Strain Development Examples

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Success for Strain Development

- Multiple challenges to overcome from a novel concept to a commercially viable production process

- BTR can assist you at every stage toward the success:
  - Help you in meeting particular needs at one or more stages
  - Help you in accelerating the entire process of your technology development

- Highlighted here are three examples of metabolic engineering for microbial strain development

- More examples are provided in PowerPoint slide show "Success Stories of Integrated Strain and Process Development"

Yeast Metabolic Engineering for Isoprenoid Production

- **Isoprenoids:** derived from 5-carbon molecule isopentenyl pyrophosphate (IPP)
- **IPP monomers are condensed to form isoprenoids of different lengths:** carotenoids, ubiquinones, steroids, precursors for vitamin synthesis, and pharmaceuticals (CoQ10)
- **BTR's focus:** production farnesol and geranylgeraniol
  - Generated Squalene Synthase mutants (*erg9*) using classical mutagenesis and screening
  - Over-expressed GGPP synthase and FPP synthase
  - Expressed de-regulated HMG CoA reductase
  - Amplified genes for the first three steps of isoprenoid pathway
  - Achieved high level production of various isoprenoids and gained valuable insights on regulation of the pathways
- For more details, please see PowerPoint slide show "Production of Farnesol and Geranylgeraniol by Strains of *Saccharomyces cerevisiae*"

Strain and Process Development for Glucosamine Production

- **BTR internal R&D program**: Develop *E. coli* strains and fermentation process for production of glucosamine
- **Program highlights:**
  - *E. coli* was metabolically engineered to increase production of glucosamine through gene knockouts and gene overexpression
  - Titers up to 18 g/L glucosamine were achieved through strain and fermentation process development
  - Further improvement was difficult:
    - Glucosamine is labile at neutral pH
    - Glucosamine and its degradation products are inhibitory
  - These challenges were overcome by a new strain development strategy:
    - Extending the pathway to NAG (*N*-acetylglucosamine)
    - NAG is a stable, and non-inhibitory derivative
    - NAG is easily hydrolyzed to glucosamine
  - The new strategy immediately resulted in a titer of 55 g/L NAG; further fermentation development resulted in titers >110 g/L within 60 hr
- For more details, please see PowerPoint slide show "*E. coli* Metabolic Engineering for Glucosamine and *N*-Acetylglucosamine Production"

Exploring a Catabolic Enzyme to Construct a Biosynthesis Pathway

- High level *N*-acetylglucosamine production achieved previously in *E. coli* through overexpression of two biosynthesis enzymes, GlmS and GNA1:
  - GlmS (glucosamine synthase) converts fructose-6-P to glucosamine-6-P using glutamine as the amino donor
  - Yeast GNA1 (glucosamine-6-P acetyltransferase) transforms glucosamine-6-P to *N*-acetylglucosamine-6-P
- A novel pathway engineered by combination of overexpressed NagB and GNA1:
  - Glucosamine-6-P deaminase (NagB), a catabolic enzyme, breaks down glucosamine-6-P into fructose-6-P and ammonia. Its reversal reaction is kinetically unfavorable
  - GNA1 quickly removes glucosamine-6-P, driving a efficient metabolic flux through NagB, leading to high level production of *N*-acetylglucosamine
- A kinetically unfavorable catabolic enzyme can be used in constructing a desired metabolic pathway by coupling with an efficient downstream enzyme
- For details, see PowerPoint slideshow "Engineering a Novel Pathway for *N*-Acetylglucosamine Synthesis in *E. coli*"