.
 - ppm ppb ppt
 - Testosterone and Estradiol will only increase small amounts (roughly 10x) during puberty (a few ppb and ppt)



BIZARRO.COM
© 2004 Bizarro

"China farmers face 'exploding' watermelon problem." -BBC Headline-

- 2011
- Chinese farmers applied too much plant growth regulator/accelerator
 - Forchlorfenuron
- When it comes to hormones, a little goes a long way
- Biofeedback/Nature knows best



<http://www.theguardian.com/world/2011/may/17/exploding-watermelons-chinese-farming>

<http://www.bbc.co.uk/news/world-asia-pacific-13421374>


Hormones

Plant Growth Hormones		PCR	PCR	References
		<i>Arabidopsis thaliana</i> T1	Indole-3-acetic acid, ethylene	Prishchak et al. (2002)
		<i>Phaseolus vulgaris</i> sp. L26		
		<i>Rhizobium</i> C2A11 S80		
		<i>Escherichia coli</i> sp.		
Abscisic acid	Maintains seed dormancy and winter dormancy; closes stomata	<i>Aspergillus terreus</i>	Indole-3-acetic acid	Zelik et al. (1998), b. 2000, Chalkó et al. (2001)
		<i>Aspergillus</i> sp.	Indole-3-acetic acid	Vera et al. (2010)
Auxins	Promote stem elongation, adventitious root initiation, and fruit growth; inhibit axillary bud outgrowth and leaf abscission	<i>Aspergillus</i> sp.	Gibberellic acid	Lacourte and Bédouin (1997)
		<i>Aspergillus</i> sp.	Indole-3-acetic acid	Dobereiner et al. (2001)
		<i>Aspergillus</i> sp.	Indole-3-acetic acid	Luthe et al. (2000)
		<i>Bacillus licheniformis</i>	Ethylene	Fukuhara et al. (1989)
		<i>B. licheniformis</i>	Physiologically active gibberellins	Correa-Matias et al. (2000)
Brassinosteroids	Promote stem and pollen tube elongation; promote vascular tissue differentiation	<i>B. subtilis</i>	Indole-3-acetic acid	Correa-Matias et al. (2000)
		<i>B. subtilis</i>	Ethylene	Mauceri and Branch (1988)
		<i>Bacillus megaterium</i> BHE.P5B14	Indole-3-acetic acid	Vera et al. (2010)
Cytokinins	Inhibit leaf senescence; promote cell division and axillary bud outgrowth; effect root growth	<i>Aspergillus</i> sp.	Auxin	Mauceri and Branch (1989)
		<i>Aspergillus terreus</i>	Ethylene	Pal et al. (2005)
		<i>Aspergillus terreus</i>	Ethylene	Mauceri and Branch (1989)
		<i>Aspergillus terreus</i>	Ethylene	Simonin et al. (1979)
		<i>Aspergillus terreus</i> G20-18	Indole-3-acetic acid, auxin, ethylene, gibberellins	García de Salazar et al. (2004)
		<i>Aspergillus terreus</i> BHE.P5B16	Indole-3-acetic acid	Vera et al. (2010)
Ethylene	Promotes fruit ripening and leaf abscission; inhibits stem elongation and gravitropism	<i>Aspergillus terreus</i>	Ethylene	Pozner et al. (1981), Prishchak et al. (1999)
		<i>Aspergillus terreus</i>	Ethylene	Sato et al. (1997), Simonin et al. (1979)
		<i>Aspergillus terreus</i>	Ethylene	Simonin et al. (1979)
Gibberellins	Promote seed germination, stem growth, and fruit development; break winter dormancy; mobilize nutrient reserves in grass seeds	<i>Aspergillus terreus</i>	Auxin	Angier et al. (2008, 2002)
		<i>Aspergillus</i> sp.	Indole-3-acetic acid, auxin, ethylene	Strawner and Tallier (1989)
		<i>B. licheniformis</i>	Indole-3-acetic acid	Bokros et al. (1993)
		<i>B. licheniformis</i>	Indole-3-acetic acid	Datta et al. (2000)
		<i>B. licheniformis</i>	Indole-3-acetic acid, auxin, ethylene	Wang et al. (1993)
		<i>B. licheniformis</i>	Indole-3-acetic acid	Talier and Strawner (1991)
		<i>B. licheniformis</i>	Indole-3-acetic acid	Vera et al. (2010)


<http://ocwbiology10.wikispaces.com/file/view/37001.jpg/3908859/37001.jpg>

Research shows that gut flora impact hormone levels

Control



With Bacteria



Jay Prakash Verma, Jaganathan Yadav, Kavindra Nath Tiwari, Lavakush and Vimal Singh, 2010. Impact of Plant Growth Promoting Rhizobacteria on Crop Production. International Journal of Agriculture Research, 6, 924-933.

Credit: Saraswati Nepuane

“Rhizobacteria Capable of Producing ACC-deaminase May Mitigate Salt Stress in Wheat”

Control EC: 15 dS/m W17 EC: 15 dS/m

P. fluorescens and *P. putida*

SAM = S-adenosylmethionine
ACC = 1-aminocyclopropane-1-carboxylic acid

Naderi S., et al. 2010. Rhizobacteria Capable of Producing ACC-deaminase May Mitigate Salt Stress in Wheat Soil Sci. Soc. Am. J. 2010. 74:533-542.

Challenge	Drought	
Pre-treatment	<i>Paenibacillus polymyxa</i>	Control
A		B

P. polymyxa B2 inoculated plants (A) survived drought stress two weeks longer than untreated control plants (B). Image after three day drought exposure is shown. Plants were grown and inoculated and subsequently exposed to drought stress as described by Timmusk and Wagner (1999).

Timmusk, S., Timmusk, K., & Behers, L. (2013). Rhizobacterial Plant Drought Stress Tolerance Enhancement: Towards Sustainable Water Resource Management and Food Security. *Journal of Food Security*, 1(1), 6-9.

Iron

- Siderophores chelate iron
- Plant feeding
- Pathogen control

http://www.alive.com/articles/view/21560/clean_up_with_chelation

Figure 1. Screening of siderophore-producing bacterial isolates using CAS agar plates. The arrows indicated the isolates PC-1012 (the left one), and G-2-17-1 (the right one) which couldn't grow on CAS agar plates. The circles in the center of the orange halos were filter papers soaked up with bacterial supernatant.

Bakker, P.A.H.M., J.G. Lamers, A.W. Bakker, J.D. Manugg, P.J. Weisbeek and B. Schippers. 1986. The role of siderophores in potato tuber yield increase by *Pseudomonas putida* in a short rotation of potato. *Eur. J. Plant Pathol.*, 22: 249-256

Protection/Biocontrol

“If you have a healthy soil, you will not need soil fumigants.”

- G.W. Bird MSU

Published in Lundberg, D.S., Lebes, S.L., Paradise, Yourstoria, S., Gehring, J., Mallatt, S., Tremblay, J., Engabretsson, A., Kunin, V., Glavinia del Rio, T., Edgar, R.C., Eckhorst, T., Ley, R.E., Hugenholz, P., Tringe, S.G., and Dangl, J.L. 2012. *Nature* 488: 86-90

Plant roots secrete a wide range of compounds, among those sugars and amino acids are engaged in attracting (chemotaxis) microbes (1), flavonoids act as signaling molecules to initiate interactions with mycorrhiza (AM fungi) (2), rhizobium (3) and pathogenic fungi (oomycetes) (4), aliphatic acids (e.g. malic acid) are involved in recruiting specific plant growth promoting rhizobacteria (*Bacillus subtilis*) (5), nematodes secrete growth regulators (cytokinins) that are involved in establishing feeding sites in plant roots (6) and nematodes secrete other compounds (organic acids, amino acids and sugars) involved in attracting bacteria and in bacterial quorum sensing (7). Knowledge of the roles of other types of compounds, such as fatty acids (8) and proteins (9), secreted by roots in the rhizosphere and other multi-partite interactions (10) remains unknown.

<http://www.scribd.com/doc/6690001/282>

"When under attack, plants can signal microbial friends for help" - Bryant, Univ. Delaware, 2008-

A plant root surrounded by a film of *Bacillus subtilis* (green fluorescence) in response to an infection by a plant pathogen. (Thimmareju Rudrappa, University of Delaware)

McNear Jr., D. H. (2013) The Rhizosphere - Roots, Soil and Everything in Between. *Nature Education Knowledge* 4(3):1 <http://www.udel.edu/udahl/2008/oct/bais101708.html>

Double Whammy

- Benzoxazinoids can act as powerful antibiotics
- Protects the plant directly
- Research has shown *P. putida* is attracted
- Once attracted, help protect the plant

Neal, A. et. al. found a corn antibiotic (benzoxazinoid) to encourage chemotaxis and growth of *Pseudomonas putida*.

EPA Biopesticides

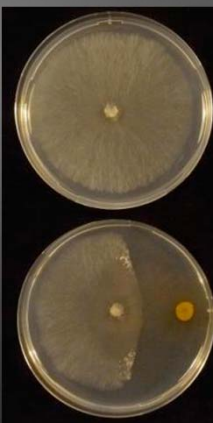
- Partial List:
 - Bacillus thuringiensis*
 - B. sphaericus*
 - B. subtilis*
 - B. cereus*
 - B. pumilus*
 - B. firmus*
 - B. amyloliquefaciens*
 - Pseudomonas fluorescens*
 - Streptomyces griseoviridis*
 - S. lydicus*
 - Trichoderma harzianum*

Crop	Disease/pathogen/insect	PGPR	References
Barley	Powdery mildew	<i>B. subtilis</i>	Schobeck et al. (1980)
Beans	Halo blight	<i>Pseudomonas fluorescens</i> strain 97	Altrona (1991)
	Scirpus rosetta	<i>Pseudomonas cepacia</i>	Fridlander et al. (1993)
Carnation	Fusarium wilt	<i>Pseudomonas</i> sp. (WCS 417)	Van Peer et al. (1991)
Cotton	Damping off	<i>Pseudomonas fluorescens</i>	Hotell and Saporito (1979, 1980)
	<i>Motacillomyces invicinus</i>	<i>B. subtilis</i>	Sikora (1998)
	<i>M. Aromatis</i>		
	<i>Rhizoctonia solani</i>	<i>Pseudomonas cepacia</i>	Fridlander et al. (1993)
	<i>Helicoverpa armigera</i>	<i>Pseudomonas gladioli</i>	Quingwen et al. (1998)
Cucumber	Cucumber anthracnose	<i>Pseudomonas putida</i> (89B-27)	Wei et al. (1991, 1996)
		<i>Serratia marcescens</i> (90-166)	
		<i>Pseudomonas cepacia</i>	Fridlander et al. (1993)
		<i>Pseudomonas putida</i> (89B-27)	Klopper et al. (1993)
		<i>S. marcescens</i> (90-166)	
	Bacterial angular leaf spot	<i>P. putida</i> (89B-27), <i>Flavobacterium oryzihabitans</i> INR-5, <i>S. marcescens</i> (90-166), <i>Bacillus pumilus</i> (INR-7)	Klopper et al. (1993)
	Cucumber mosaic virus	<i>P. putida</i> (89B-27), <i>S. marcescens</i> (90-166)	Raupach et al. (1996)
	Striped Cucumber beetle	<i>P. putida</i> (89B-27), <i>Flavobacterium oryzihabitans</i> INR-5, <i>Pseudomonas</i> sp.	Zelander et al. (1997a)
Green gram	<i>Aspergillus</i> sp., <i>Curvularia</i> sp., <i>Fusarium oxysporum</i> , <i>Rhizoctonia solani</i>		Siddin et al. (1999)
Mung bean	Root rot, Foot rot	<i>P. aeruginosa</i> , <i>B. subtilis</i>	Siddiqui et al. (2001)
Rice	Rice sheath blight	<i>Streptomyces</i> spp. and <i>Bacillus cereus</i> in combination with <i>P. fluorescens</i> and <i>Bordetella</i>	Sung and Chung (1997)
	Rice sheath blight	Combination of <i>P. fluorescens</i> strains Pf1 and Pf7	Nandakumar (1998)
	<i>Rhizoctonia solani</i> (sheath blight pathogen)	<i>P. fluorescens</i> Strains Pf1 and Pf7	Vidhyasekaran and Mathamalar (1999)
	Blue mold	<i>S. marcescens</i> 90-116, <i>B. pumilus</i> SE 34, <i>P. fluorescens</i> 89-6 1, <i>B. pumilus</i> T4, <i>B. pasteurii</i> C-9	Zhang et al. (2002)
Tomato	Tomato mottle virus	<i>B. subtilis</i> strain IN 937b	Murphy et al. (2000)
		<i>B. amyloliquefaciens</i> strain IN 937a	
		<i>B. subtilis</i> strain IN 937b	
		<i>Bacillus pseudomonas</i> , <i>Penicillium</i> , <i>Beauveria</i> , <i>Rhizoglyphus</i>	Benwick et al. (1991)
Wheat	Take all disease	<i>Bacillus subtilis</i>	Borison and Waller (1994)

Jay Prakash Verma, Jambhori Yashy, Kishore Kishor, Lakshmi Devi, et al. (2004). Growth Promoting Rhizobacteria on Crop Production. International Journal of Agricultural Research, 5: 954-963.

Botrytis

- *Bacillus brevis*
- *Bacillus subtilis*
- *B. pumilus*





Latter G, Li H, Choudhury S, Hampson S, Workman S, Digi D, Epton HAS, Harbour A (1999). Antibiotic production and biocontrol activity by *Bacillus subtilis* CL 27 and *Bacillus pumilus* CLAS. J Appl Bacteriol 78: 97-108

Edwards S.G., 1990. Biological control of Botrytis cinerea by *Bacillus brevis* on protected Chinese cabbage. PhD Thesis, University of Aberdeen, UK.

Downy Mildew

- *Bacillus subtilis*
- *Pseudomonas fluorescens*
- *Streptomyces lydicus*
- *Trichoderma harzianum*



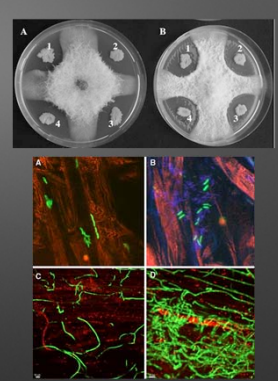


Niranjana-Raj, S., Shetty, N. P., and Shetty, H. S., 2004. Seed biopriming with *Pseudomonas fluorescens* isolates enhances growth of pearl millet plants and induces resistance against downy mildew. Intern. J. Pest Manag. 50: 41-46

<http://dx.doi.org/10.1080/02601920410001638802>

Fusarium

- *Azospirillum brasilense*
- *Bacillus subtilis*
- *Pseudomonas fluorescens*
- *Trichoderma harzianum*



Reddy KRN, Choudhary KA and Reddy MS. 2007. Antifungal metabolites of *Pseudomonas fluorescens* isolated from rhizosphere of rice crop. J Mycol Pl Pathol 37(2):

UNLAP C. A., SCHLESER D. A., PRICE N. P., VAUGHN F. Cyclic lipopeptide profile of three *Bacillus subtilis* strains antagonistic of *Fusarium head blight*. J. Microbiol. 49, (6), 602-2011

Cao Y., Zhang Z., Ling N., Yuan Y., Zheng X., Shen B., Shen Q. (2011) *Bacillus subtilis* S290 can control *Fusarium* wilt in cucumber by colonizing plant roots. Biol Fertl Soils 47: 495-506

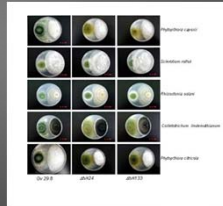
Chen L., Huang X., Zhang F., Zhao D., Yang X., Shen Q. (2012) Application of *Trichoderma harzianum* SQR-1037 bio-organic fertilizer significantly controls *Fusarium* wilt and affects the microbial communities of continuously cropped soil of cucumber. J. Soil Food Agric 22:2456-2470

Bashan A. et al., 2010. How the Plant Grows—A Critical Assessment Advances in Agronomy, Volume 108

Benhamou, N., Bittenger, R. R., and Paulitz, T. C., 1996b. Induction of differential host responses by *Pseudomonas fluorescens* in R1, T-DNA-transformed pearl millet after challenge with *Fusarium oxysporum* f. sp. *psii* and *Pythium ultimum*. Phytopathology, 86: 1174-1185.

Phytophthora

- *Bacillus amyloliquefaciens*
- *B. cereus*
- *B. subtilis*
- *Pseudomonas fluorescens*
- *Streptomyces griseovirdis*
- *Trichoderma*



T. vires

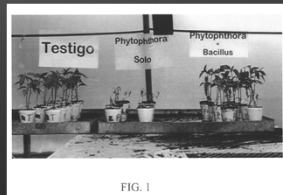


FIG. 1

Aguiar L, Bonatera A, Moragrega C, Camps J, Montesinos E. Biocontrol of root rot of strawberry caused by phytophthora cactovarum with a combination of two pseudomonas fluorescens strains. J Plant Pathol. 2011;93(2):363-72.

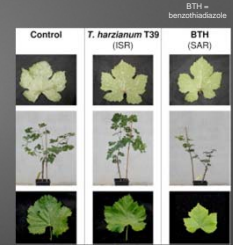
Vakib, D., Fayad, K., Barbasubay, T., Garon, M., Diery, C., Brzezinski, R., and Beaulieu, C. 1996. Glucanolytic actinomycetes antagonistic to Phytophthora fragariae var. rotii, the causal agent of crown rot of strawberry. Appl. Environ. Microbiol. 62: 1630-1635

Diby, P., Saha, K.A., Jaha, P.J., Sena, Y.R., Kumar, A., Mondal, M., 2005. Mycoparasitism induced by *Pseudomonas fluorescens* and *Trichoderma* spp. against *Phytophthora capsici*, the foot rot pathogen of black pepper (*Piper nigrum* L.). Annals of Microbiology 55, 129-133. PMID: 16589524

Smith, V. L., Wilcox, W. F., and Haman, G. E. 1990. Potential for biological control of Phytophthora root and crown rots of apple by *Trichoderma* and *Gliocladium* spp. Phytopathology 80:880-885.

Powdery Mildew

- *Bacillus subtilis*
- *Streptomyces lydicus*
- *Trichoderma harzianum*



http://www.sciencedirect.com/science/article/pii/S1049964411000909

Sample	Dilution ratio and concentration (mg/d)	Control value (%)	
		The 1 st Exp.	The 2 nd Exp.
<i>Bacillus subtilis</i> EB120	1:10 diluent	86 ± 5.6	94 ± 3.3
	1:25 diluent	84 ± 5.0	91 ± 2.7
	1:50 diluent	76 ± 1.1	86 ± 3.0
	1:100 diluent	21 ± 9.2	84 ± 6.7
Topseed	1:200 diluent	16 ± 5.5	72 ± 2.0
	1:10 diluent	87 ± 3.9	92 ± 5.4
	1:2.5 diluent	69 ± 7.8	82 ± 6.7
	1:50 diluent	56 ± 9.3	48 ± 1.1
Phthalazole	1:10 diluent	32 ± 2.3	14 ± 3.9
	1:200 diluent	29 ± 1.3	45 ± 3.0
	10 µg/ml	97	97
	10 µg/ml	99	99

Patent: WO 2005090553 A1

Schobeck, F., H.W. Dehne and W. Beicht, 1980. Untersuchungen zur Aktivierung unspaltbarer Resistenzmechanismen in Pflanzen. Z. Pflk. Pflschutz, 87: 654-666

<http://www.trichoderma.org/links/links.html>
<http://www.trichoderma.org/links/links.html>
<http://www.trichoderma.org/links/links.html>

Pythium

- *Azospirillum brasilense*
- *Bacillus amyloliquefaciens*
- *Bacillus subtilis*
- *Bacillus cereus*
- *Pseudomonas fluorescens*
- *Trichoderma harzianum*



T. harzianum parasitizing Pythium

Basham et al., 2010. How the Plant Growth-Promoting Bacterium Azospirillum Promotes Plant Growth—A Critical Assessment. Advances in Agronomy, Volume 108

Leiper, J. E. 1988. Role of fluorescent siderophore production in biological control of Pythium ultimum by a Pseudomonas fluorescens strain. Phytopathology, 78: 166-172.

Berhamou N., Belanger R. R., and Paulitz T. C., 1996a. Pre-implantation of Ri T-DNA transformed pea roots with Pseudomonas fluorescens inhibits colonization by Pythium ultimum. Travaux de l'Institut National de la Recherche Scientifique, Plantes, 108: 105-117.

Berhamou N., Belanger R. R., and Paulitz T. C., 1996b. Induction of differential host responses by Pseudomonas fluorescens in Ri T-DNA-transformed pea roots after challenge with Fusarium oxysporum f. sp. pis and Pythium ultimum. Phytopathology, 86:1174-1180.

Chang H., Chen J., Handelsman J., Goodman R.M., 1999. Behavior of Pythium torulosum zoospores during their interaction with tobacco roots and Bacillus cereus. Current Microbiology 38: 199-204.

Abo-Elnaga HIG (2006). Bacillus subtilis as a biocontrol agent for controlling sugar beet damping-off disease. Egypt J Phytopathol 34: 51-59.

Fiddaman, P.J. and Rossall, S. 1993. The production of antifungal volatile by Bacillus subtilis. J. Appl. Bacteriol., 74: 119-126.

Gurudatta, S., Weller, D.M., Sengar, A., Goode, J.R. 1996. Characterization of an antibiotic produced by a strain of Pseudomonas fluorescens inhibitory to Gaeumannomyces graminis var. tritici and Pythium spp. Antimicrob. Agents Chemother. 29: 488-490.

Rhizoctonia

- *Azospirillum brasilense*
- *Bacillus subtilis*
- *Pseudomonas putida*
- *Streptomyces griseus*
- *Trichoderma harzianum*



Antagonistic activity of *Pseudomonas putida* strain BHL-PSB04 (accession number GU124834) against *Rhizoctonia solani*

S. Aboysinghe, 2009. Effect of Combined Use of Bacillus subtilis CA32 and Trichoderma harzianum RU01 on Biological Control of Rhizoctonia solani on Solanum melongena and Capsicum annum. Plant Pathology Journal, 8: 9-16.

Eilat, Y., Chet, I., & Katan, J. (1980). Trichoderma harzianum: A biocontrol agent effective against Sclerotium rolfii and Rhizoctonia solani. Journal of Phytopathology 10, 119-121.

Nagaraju mar, M., Bhaskaran, R., Velazha ba n, R., 2004. Involvement of secondary metabolites and extracellular lytic enzymes produced by Pseudomonas fluorescens in inhibition of Rhizoctonia solani, the rice sheath blight pathogen. Microbiological Research, 159, 73-81.

Merriman, P.R., R.D. Pico, J.F. Kollmann, T. Peggott and E.H. Ridge, 1974. Effect of seed inoculation with Bacillus subtilis and Streptomyces griseus on the growth of cereals and canna. Aust J Agric Res. 25: 219-225.

Anka O. Shadi M (1996). Biocontrol of Rhizoctonia damping-off of tomato with Bacillus subtilis RB14. Appl Environ Microbiol 62: 4081-4085.

Nandakumar, R., Babu, S., Radjagopalan, R., Rajagundhar, T., Samiyappan, R., 2002. Pseudomonas fluorescens mediated antifungal activity against Rhizoctonia solani causing sheath blight in rice. Phytopathologia Medicinaria 41, 109-119.

Fiddaman, P.J. and Rossall, S. 1993. The production of antifungal volatile by Bacillus subtilis. J. Appl. Bacteriol., 74: 119-126.

Basham et al., 2010. How the Plant Growth-Promoting Bacterium Azospirillum Promotes Plant Growth—A Critical Assessment. Advances in Agronomy, Volume 108.

Sclerotium

- *Azospirillum brasilense*
- *Bacillus subtilis*
- *Trichoderma harzianum*
- Dancing- Ergot/St. Anthony's Fire - 1518



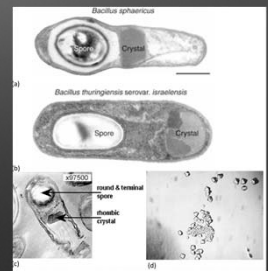
Moustafa E. Shalaby, Kamal E. Ghoniem, Mohamed A. El-Diethi Biological and fungicidal antagonism of *Sclerotium cepivorum* for controlling onion white rot disease December 2013, Volume 63, Issue 4, pp 1579-1589

Bashan El et al., 2010. How the Plant Growth-Promoting Bacterium *Azospirillum* Promotes Plant Growth—A Critical Assessment. *Advances in Agronomy*, Volume 106.

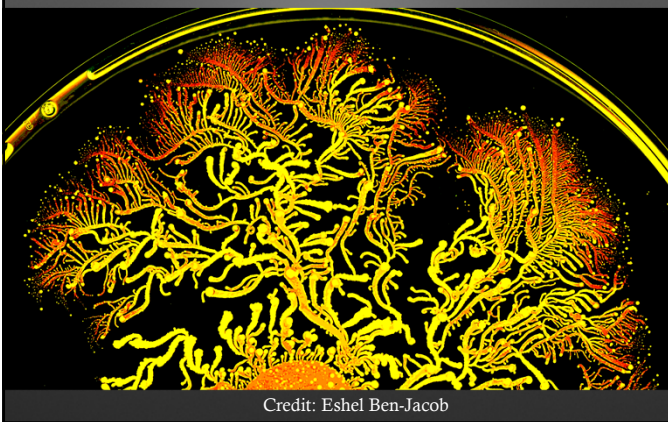
Elsal, Y., Chet, I., & Katan, J. (1980). *Trichoderma harzianum*: A biocontrol agent effective against *Sclerotium rolfii* and *Rhizoctonia solani*. *Journal of Phytopathology* 70, 119-121

And More...

- Fungicidal
- Bactericidal
- Nematicidal
- Insecticidal
- Antiviral
- Etc...
- Long story short, these beneficial organisms have a major impact on plant nutrition, growth and health



Examples of PGPR in the Field:



Credit: Eshel Ben-Jacob

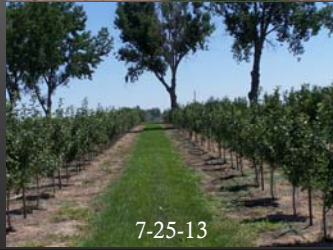
Established fescue side by side comparison shows results 3 weeks post application



Result is improved: soil/plant digestive system, plant stress management, nutrient retention, water retention, biodiversity, and the list goes on...

Stress Reduction

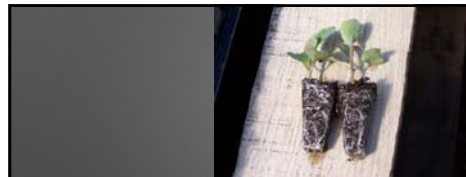
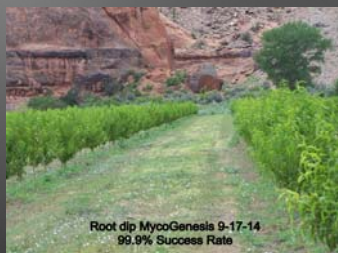
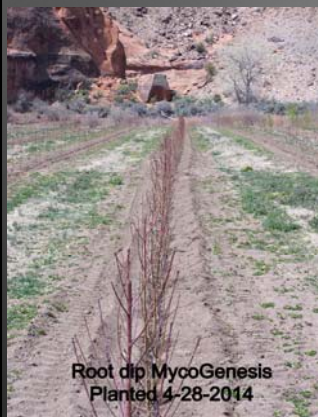
- 80 Degree day
- 30 mph wind
- Bare root trees dipped and laid on the ground next to hole for planting
- No water until that evening



Control



Treated

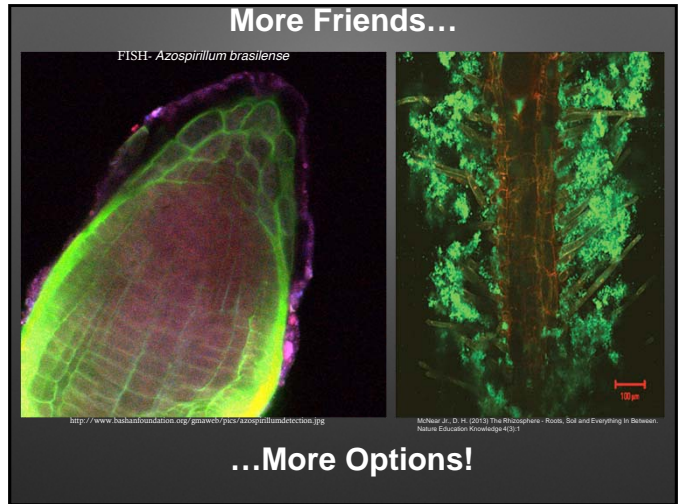


Treated:
~10 skips



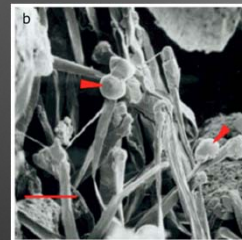
Control:
~50 skips





Lessons from an Efficient "Farmer"

- Earth worms:
 - Consume low to no protein leaves
 - Process in gizzard
 - Mix with mucilage
 - Regurgitate
 - ⊗ Have now created microbe "agar"
 - ⊗ Microbes grow on "agar"
 - ⊗ Microbes have a 5:1 C:N
 - ⊗ Worms have taken low to no protein food and created a protein food source for themselves!



b) Red arrows point to droplets of root exudates released from the tips of root hairs on the surface of broom corn (*Sorghum* sp.).



<http://www.habanfoundation.org/gmweb/picv/azospirillumdetection.jpg>

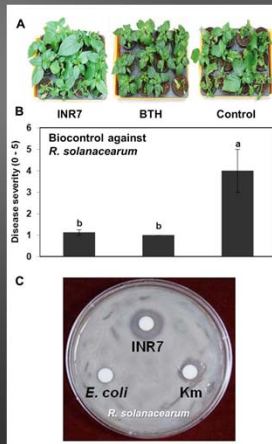
FISH- *Azospirillum brasilense*

Andrew L. Neal, Shakoor Ahmad, Ruth Gordon-Weeks, and Jurriean Ton. (2012). Benzoxazinoids in Root Exudates of Maize Attract *Pseudomonas putida* to the Rhizosphere. *PLoS ONE*: doi:10.1371/journal.pone.0035498

2012 Nature Education McGuffey, M. The rhizosphere: the key functional unit in plant/microbial interactions in the field: implications for the understanding of allelopathic effects. Division of Plant Industry, Forth World Congress on Allelopathy. The Regional Institute Ltd. All rights reserved.

Indirect: ISR

- "The term induced resistance is a generic term for the induced state of resistance in plants triggered by biological or chemical inducers, which protects non-exposed plant parts against future attack by pathogenic microbes and herbivorous insects." - Pieterse, Utrecht Univ.



<http://www.studierreviews.org/cpint/7gPVjkzmlT1epZXL1WRKZ/8/doi/10.1146/annurev-phyto-082712-102240>

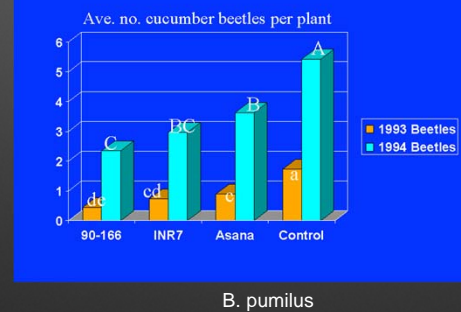
Ryu CM, Farag MA, Hu CH, Reddy MS, Wei HX, Para PW, Kloepper JW (2003) Bacterial Volatiles Promote Growth in Arabidopsis. *Proc Natl Acad Sci USA* 100:4927-4932

Ryu CM, Farag MA, Hu CH, Reddy MS, Kloepper JW, Para PW (2004a) Bacterial volatiles induce systemic resistance in Arabidopsis. *Plant Physiol* 134:1017-1026

Ryu CM, Murphy JF, Mysore KS, Kloepper JW (2004b) Plant growth-promoting rhizobacteria protect systemically Arabidopsis thaliana against cucumber mosaic virus by a salicylic acid and NPR1-independent and jasmonic acid dependent signaling pathway. *Plant J* 39:381-392

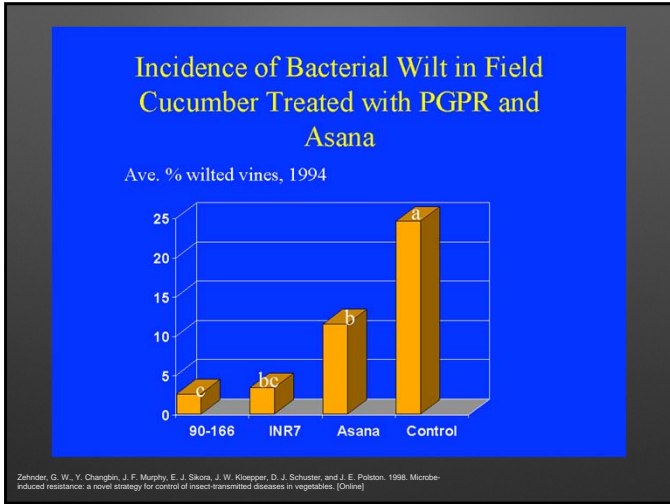
<http://journal.floriculture.org/doi/10.3389/fgs.2013.00122/full>

Control of Cucumber Beetles in Field Cucumber with PGPR and Asana



B. pumilus

Zehnder, G. W., Y. Changbin, J. F. Murphy, E. J. Sikora, J. W. Kloepper, D. J. Schuster, and J. E. Polston. 1998. Microbe-induced resistance: a novel strategy for control of insect-transmitted diseases in vegetables. [Online]



What about Microbes and Carbon?

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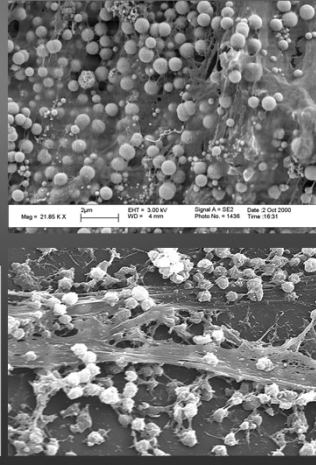
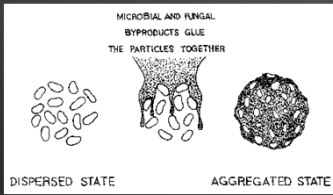
    graph TD
      L[Lignin] -- "attack by microorganisms" --> PA[Phenolic aldehydes and acids]
      L -- "Cellulose and other non-lignin substances" --> C[Cellulose and other non-lignin substances]
      C -- "utilization by microorganisms" --> P[Polyprenols]
      P -- "phenoloxides" --> Q[Quinones]
      Q -- "amino compounds" --> HA[Humic acids]
      Q -- "amino compounds" --> FA[Fulvic acids]
      PA -- "Further utilization by microorganisms and oxidation to CO2" --> CO2[CO2]
      HA -- "The polyphenol theory of humus formation (Stevenson 1982)" --> HA
      FA -- "The polyphenol theory of humus formation (Stevenson 1982)" --> FA
  
```

Residue processing

Soil Organic Matter Extraction Test		
LOW	MEDIUM	HIGH
1.0-1.5 %	2.0-2.5 %	3.0-3.5% *

Microbe "glue"

- Microbes produce a wide variety of materials that help bind soil together
- Aggregation = gas exchange and water storage
- Mucilaginous carbohydrates (EPS), biofilms mycelium, hyphae, etc... all help hold soils together

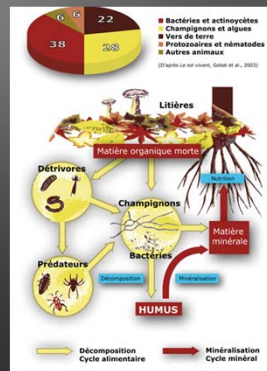


Glomalin



Organic Matter

- OM Levels:
 - CEC of 100 or more
 - Organic matter is important for the microbes as a source of carbon and nutrients
 - 4 or more: Can mean slow microbial breakdown or low microbial counts
 - 3-4: Often times Ideal levels
 - 2-3: Good Level
 - 0-2: Low



Carbon:Nitrogen

- C:N "microbial suitability" and sustainability
 - 30:1+ High levels of Carbon
 - Dr. Clapperton's research is suggesting high C:N can have long term support characteristics
 - 20:1 to 30:1 Excellent
 - 15:1 to 19:1 Some Carbon needed
 - 1:1 to 14:1 High quantities of Carbon needed
 - Depending upon form, remember that C is necessary for N storage
- Photosynthesis and exudates = long term building

REPORT NUMBER: **11-354-0066**
 DATE: Dec 29, 2011 12:34
 DECISION DATE: Dec 20, 2011

Midwest Laboratories Inc.
 13871 18th Street • Crystal Lake, IL 60154 • 815.302.3770 • FAX 815.302.3443
 WWW.MIDWESTLABS.COM

PAGE 1/1

CARBON NITROGEN RATIO REPORT

Sample Id	Total Carbon	Total Nitrogen	C/N Ratio
1 N	1.90 %	0.20 %	9.3

Drop Test



Graham Shepherd VSA Vol. III

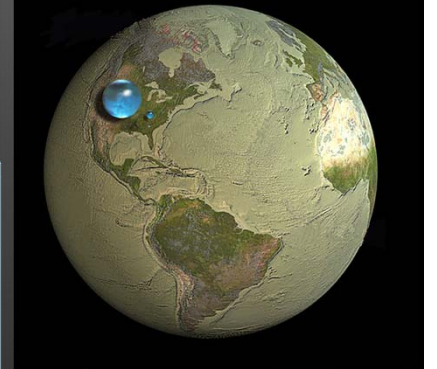
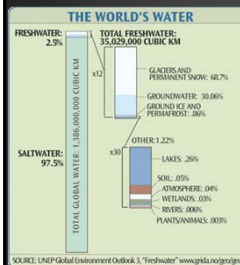
Porosity



Graham Shepherd VSA Vol. III

What's the big deal about Carbon?

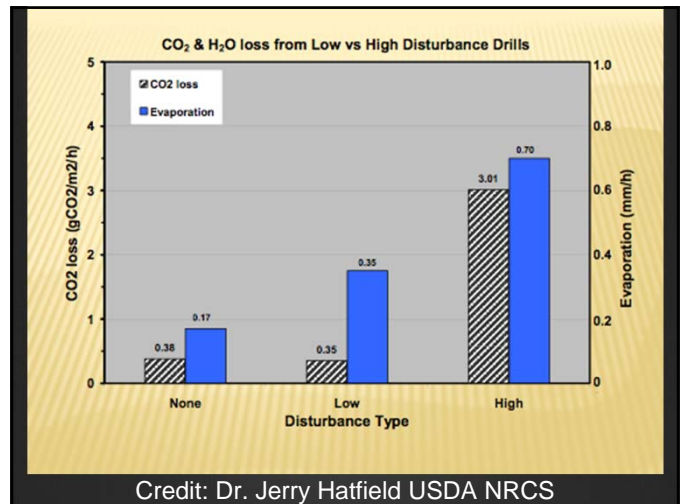
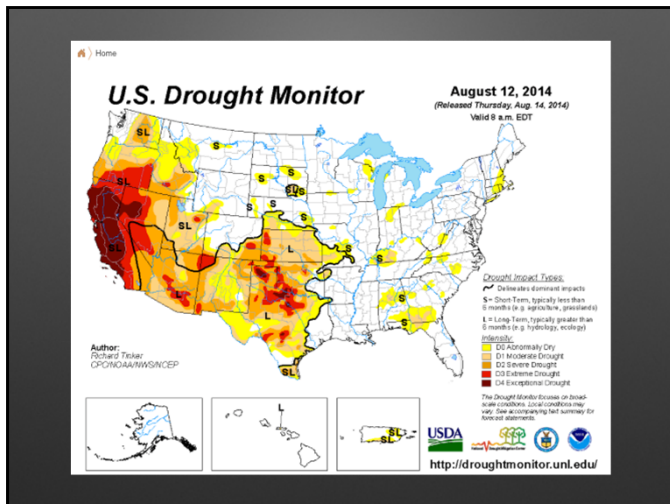
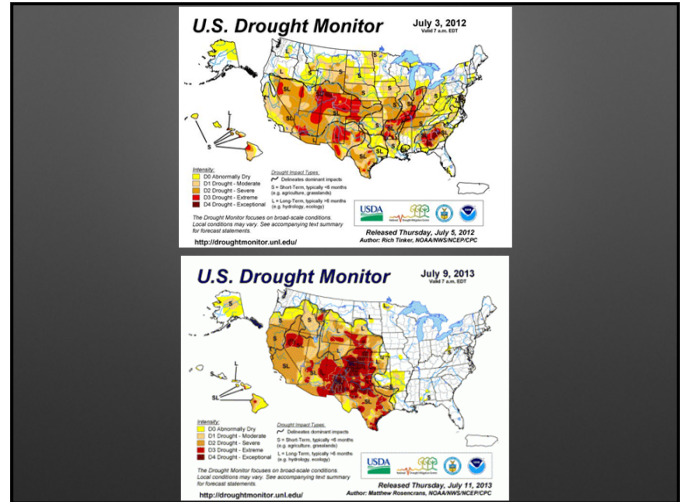
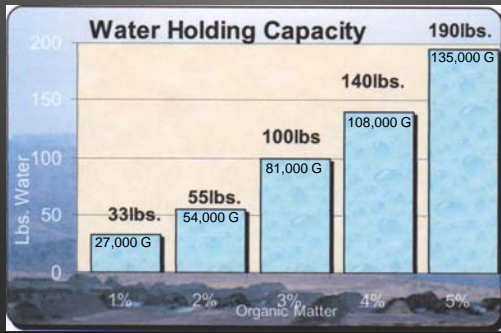
- All the water in the world (1.4087 billion cubic kilometers) including sea water, ice, lakes, rivers, ground water, and clouds
- 860 mile



NRCS:

Organic matter builds as tillage declines and plants and residue cover the soil. Organic matter holds **18-20 times** its weight in water and recycles nutrients for plants to use.

One percent of organic matter in the top six inches of soil would hold approximately **27,000 gallons** of water per acre!



Credit: Dr. Jerry Hatfield USDA NRCS

TEMPERATURE CHANGE BY DECADE

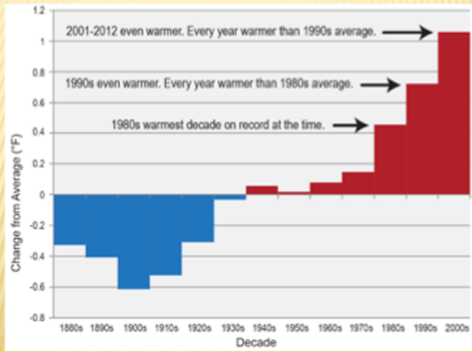
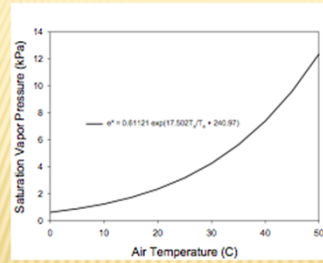


Figure source: NOAA NCEP

Credit: Dr. Jerry Hatfield USDA NRCS

TEMPERATURE EFFECTS ON EVAPORATION

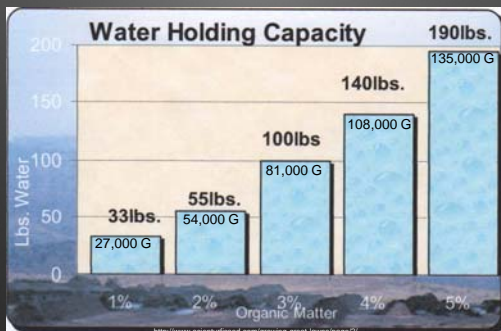


$$ET = \rho c_p (T_{i0} - T_s) / r_{la} + \rho c_l p [e_s(T_{i0}) - e_a] / \gamma (1 + r_{is} / r_{la}) r_{la}$$

Credit: Dr. Jerry Hatfield USDA NRCS

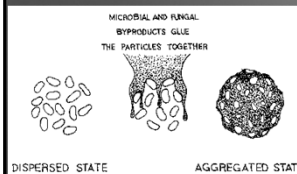
NRCS:

OM holds 18-20 times its weight in water
One percent OM = 27,000 gallons



<http://www.scientificsoil.com/growing-great-soils/page/2/>

Build Carbon or Wave Goodbye



Download from
Dreamstime.com

Tools to build Carbon:

"Passive protective blanket"

"Active protective blanket"

Credit: Dr. Jerry Hatfield USDA NRCS

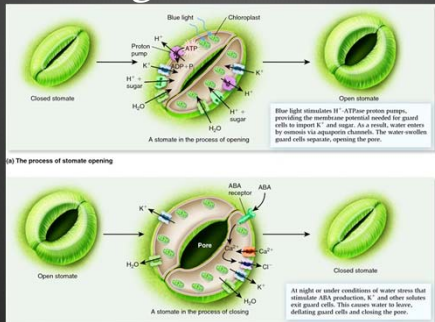
Carbon Fertilizer

IF YOU SOIL THIS VEHICLE A CHARGE WILL BE MADE

Microbes, Carbon, and Water

- Biological soils help both plant and soil during drought times:
 - Pore spaces
 - Aggregation
 - Humus/Soil Carbon
 - Hormones
 - Nutrient Osmotic Control

Ring the Alarm!



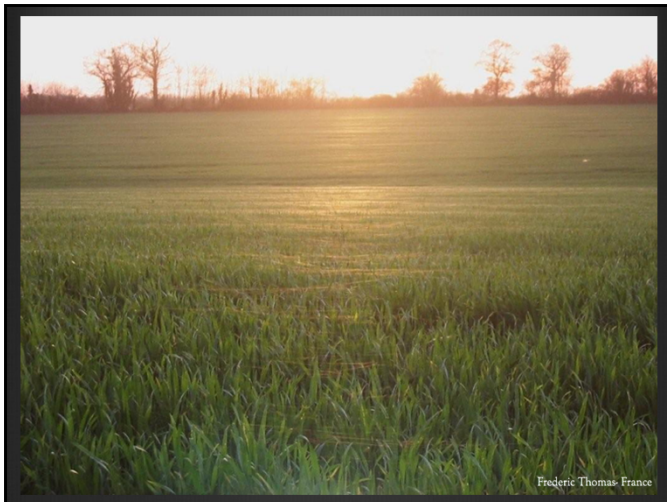
<http://biology-forums.com/index.php?action=gallery-as=view;id=1030>

- PGPR produce plant hormones that help control plant growth.
- During drought, the abscisic acid (ABA) is produced which translocates to leaves to regulate stomata.

How do we build Carbon and maintain moisture?



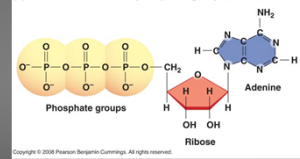
Short answer...
On Purpose



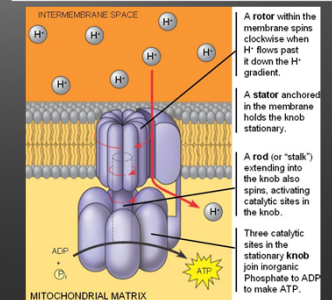
Mighty Mito

- Mitochondria turnover approximately 60 kg of ATP per day/healthy adult.
- 507.18 g/mol
- 6.022×10^{23} molecules or atoms per mole
- 118.3 moles of ATP turned over
- 7,124,026,000,000,000,000,000 (Sept.)

(a) ATP consists of three phosphate groups, ribose, and adenine.



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People are incredible, but...

- Pretty much everything humans can do, a creature in nature can do better.

EGO vs **NATURE**

#1 Bears have 2,100 times better sense of smell than humans!

HOW SENSITIVE ARE LIVING SYSTEMS?

Silent Mutations

ATG	GA	GCA	CGT
Met	Glu	Ala	Gly
ATG	GAC	GCA	CGT
Met	Glu	Ala	Gly

- Pain sensitivity is affected by a silent mutation as are 50+ other human disorders.
- bun—patty—cheese—pickle—mustard—ketchup—lettuce—bun
- Different than
- bun—patty—cheese—pickle—mustard—ketchup—lettuce—bun

Vibrio fischeri

- Marine Bacteria
- Symbiotic
 - Bobtail Squid
 - Bioluminescence

To Glow or not to Glow

- " Instead of language, bacteria use signalling molecules which are released into the environment." -Atkinson-
- Quorum sensing enables bacteria to co-ordinate their behavior.
- As environmental conditions often change rapidly, bacteria need to respond quickly in order to survive.
- These responses include adaptation to availability of nutrients, defense against other microorganisms which may compete for the same nutrients and the avoidance of toxic compounds potentially dangerous for the bacteria.
- Autoinducers
- Bonnie Bassler

Intra-Species Communication

Serratia liquefaciens
Vibrio harveyi
Vibrio fischeri
Agrobacterium tumefaciens
Pseudomonas aeruginosa

Multi-Lingual Bacteria

Intra-Species
Inter-Species
Signal 1
Signal 2
Group Behavior Genes

Erwinia carotovora

- Quorum sensing
- Waits until sufficient numbers are present and then... attack!
 - Emits virulence factor
 - Spits out enzymes to create wound/infection site
 - Simultaneously releases protective molecules to keep all other pathogens away



SNEAK ATTACK
You Never See It Coming
http://em-beats-images.s3.amazonaws.com/135205721_sneak_attack.jpg

Long Term

- Dr. James Collins - Boston Univ.
- "In studying the development of antibiotic-resistant strains of bacteria, the researchers found that the populations most adept at withstanding doses of antibiotics are those in which a few highly resistant isolates sacrifice their own well being to improve the group's overall chance of survival."
 - Indole — Signal Flare
 - Encourages biofilm and stationary phase
 - Hurts/costs individual
 - Over 85 species have been found
 - "Single shot" approaches may miss "sleepy" individuals
 - Biological approach



https://experimedi.com/wp-content/uploads/2011/11/8cp11_Bact1.jpg



<http://thewestchestview.com/files/2009/10/1camworkSuccessPicuk.jpg>

Lee J. H. & Lee J. (2009). Indole as an intercellular signal in microbial communities. *ESDM Microbiology Reviews* 74:61-64.
Boston University College of Engineering. "Chaotic behavior found in bacteria." *ScienceDaily*. www.sciencedaily.com/releases/2008/09/08091132107.htm (accessed January 28, 2015).
Henry J. Lee, Michael N. Mello, Charles E. Curtis, James J. Collins. Bacterial diversity weakens in population-wide resistance. *Nature*, 2010, 467 (7313): 82-84. DOI: 10.1038/nature09354