Large Scale Fermentation Manufacturing Services for the Biocontrol Industry

Thomas Riedel, Global Marketing, Business Unit (BU) Agro Ingredients
October, 20th, 2015, ABIM Basel, Switzerland
Lonza Overview

- Trusted supplier to the pharmaceutical, biotech and specialty ingredients markets
- Founded in 1897 with headquarters in Basel, Switzerland
- Sales of CHF 3.64 billion in 2014
- Global operations:
  - Located in more than 40 major sites
  - Employs approximately 9,800 people

- Our service and product range addresses large number of markets:
  - Pharma and Biotech Markets
  - Water Treatment
  - Consumer Care
  - Wood Protection
  - Industrial solutions
  - Agro Ingredients
BU Agro Ingredients Service and Product Offerings

- Advanced Chemical Manufacturing in Visp, CH
  - Exclusive manufacturing of intermediates and active ingredients
  - Supply of non-exclusive key building blocks

- Global Molluscicide Business
  - Metaldehyde as active ingredient
  - Own formulated products

- Regional Specialties in South America

“Science and Technology for securing food in a growing world”
Agro Formulation Ingredients

**Activator Adjuvants**
Increase Effectiveness
Reduce Active Level

**Compatibility Adjuvants**
Improve Compatibility with Electrolytes, Cationics, and Acidic Actives

**Preservatives**
Maintain Integrity of Formula
Increase Shelf Life

**Naturally Based Surfactants**
Sustainability
Environmentally Friendlier

**Specialties for Seed Treatment**
Antifreeze, Emulsifier, Flowability Enhancer, Binder, Stabilizer, Preservatives

Lonza Agro Ingredient Products
Designed for Crop Protection Formulations
Proxel® – Wet State Preservation

- Lonza’s cost effective preservative based on well-established active ingredient 1,2-Benzisothiazolin
  - Broad spectrum of activity
  - Stable over wide pH range
  - Excellent thermal stability (157°C)
  - Long term effect
  - Strong toxicology package & broad regulatory approvals

- In combination with excellent consultancy and service support to guarantee best application results in your products

- Broad application today in many final formulations
  - Bacillus Thuringensis species
  - Rhizobium based Inoculants
Biotechnology @ Lonza

Fermentation scale-up and manufacturing in Kouřim, Czech Republic

~ 200 people in all different functions at Kouřim and Visp

Fermentation development and optimization in Visp, Switzerland
From Feasibility to Manufacturing

- A one-stop-shop at every stage of your project along the value-chain
- Full life cycle management from product launch to maturity
- Continuous process optimization from the beginning

- Classical Mutation
- Metabolic Engineering
- Diverse & Advanced Microbial Expression Toolbox
- Biocatalytic toolbox

- Scale-down Studies Optimization
- Fermentation & Media Optimization
- Integration into a Multi-Steps Chemical Process

- DSP Development & Optimization
- Bioprocess Integration
- Formulation Development

- Pilot Validation Process
- Scale-up Criteria Definition for Industrial Tech Transfer
- Equipment Selection for best fit to Manufacturing

- Process Tech Transfer
- Engineering Batch
- Support Regulatory Registration
- Commercial Production
Optimization of Fermentation Processes

- Dedicated team of R&D, QC, and production with support from any other organization unit required, e.g. engineering, expert teams, sourcing, …
- Detailed production process analysis as starting point
- Definition of optimization plan in close cooperation with our customers
  - to account for any registration impact
  - to consider impact on stability/formulation requirements
- 1:1 implementation in production scale, otherwise full lab/pilot support available (up to 75 l on lab-scale, 1.5 m³ on pilot-scale)
- Achieving the most reliable and economical solution
State-of-the-Art Manufacturing Assets in Kouřim

- 80’000 m² site including infrastructure
- 5 individually operated lines for commercial scale production with total capacity of 475 m³
  - 2 x 15 m³ (Bio Safety Level 2)
  - 3 x 15 m³
  - 2 x 50 m³
  - 3 x 50 m³
  - 2 x 75 m³
- Ex-Proof DSP facilities for solvent handling
- On-site waste water treatment plant
- 3’400 m² warehousing, storage conditions under ambient, 2 to 8 °C, and -20°C
State-of-the-Art Manufacturing Assets in Kouřim

Associated downstream process equipment
- Storage tanks
- Electrodialysis
- Frewitt mill
- Chromatography columns
- Crystallizer
- Centrifuges
- Vacuum dryer
- Spray dryer
- Liophilization
- Chromatography columns
- Filtration units (depth, ultra, nano, micro)
- Evaporator
- Homogenizer
- Filling lines

QC and Microbiology lab supporting production
- HPLC / UPLC / GC
- Spectroscopy (UV, IR, NIR)
- Titration (Karl-Fischer, …)
- ELISA
- Bioprofile – IPC for fermentation
- SDS-Page
- Enzyme activity assays
- Testing of microbial contaminants
- Particle size distribution
Fermentation Processes and Microorganisms

**Bacteria**

- Bacillus (lentus, subtilis) (GMO)
- Gluconobacter
- Rhizobium
- Pseudomonas
- Streptomyces sp.
- Burkholderia sp
- Acetobacter sp. (mutated)
- Nonomuracea sp
- Heamophillus sp. (BSL 2)
- E. Coli (K12, CMG 2576, … (GMO)

**Fungi**

- Aspergillus sp.
- Penicilium sp.
- Trichoderma
- Phanerochaete
- Chrysoporium
- Phichia sp. (GMO)
- S. cerevisiae

**Microalgea**

- Ulkenia (mutated)

More than 40 processes transferred to industrial scale within last 10 years
Examples of Biotechnology @ Lonza
Strain Improvement / Fermentation Development
Sec. Metabolite Production with Actinomycete

Strain Improvement

1. Classical mutation (non GMO)
   - Rational & random mutagenesis
   - Genome shuffling (protoplast fusion)

2. HTP mutant screening
   - Identification of improved mutants in 48 or 96 microwell plate scale

3. Improved strain testing @ bioreactor scale
   - Selection of potential strain candidates

Process Optimization

Titer improvement ~ 75 times within 2 years
Process Technology Transfer
Scale-up of an *E. coli* fermentation

Bioreactors at Different Scales

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Reactor volume</th>
<th>1L</th>
<th>20L</th>
<th>1000L</th>
<th>15 000L [1]</th>
<th>15 000L [2]</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stirrer speed</td>
<td>[rpm]</td>
<td>1200</td>
<td>1100</td>
<td>275</td>
<td>145</td>
<td>145</td>
</tr>
<tr>
<td>Liquid volume</td>
<td>[L]</td>
<td>0.5</td>
<td>15</td>
<td>800</td>
<td>10 000</td>
<td>10 000</td>
</tr>
<tr>
<td>Gas flow</td>
<td>[L/min]</td>
<td>0.5</td>
<td>12</td>
<td>480</td>
<td>8000</td>
<td>8000</td>
</tr>
<tr>
<td>$P_{\text{g}}, 1, 2, 3 , / , V$</td>
<td>[W/m$^2$]</td>
<td>7310, 12 190, 14 620</td>
<td>3 790, 6 320, 7 580</td>
<td>2 710, 4 520, 5 420</td>
<td>1 910, 2 420, 5 150</td>
<td>1 010, 1 680, 2 020</td>
</tr>
<tr>
<td>$K_{\text{L}a}, 1, 2, 3$</td>
<td>[1/s]</td>
<td>0.27, 0.38, 0.44</td>
<td>0.23, 0.33, 0.38</td>
<td>0.22, 0.31, 0.35</td>
<td>0.25, 0.36, 0.41</td>
<td>0.13, 0.19, 0.21</td>
</tr>
<tr>
<td>Mixing time</td>
<td>[s]</td>
<td>1.4</td>
<td>3</td>
<td>7</td>
<td>12</td>
<td>23</td>
</tr>
</tbody>
</table>
Process Technology Transfer
Scale-up of an *E. coli* fermentation

Project 1

Protein 1 (P1) [mg/L] (0-100)

- Scale: 1 L
- Scale: 20 L
- Scale: 1000 L

Batch

0 2 4 6 8 10 12 14

Project 2

Protein 2 (P2) [mg/L] (0-100)

- Scale: 20 L
- Scale: 1000 L

Batch

0 5 10 15 20 25 30 35

Project 3

Protein 3 (P3) [mg/L] (0-140)

- Scale: 20 L
- Scale: 1000 L

Batch

0 2 4 6 8 10 12 14

Project 4

Protein 4 (P4) [mg/L] (0-70)

- Scale: 20 L
- Scale: 15000 L

Batch

0 2 4 6 8 10 12 14

Project 5

Protein 5 (P5) [mg/L] (0-50)

- Scale: 20 L
- Scale: 15000 L

Batch

0 5 10 15 20 25 30 35

J. Wenger, B. Sommer, S. Núñez, H. Engelking, T. Bartek, M. Funke, M. Eiting, T. Schmidt, Lonza Ltd, Visp, Switzerland
Manufacturing Optimization
Focus: Yield and Fermentation Time

- Detailed process analysis with focus on
  - Yield generation and yield loss
  - Fermentation time
  - Energy consumption

- Defined optimization plan
  - Increase biomass yield in seed step
  - Optimize fermentation media
  - Modification of feeding profile

- Stepwise and direct implementation on large scale fermenter
Manufacturing Optimization
Focus: Reduction of Cycle Time

- Detailed process throughput analysis and identification of bottle-necks
- Defined optimization plan
  - Process adaptation from centrifuge to membrane filtration
  - Optimize fermentation media preparation procedure
  - Re-arrangement of number of fermenters and equipment scale
- Stepwise and direct implementation on large scale

More than 50% reduction in cycle time achieved within an optimization project of 6 months
Lonza’s Polypass™ - From Shake-Flask to Formulated Product within One Year

- **Starting point:** A cryo vial and a patent application
  - *Lactobacillus reuteri* (probiotic strain) has the ability to co-aggregate with the pathogenic *Helicobacter pylori* (causes gastritis and gastric ulcer) under physiological conditions (stomach)
  - Translate Market needs into defined product applications and appropriate product formulation

- **Process development at lab scale, Lonza LSI R&T Visp, Switzerland**
  - Proof of concept and feasibility study for large scale production

- **Scale-up with pilot trials at Lonza Kourim, Czech Republic**
  - Proof of technical feasibility and demonstration at pilot scale
    - Optimized fermentation conditions
    - Carrier screening to avoid auto-aggregation in formulated product
    - Spray drying optimization
  - Providing final product samples (registration and application test)

- **Commercial production and market launch**
  - Implementation at large scale production
  - Confirmation of defined requirements (technical, regulatory, customer specs)
  - Appropriate packaging, storage and logistics / technical support for customers

[L. reuteri (Pylopass™) = blue
H. pylori = red
SEM magnification = 13'000 x]
**Exclusive Biotechnological Manufacturing**

- *Pasteuria nishizawae* is a naturally, via fermentation derived nematicide against the Soybean Cyst Nematode (SCN)

- US market launch by Syngenta in summer 2013

- Successful execution of technology transfer and production of this biopesticide at Lonza’s Kouřim site (CZ) since 2012

- Continuous process improvement on-going in joint-cooperation with our customer Syngenta
Lonza’s Large Scale Fermentation Manufacturing Service

- Final fermentation and down stream process development starting with any initial lab process package by our customer

- Technology transfer into Lonza’s assets based on any development stage
  - Customer lab process
  - Customer pilot trials
  - Large scale experience

- Proposal for potential process adjustments (process wise, and technology wise) to further improve economically attractiveness

- Full life cycle management from the initial market launch volumes until large volumes at maturity

- Thorough, continuous process improvement in close cooperation with customer
Why Outsource with Lonza

- Proven track record in the agricultural industry as reliable and trustworthy partner, and experience from more than 30 years of commercial fermentation
- Strong expertise in regulatory requirements for the agricultural industry
- Excellent know-how in prevention of cross contamination
- Avoid large investments in your own capacity
- Speed to market, and high flexibility in your order volumes
- Full guarantee of your know-how and IP
- Full access to Lonza’s process optimization results for your own manufacturing

Security of Your Supply Chain
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- Our service and product range addresses large number of markets:
  - Chemical and biological active pharmaceutical ingredients
  - Stem-cell therapies
  - Pool treatment chemicals & drinking water sanitizers
  - Cosmetic & nutritional ingredients
  - Agrochemical products
  - High-performance materials
  - Microbial control solutions
  - Wood preservatives
Accounting for the global megatrends and leveraging Lonza’s broad technology base and our long-year track record in the agricultural industry, a new **BU Agro Ingredients** was founded in 2013.

“Science and Technology for securing food in a growing world”
Advanced Chemical Manufacturing

- Exclusive manufacturing of advanced intermediates and active ingredients
  - Combined with development, scale-up and optimization services
- Supply of non-exclusive key building blocks

Backward integrated site in Visp, Switzerland, with complex multi-purpose plants, broad technology portfolio, and integrated waste management facilities
Continuous expansion of Hastelloy capacity to account for F-chemistry

Inside Lonza multi purpose plant

16 m³ Hastelloy batch reactor, up to 16 bar,

Inside multi-product Hastelloy divided wall column for highest purity products

Coupling station for connection to glass line, and stainless steel line

Unloading station

Halar centrifuge for solid separation
Start-up of continuous nitrosation plant in 2014 to manufacture Butyl nitrite and other nitrites
N-Fertilizer Production in Visp

Sales of N-based fertilizer in Switzerland via Agroline
Molluscicide - Global Market Player for 60 Years

Market leader for supply of Meta® metaldehyde as active ingredient

Global launch of Lonza’s formulated products

Manufacturing plant for Lonza Slug Pellets in Visp

Meta® metaldehyde and its derived product offer fit into the IPM strategy and offer an efficient control to hobby gardeners professionals, and farmers, e.g. in rice treatment against Golden Apple Snail.
**Frexus® - Application Brand**

Pre- and post-harvest treatment
- Coffee beans
- Fruit & vegetables

Adjuvants for tank mix
- pH-reducer
- Surfactant

Biocides for fighting plant diseases

![Image of Frexus product with corn field]

White Spot on Corn Leaves

*Improvements through application of Frexus® pH Agro*

*Frexus® product line is not registered in USA*
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What drives increasing interest in biologicals

CAGR > 15%

Global Biologicals Market Value


- Biological demand fails to materialize
- Influx of startup companies
- "Big 6" begin acquiring access to biologicals
- Co's increase biologicals research
- Co's closed, downsized or diversified...biologics were relegated to niche markets

- Market Value Trend
- 1998 ~$2B
- 2014 $4-8B

- Global Bio Pesticides Market
- BASF
- Syngenta
- Becker Underwood
- AgraQuest
- Bayer CropScience
- Certis USA
- Koppert
- Marrone Bio Innovations

Lonza
## What drives increasing interest in biologicals

<table>
<thead>
<tr>
<th>Public perception</th>
<th>Resistance management</th>
</tr>
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<td>Reduced chemical residues</td>
<td>Performance</td>
</tr>
<tr>
<td>Sustainability</td>
<td>Less high development costs</td>
</tr>
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<td>Worker safety / Short re-entry</td>
<td>Fast growing segment</td>
</tr>
<tr>
<td>Registration advantage</td>
<td>Short-Cut for chemical pesticides</td>
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</table>
# What drives increasing interest in biologicals

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<th>Public Perception</th>
<th>People believe that bio-based products are of advantage in terms of health, wellness and sustainability if compared to chemical-based ones</th>
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<tr>
<td>Reduced Chemical Residues</td>
<td>Retailers demand lower residue levels than regulatory requires, setting secondary standards for marketing purposes - MRL; SYSCO, Wal-mart and other food companies develop sustainable farming requirements; Strong impact e.g. on coffee bean, and tea farmers</td>
</tr>
<tr>
<td>Sustainability</td>
<td>Natural fit, reduction of environmental impact and optimisation of natural resource efficiency; few, if any, adverse impacts on non-target organisms, like pollinators. Companies and Politics demand sustainable agriculture (McDonald, Unilever, EU's Sustainable Use Directive ...)</td>
</tr>
<tr>
<td>Worker safety / Short re-entry</td>
<td>Very short worker re-entry periods, allowing greater flexibility. Zero day pre-harvest interval, crops can be harvested on the same day.</td>
</tr>
<tr>
<td>Registration Advantages</td>
<td>Tightened registration for chemicals, potential for fast registration of bio-based products. Limited human and environmental safety data required, special governmental biopesticide initiatives, e.g. programs to accelerate biologicals in US, Brazil, India, others</td>
</tr>
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## What drives increasing interest in biologicals

<table>
<thead>
<tr>
<th>Resistance Management</th>
<th>Alternative mode of actions to control pests, diseases and to overcome resistance build-up. Fit in where only few/no chemicals exist. Use in Integrated Pest Management, and Resistance Management</th>
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<tr>
<td>Performance</td>
<td>New solutions for growers/farmers. No general replacement of chemicals, but in combination with chemicals for high performance programs.</td>
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<tr>
<td>Less High Development Costs</td>
<td>Total costs for development of biopesticides are much lower (5-10 Mio US$, 3-4 years time-to-market) compared to chemical pesticides (~250 Mio US$, 10 years time-to-market)</td>
</tr>
<tr>
<td>Fast Growing Segment</td>
<td>Bio-based products are one of the fastest growing segments with CAGR of 10-16%, however, still having a share of &lt;&lt; 10% on the over-all pesticide market</td>
</tr>
<tr>
<td>Short cut for Chemical Pesticides</td>
<td>Biologically derived intermediates, and biotransformation catalysts can help to replace complex chemical route to same end-molecule. Large market for biologically derived products like Spinosad, which are no biopesticides, but experience certain registration advantages.</td>
</tr>
</tbody>
</table>
Biopesticides against nematodes in seed treatment of large crops (soy-bean, maize, …) as most fast growing segment.

Most important growing fungi is Trichoderma e.g. for control of seeding diseases.

Source: CPL 2013
Recent Acquisitions

- Syngenta
  - $523 million
- BASF
  - $123 million
  - $1 billion
- Bayer CropScience
  - $425+ million
- novozymes
- TJ Technologies
- Natural Industries
- FMC
  - JV
  - Center for Agricultural and Environmental Biosolutions
- Chr. Hansen
- Monsanto
  - Acquires Multiple RNAi Providers
- Gowan
  - ... becomes exclusive distributor of Polyversum biofungicide
- American Vanguard Corporation
  - TyraTech
  - American Vanguard Invests in TyraTech Natural Product Technologies
Market summary

- Governmental initiatives, and food companies are pushing farmers to apply biological solutions (chemical pesticide free solutions)

- Strong reaction of all big Ag companies via recent and still on-going acquisitions and partnerships with innovative biocontrol companies

- Still niche character, < 5% share within global plant protection market
  - 2-3 bil US$ market for fermentation derived products

- Over-proportional high growth rates with CAGR > 15%

- Further strong push expected through step-in of big Ag companies

- Increasing demand for fermentation capacity
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- Full life cycle management from product launch to maturity
- Customer focused project set-up and execution
R&D Team Visp

- 30 scientists, strong support from chemistry and process engineering team
- Broad and well-established external network
- Focus on initial process development and support of technology transfer to Kouřim
R&D Team Visp – Lab Equipment

Microbiology equipment
- shakers, thermostats, laminar flow
- MTP incubators

Fermentation equipment
- Parallel bioreactor systems (Dasgip)
- Several 3.5L, 20L, 30L, 75L scale bioreactors

Analytical equipment
- HPLC, GC, UV-VIS spectrophotometer, ELISA, centrifuges
- GC-FID, HPLC, UPLC,
- Ion Chromatography
- Glucose Analyzer
- Spectrophotometers
- Microscope

Downstream equipment
- Laboratory MF / UF / NF units, evaporators, crystallizers
- Lyophilizer, spray dryer, vacuum dryer
- Electro dialysis, preparative chromatography
- continuous centrifuge, homogenizer
Manufacturing Service and Technology (MSAT) Team in Kouřim

Dedicated team of 20 scientist ensuring full production life-cycle management

- Equipment available
  - 10 x 20L, 2 x 75L lab scale fermenters
  - 1.5 m³ pilot fermenter
  - Associated downstream processing to mirror original customer processes at lab scale and to propose alternative options

- Existing collaborations with universities and external R&D institutes (equipment and services, e.g. special analytics)

- Focused on:
  - Process take-over from customer and from Visp R&D
  - Technology Transfer into production assets
  - Continuous process support and optimization
MSAT Kouřim
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  - Crystallizer
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  - Vacuum dryer
  - **Filtration units**
    - (depth, ultra, nano, micro)
  - Spray dryer
  - Liophilization
  - Evaporator
  - Homogenizer
  - Filling lines

- **QC and Microbiology lab supporting production**
  - HPLC / UPLC / GC
  - Spectroscopy (UV, IR, NIR)
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- E. Coli (K12, CMG 2576, … (GMO)

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- Phanerochaete
- Chrysoporum
- Phichia sp. (GMO)
- S. cerevisiae

Microalgea
- Ulkenia (mutated)

More than 40 processes transferred to industrial scale within last 10 years
State-of-the-Art Manufacturing Assets in Kouřim

- Fermenter Line
- Down Stream Process Plant
- Ultra Filtration Unit
- Centrifugation Unit
- Spray Dryer (Upper part)
- Control room
Examples of Biotechnology @ Lonza
Focus on Strain and Fermentation Improvement at the Same Time

Strain Improvement

1. Classical mutation (non GMO)
   - Rational & random mutagenesis
   - Genome shuffling (protoplast fusion)

2. HTP mutant screening
   - Identification of improved mutants in 48 or 96 microwell plate scale

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Process Optimization

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Scale-up of an *E. coli* fermentation for a dietary ingredient production in 30L scale

Fermentation Verification and Scale-up runs
- Validation runs at 1L and 30L scale to test the process robustness
- Material production for primary recovery optimization and validation runs
Scale-up of an *E. coli* fermentation

Different scales of bioreactors at Lonza Production site in Visp (CH) and their comparison based on the gassed stirrer power consumption, liquid mass transfer coefficient, mixing time and hold up at similar conditions.

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<td>Stirrer speed [rpm]</td>
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<td>Liquid volume [L]</td>
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<td>$P_{g, 1, 2, 3}$ [W/m²]</td>
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<tr>
<td>Hold up [h]</td>
<td>6 (4)</td>
</tr>
</tbody>
</table>

J. Wenger, B. Sommer, S. Núñez, H. Engelking, T. Bartek, M. Funke, M. Eiting, T. Schmidt, Lonza Ltd, Visp, Switzerland
Scale-up of an *E. coli* fermentation

Five examples of the fermentation results for different proteins and different processes, which were scaled-up from different laboratory (1 – 20L) to different production (20 – 15000L) scales and box plot diagram summarizing results shown in each example.
Spray drying of biologic material - Development program (High level)

0. Starting point: e.g. Idea of cost effective Spray drying instead of more expensive methods
   - Assumption: 20%wt solution of biomass from last DSP step before drying step
   - Evaluation of technical information package from customer (analytical methods, spec, etc.)

1. Define the requirements of final product (basic formulation for AI) → Lead: Lonza R&T, Switzerland
   - Technical requirements: Specification for final product formulation (water content, specific activity, etc.)
   - Product stability requirements (Carrier y/n, packaging material, etc.)
   - Regulatory requirements (ISO, Food, GMP, Halal, Kosher, etc.)
   - Estimation of costs for drying method (e.g. Spray drying, different process scenarios)

2. Process development at Laboratory scale → Lead: Lonza R&T, Switzerland
   - Proof of concept (performing 1st lab trials, establish analytics, check thermal product stability)
   - Parameter screening for main process parameters (basic temperature profile, etc.)
   - Process parameter optimization (Feed concentration, yield, water content, stability, activity, Particle size, addition of stabilizers / adjuvants, etc.), approx. 10 - 20 spray drying trials

3. Scale-up with pilot trials → Lead: Lonza MSAT team, Kourim, Czech Republic
   - Technology process transfer R&T → MSAT team at Lonza’s production site (up to 3 lab runs)
   - Proof of technical Feasibility and Demonstration at Pilot Scale (up to 3 demo runs in pilot spray dryer)
   - Providing of first semi-industrial product samples (for laboratory use only)

4. Scale-up and validation at industrial scale ( > 10 m³ / fermentation batch)
   - Implementation of validated pilot process at production scale (up to 3 engineering batches)
   - Extended Product analysis (Microbial purity, storability, activity, etc.)
   - Providing of first commercial material (Final validation of technical specifications)
   - Setup of cost structure for production campaigns and define optimization program (process excellence)
Spray drying development
Process parameter scouting

- 12 spray drying runs (8 DoE runs + centerpoints + reserve) on Büchi Lab equipment
- Parameter screening of max. 4 variable parameters
- Parameters to be defined
- Supply of dried powder to formulation development / Analytics
- Evaluate and confirmation of optimal drying temperatures and time conditions
- Evaluate response at minimal and maximal parameter ranges
- Determine variance of process and analytics
- Results presented as PowerPoint slides (not including all online and offline data)

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Aspirator</th>
<th>Humidity drying gas</th>
<th>Inlet temperature</th>
<th>Spray gas flow</th>
<th>Feed rate</th>
<th>Solid concentration</th>
<th>Organic solvent instead of water</th>
</tr>
</thead>
<tbody>
<tr>
<td>Outlet temperature</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Particle size</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Humidity in final product</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yield</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Legend:
- High influence
- Moderate influence
- Minor influence
- No influence
- Increasing parameter
- Increasing variable
- Decreasing variable
- No influence

Lab Spray dryer
Manufacturing Optimization
Focus: Yield and Fermentation Time

- Detailed process analysis with focus on
  - Yield generation and yield loss
  - Fermentation time
  - Energy consumption

- Defined optimization plan
  - Increase biomass yield in seed step
  - Optimize fermentation media
  - Modification of feeding profile

- Stepwise and direct implementation on large scale fermenter

Yield and fermentation time before optimization program
Manufacturing Optimization
Focus: Yield and Fermentation time II

- 50% reduction of fermentation time to achieve same titer
- 3-times higher titer after 2/3 of fermentation time
- Significant cost reduction within 6 months of process improvement project
Manufacturing Optimization
Focus: Reduction of Cycle Time

- Detailed process throughput analysis and identification of bottle-necks

- Defined optimization plan
  - Process adaptation from centrifuge to membrane filtration
  - Optimize fermentation media preparation procedure
  - Re-arrangement of number of fermenters and equipment scale

- Stepwise and direct implementation on large scale

More than 50% reduction in cycle time achieved within an optimization project of 6 months
Starting point: A cryo vial and a patent application
- *Lactobacillus reuteri* (probiotic strain) has the ability to co-aggregate with the pathogenic *Helicobacter pylori* (causes gastritis and gastric ulcer) under physiological conditions (stomach)

Translate Market needs into defined product applications and appropriate cell formulation

Process development at Lonza LSI R&T Visp, Switzerland
- Proof of concept (performing 1st lab trials, establish analytics)
- Feasibility study for large scale at lab scale (20L lab scale)

Scale-up with pilot trials at Lonza Kourim, Czech Republic
- Proof of technical feasibility and demonstration at pilot scale
- Providing of final product samples (registration and application test)

Commercial production and market launch
- Implementation at large scale production
- Confirm defined requirements (technical, regulatory, customer specs)
- Appropriate storage and logistics / technical support for customers
From Shake-Flask to Formulated Product within One Year

- Fermentation development
  - Scale-up from flask to bioreactor
  - Medium & fermentation process optimization
  - 4 times increased yield at 10 times reduced media costs

- Harvesting
  - Biomass conditioning & concentration
  - Membrane filtration vs. centrifugation
  - Stability and storability of the biomass

- Formulation & Spray drying
  - Carrier screening to avoid auto-aggregation problem
  - Spray drying optimization: temperature profile, feed concentration
  - Pylopass™ comes as a free flowing powder, stabilized in a special matrix

- Packaging
  - Develop suitable packaging material for product stability, microbial purity, storability, activity requirements

Development time @ Lonza: 1 year
Spray Drying Carrier Screening

Screening for a suitable carrier for the stabilization and standardization of the active biomass

- **Problem to overcome**
  - Problem with auto-aggregation
  - Activity loss after spray drying
  - Formulation issues without carrier

- **Carrier selection and Screening**
  - High water soluble
  - Cheap
  - Food approved
  - Activity conservation
  - Compliance

- **Spray drying optimization**
  - Temperature profile
  - Feed concentration
  - Residual moisture

<table>
<thead>
<tr>
<th>Suitable carrier?</th>
<th>Carrier Type</th>
<th>Inlet Air Temperature</th>
<th>Outlet Air temperature</th>
<th>Feed concentration</th>
<th>Auto-Aggregation activity level</th>
<th>Co-Aggregation Activity level</th>
<th>Microscopic picture of co-aggregation</th>
</tr>
</thead>
<tbody>
<tr>
<td>NO</td>
<td>Carrier 1</td>
<td>High</td>
<td>Low</td>
<td>High</td>
<td>Negative - / 0%</td>
<td>Negative - / 0%</td>
<td></td>
</tr>
<tr>
<td>NO</td>
<td>Carrier 2</td>
<td>High</td>
<td>High</td>
<td>Low</td>
<td>Negative - / 0%</td>
<td>Neutral +/- / 25%</td>
<td></td>
</tr>
<tr>
<td>NO</td>
<td>Carrier 2</td>
<td>High</td>
<td>Low</td>
<td>High</td>
<td>Negative - / 0%</td>
<td>Positive + / 50%</td>
<td></td>
</tr>
<tr>
<td>++</td>
<td>Carrier 3</td>
<td>High</td>
<td>High</td>
<td>Low</td>
<td>Negative - / 0%</td>
<td>Positive ++ / 75%</td>
<td></td>
</tr>
<tr>
<td>+++</td>
<td>Carrier 3</td>
<td>High</td>
<td>Low</td>
<td>High</td>
<td>Negative - / 0%</td>
<td>Positive +++ / 100%</td>
<td></td>
</tr>
</tbody>
</table>
Lonza’s Large Scale Fermentation Manufacturing Service

- Final fermentation and down stream process development starting with any initial lab process package by our customer
- Technology transfer into Lonza’s assets based on any development stage
  - Customer lab process
  - Customer pilot trials
  - Large scale experience
- Proposal for potential process adjustments (process wise, and technology wise) to further improve economically attractiveness
- Full life cycle management from the initial market launch volumes until large volumes at maturity
- Thorough, continuous process improvement in close cooperation with customer
- Take-over of responsibility for specific customer requests, e.g. new raw material sources, specification adjustments, …
Why Outsource with Lonza

- Proven track record in the agricultural industry as reliable and trustworthy partner, and experience from more than 30 years of commercial fermentation
- Highest standards for process and worker safety, environment and quality assurance
- Unique combination of biotechnological and chemical platform
- Strong expertise in regulatory requirements for the agricultural industry
- Excellent know-how in prevention of cross contamination
- Excellent, state-of-the-art scientific know-how and outstanding customer focused project management
- Avoid large investments in your own capacity
- Speed to market, and high flexibility in your order volumes
- Full guarantee of your know-how and IP
- Full access to Lonza’s process optimization results for your own manufacturing

Security of Your Supply Chain
Customer Project Acquisition Process

- Increasing level of information exchange within each new stage
- Agreement on basic principles of manufacturing contract in stage 3
Customer Project Execution

One commercial key contact at Lonza

- First contact for all customer needs
- Provides technology-based proposals with shortest time to market
- Responsible for contract negotiation

Lonza’s Project Leader

- Drives projects to agreed goals, milestones and timelines in close cooperation with our customers
- Takes care on specific customer requests
- Ensures continuous process optimization

Backed by Lonza’s reliability, quality and service guarantees, we help our customers securing their supply chain and to achieve success in the market with maximizing the financial benefit of their products.