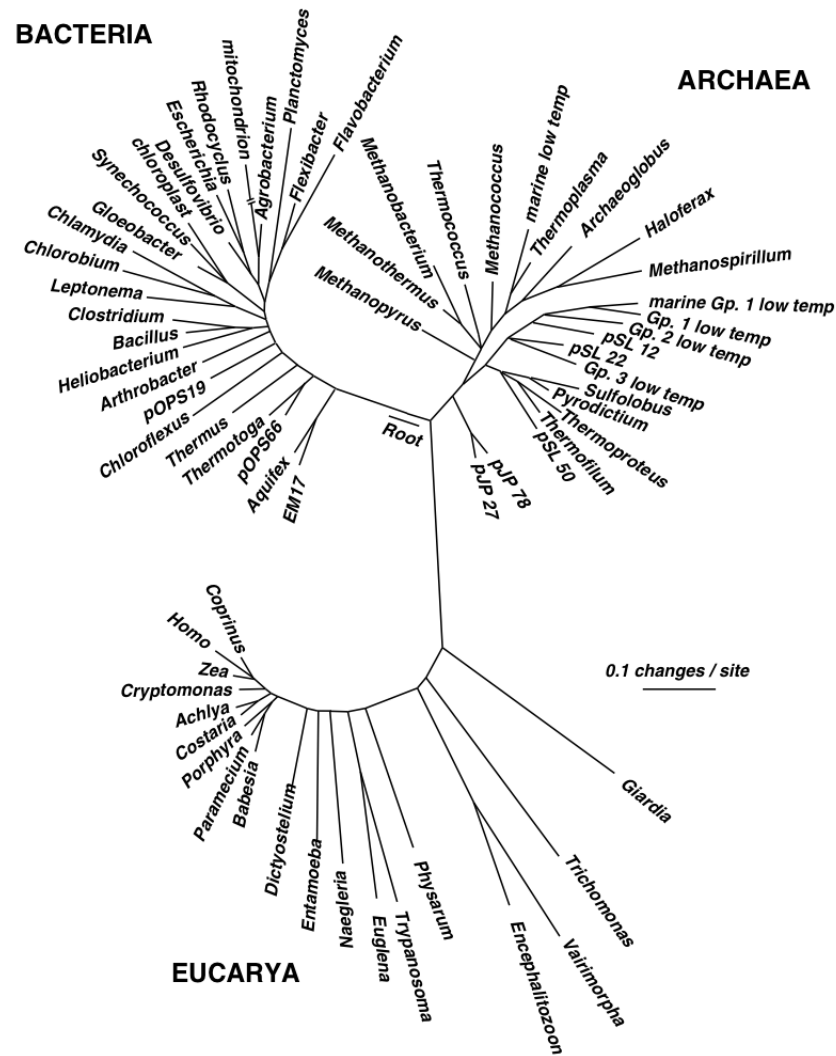


Challenges in developing microbes for industrial biotechnology

Rishi Jain, Praj Matrix, India

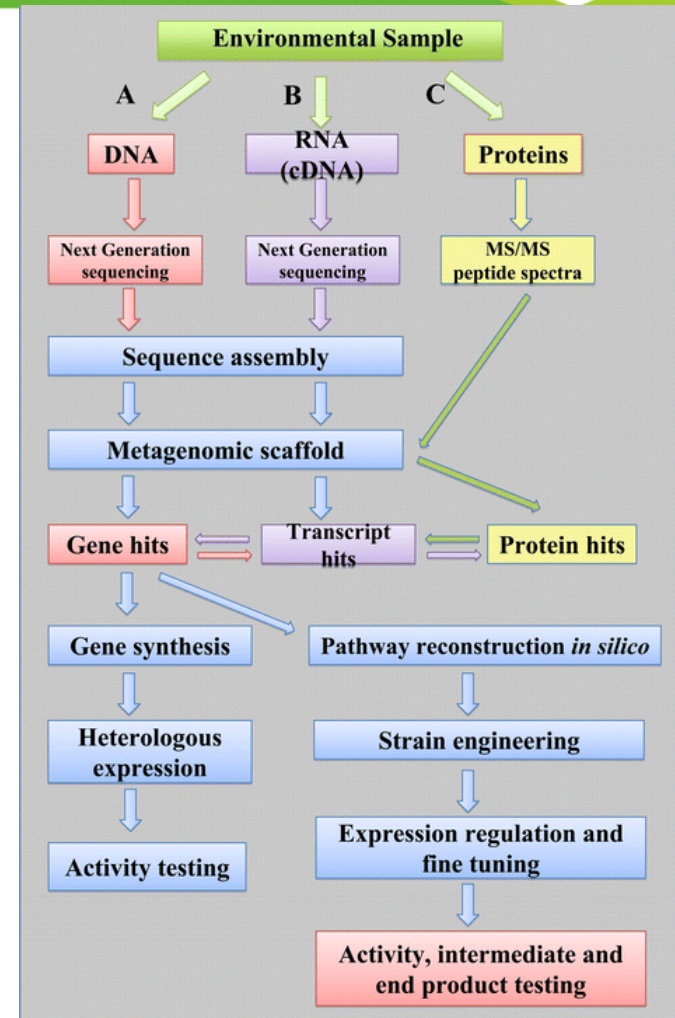
10-Oct-2012

Domains of life



http://pacelab.colorado.edu/images/Big_Tree_Bold_Letters_white.png

- Various environments
 - Thermal springs
 - Deep sea hydrothermal vents
 - Polar regions
 - Marine environments
 - Forests
 - Deserts
 - Digestive tracts of animals
- Techniques
 - Traditional cultivation
 - Metagenomics
 - Metatranscriptomics
 - Metaproteomics
 - Single cell genomics



**A) Metagenomics B) Metatranscriptomics
C) Metaproteomics**

Biotechnol Lett. 2010 Oct;32(10):1351-9

Contributors to Cost Stack

- Titrers, Yield, Productivity
- Carbon source
- Nutrient requirements
- Sterility
- Down stream processing
- Power
- Water and carbon footprints
- Energy and heat

- First generation sugars
 - Mainly C6 sugars
 - Sugarcane juice, Molasses, Beet, Cassava, Corn
- Second generation feedstocks
 - Agricultural residues, Forest biomass, Grasses
 - Polymers of C6 and C5 sugars
 - Microbial growth Inhibitors – acetic acid, furans and phenols
 - Strain development solutions
 - Bioprospecting for microbes that efficiently convert C6 and C5 sugars to product of interest
 - Targeted strain engineering approaches to introduce pathways that can efficiently utilize different types of sugars
 - Classical mutagenesis used for overcoming inhibitor tolerances
 - Reverse metabolic engineering
 - Consolidated bioprocessing

Nutrient requirements

Nutrients

- Nitrogen
- Phosphorus
- Sulfur
- Amino acids
- Vitamins
- Trace elements

Industrial sources

- Corn steep liquor
- Corn steep powder
- Cotton seed meal
- Dry brewer's yeast
- Distillery wastes
- Chicken Feather hydrolysate

- Strain development solutions
 - Targeted engineering approaches for elimination of certain auxotrophies
 - Adaptation to industrial sources to overcome inhibitor tolerances

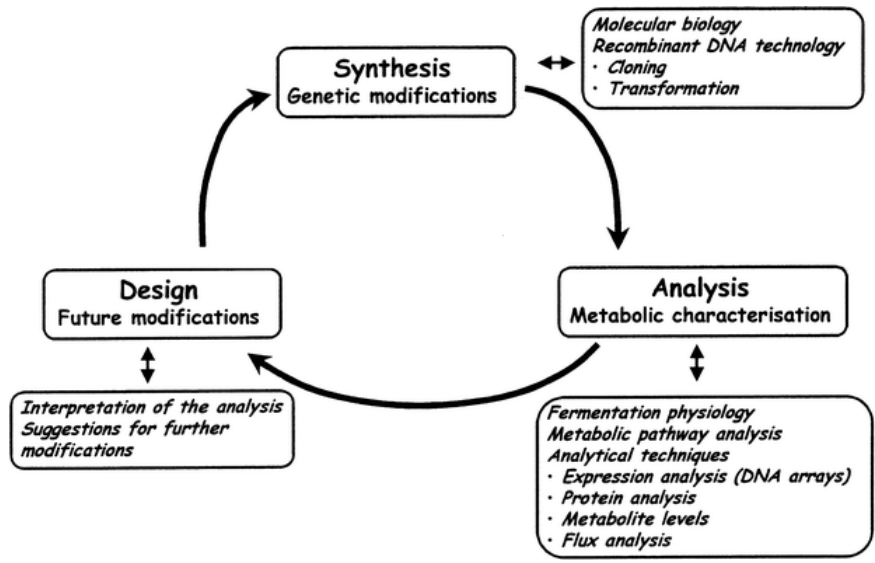
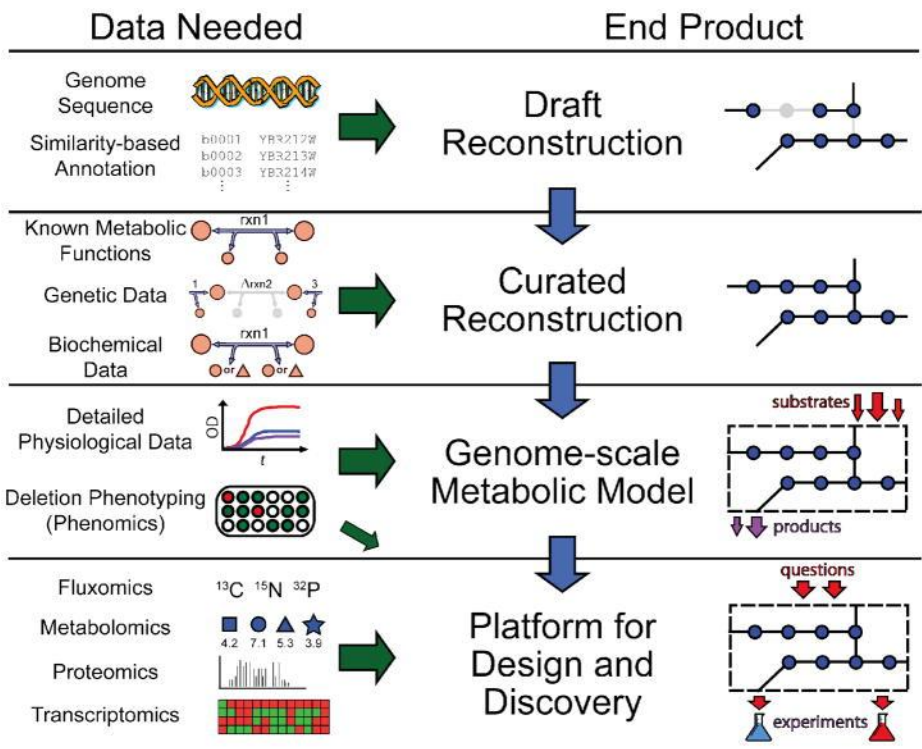
Downstream processing

- Extraction of product from the fermentation broth
 - Product titers
 - Intracellular / Extracellular
 - Byproduct profile and concentrations
- Strain development solutions
 - Targeted engineering approaches for increasing titers of the product
 - Mutagenesis and adaptation for increasing product tolerance
 - Reduction of byproducts by targeted engineering approaches
 - Targeted engineering approaches to expel intracellular products of interest out of the cell

- Highly engineered or mutated strain – growth rate decreases
- Competitive edge over other wild-type strains decreases
- In case of contamination, batch losses may occur

- Strain development solutions
 - Optimize the number of genetic changes
 - Co-culture fermentation
 - Synthetic biology for transitions between different physiological states

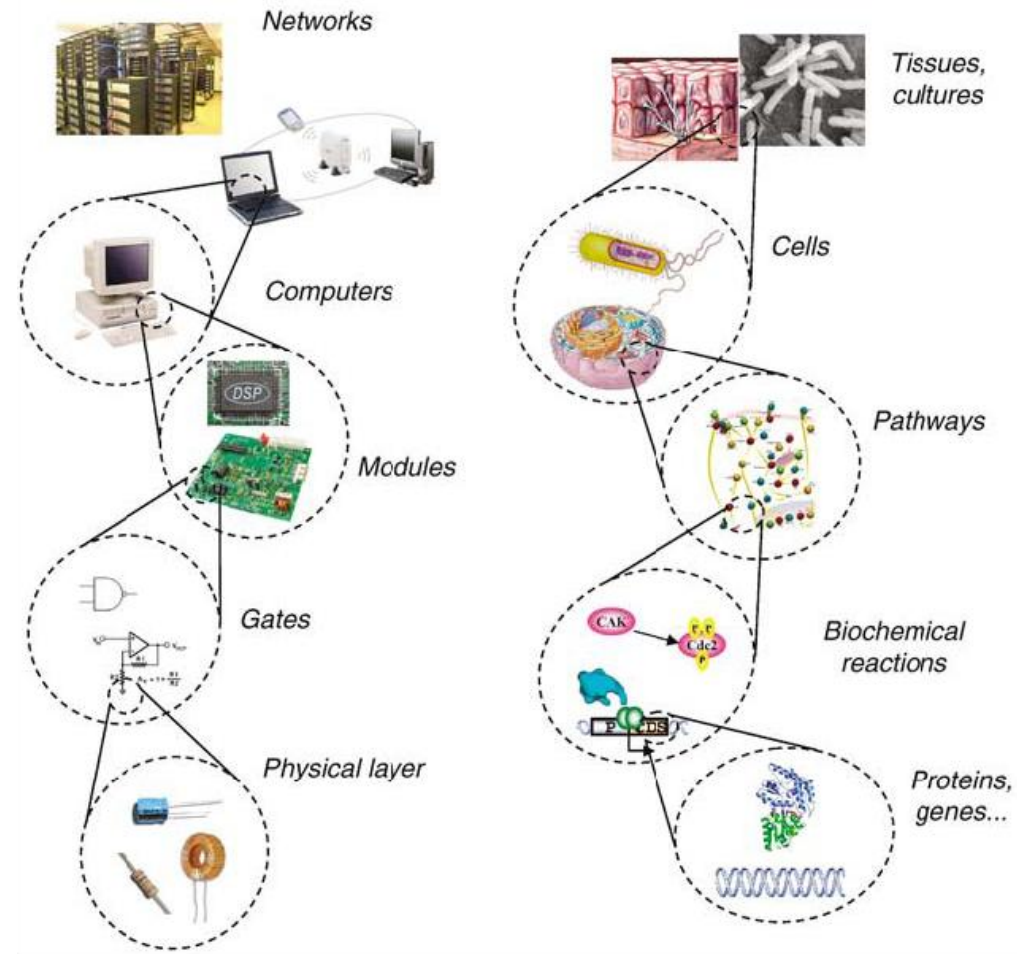
Metabolic engineering



Nat Rev Microbiol. 2009 Feb;7(2):129-43

Appl Microbiol Biotechnol. 2001 Apr;55(3):263-83

- Rational design, construction & characterization of biological systems
- Assembling of basic biological components into larger networks
- Creation of standardized biological parts, devices and systems
- These biological components are interchangeable, functionally discrete and capable of being easily combined in a modular fashion

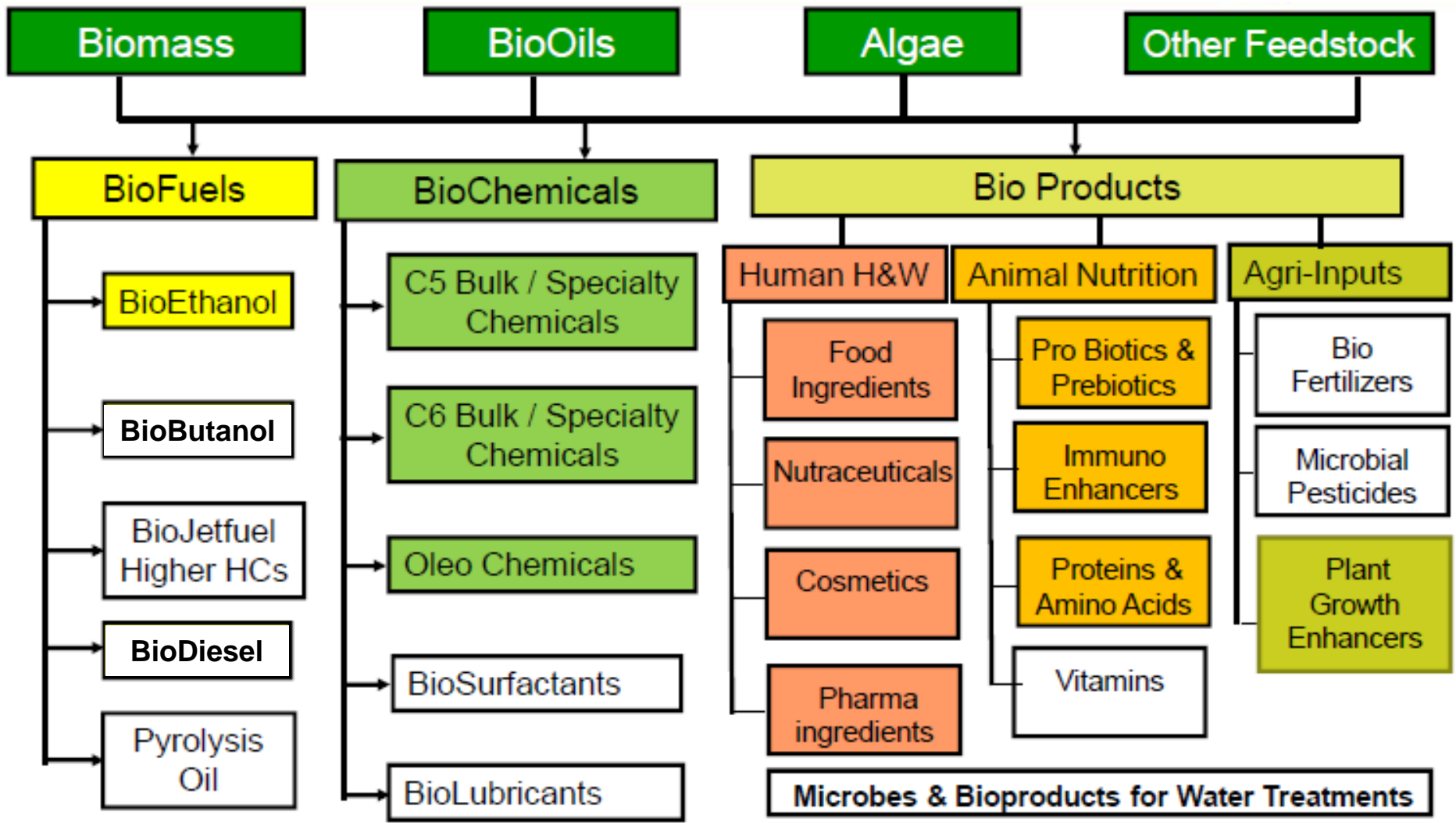


Andrianantoandro, E., *Mol. Sys. Biol.*, 2006 May

Classical methods

- Complex targets that are not well understood at the molecular level
 - Product tolerance
 - Growth inhibitor tolerance
 - Thermotolerance
- Random mutagenesis
 - Physical mutagens – UV rays, X-rays, etc
 - Chemical mutagens – Ethidium bromide, N-methyl-N'-nitro-N-nitrosoguanidine (NTG)
- Adaptation – Chemostat
- Hybrid approaches like site-directed mutagenesis and combinatorial mutagenesis
- Reverse metabolic engineering

- Millions of dollars
- Interdisciplinary requirements
- High-end instrumentation
- Collaboration
 - Industrial
 - Academic
- Government support
 - Funding
 - Collaborative platforms
 - Indo-Canadian
 - Indo-US
 - Indo-European



Unfilled Boxes Indicates areas under preliminary exploration

Thank you

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