Agro Ingredients

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

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

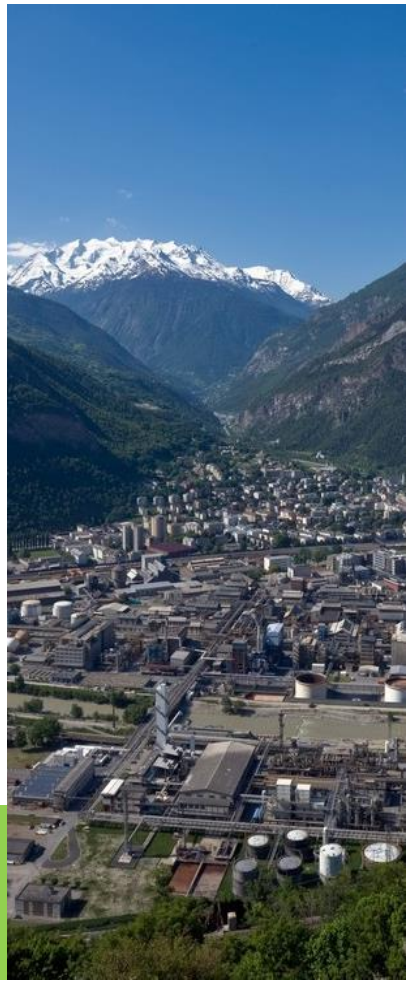
- Our service and product range addresses large number of markets:
  - Pharma and Biotech Markets
  - Water Treatment
  - Consumer Care
  - Wood Protection
  - Industrial solutions
  - **Agro Ingredients**

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”

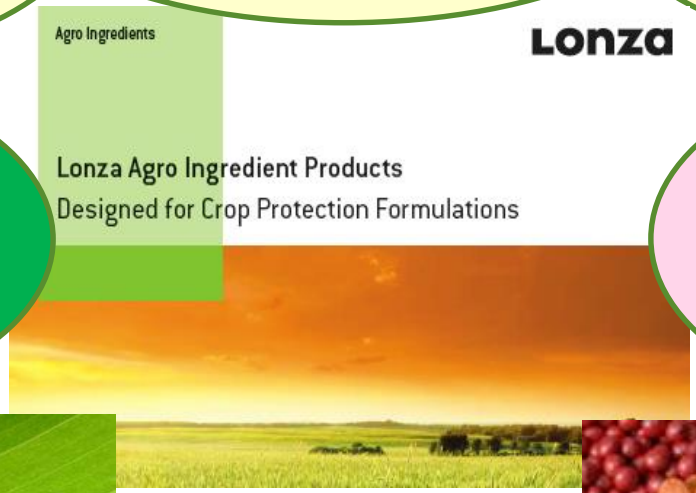
Agro Formulation Ingredients

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

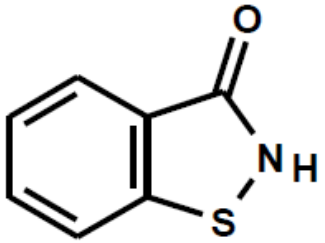
Naturally Based Surfactants
Sustainability
Environmentally Friendlier



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

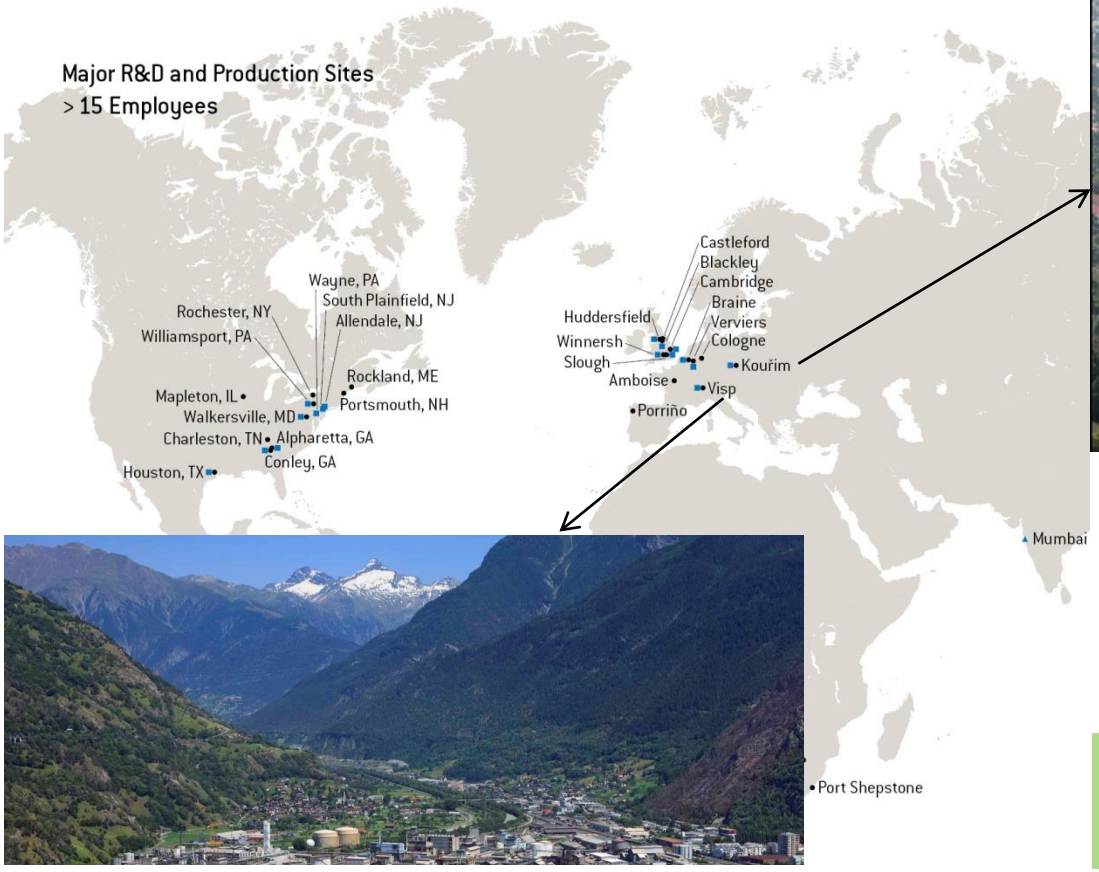
Proxel® – Wet State Preservation

- 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

Fermentation development and optimization in Visp, Switzerland

~ 200 people in all different functions at Kouřim and Visp

From Feasibility to Manufacturing

Strain Improvement	Fermentation Development	DSP & Formulation	Scale Up & Piloting	Manufacturing & Regulatory
				
▪ 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

- 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

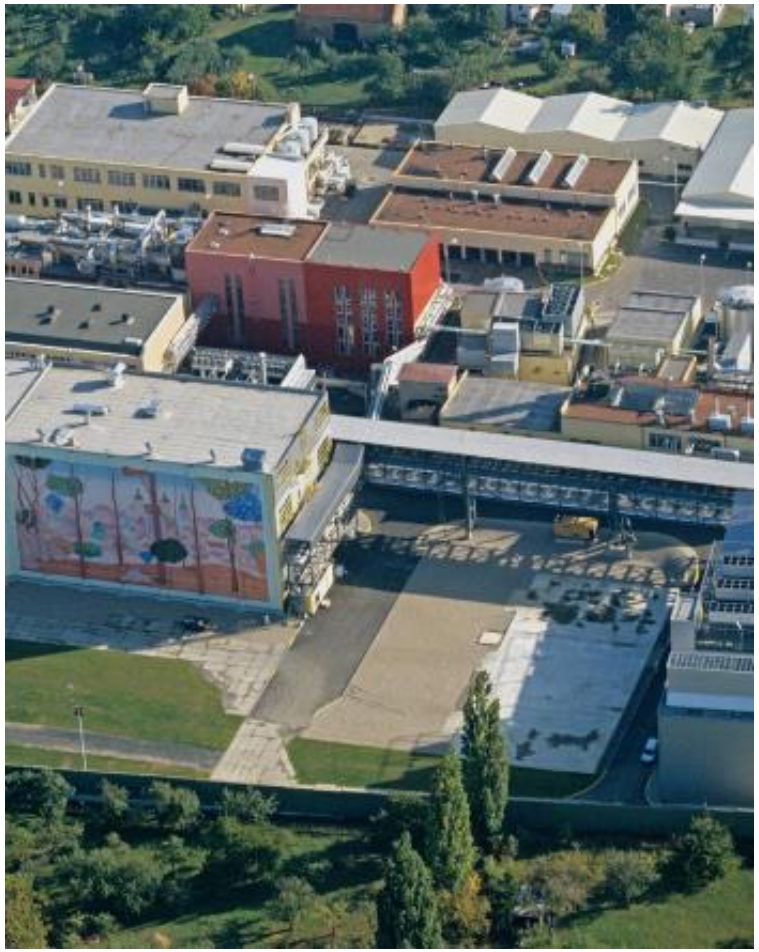
Optimization of Fermentation Processes

- 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 m^3 on pilot-scale)
- Achieving the most reliable and economical solution




State-of-the-Art Manufacturing Assets in Kouřim

- 80’000 m^2 site including infrastructure
- 5 individually operated lines for commercial scale production with total capacity of 475 m^3
  - 2 x 15 m^3 (Bio Safety Level 2)
  - 3 x 15 m^3
  - 2 x 50 m^3
  - 3 x 50 m^3
  - 2 x 75 m^3
- Ex-Proof DSP facilities for solvent handling
- On-site waste water treatment plant
- 3’400 m^2 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
- **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)
- Titration (Karl-Fischer, …)
- ELISA
- Bioprofile – IPC for fermentation
- Testing of microbial contaminants
- SDS-Page
- Enzyme activity assays
- Particle size distribution

Lonza

Fermentation Processes and Microorganisms

Bacteria

Bacillus (lentus, subtilis) (GMO)

Gluconobacter

Rhizobium

Pseudomonas

Streptomyces sp.

Burkholderia sp

Acetobacter sp. (mutated)

Nonomuraea 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

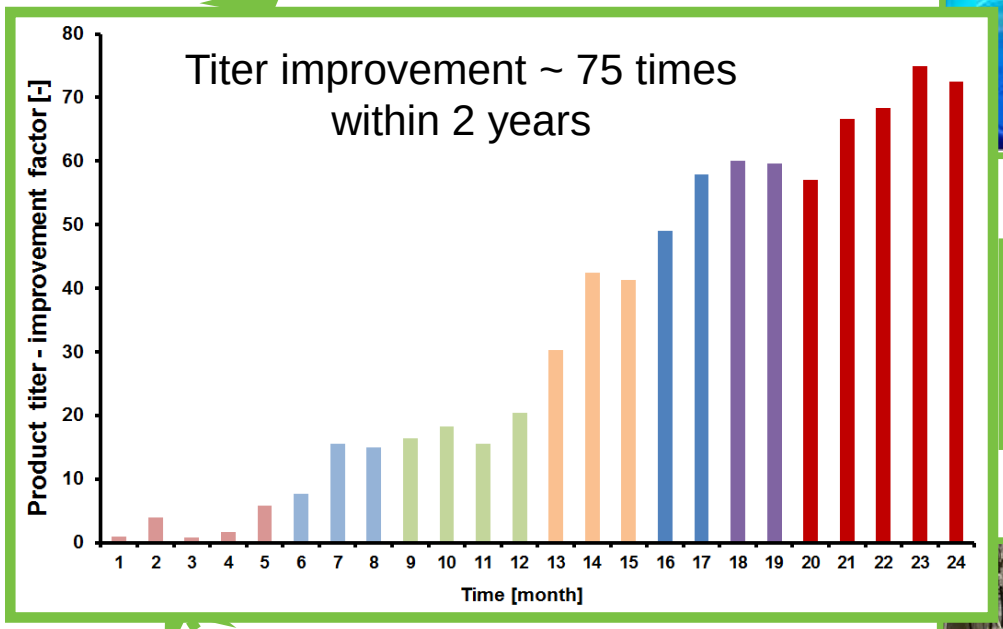
Ulkenia (mutated)

More then 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

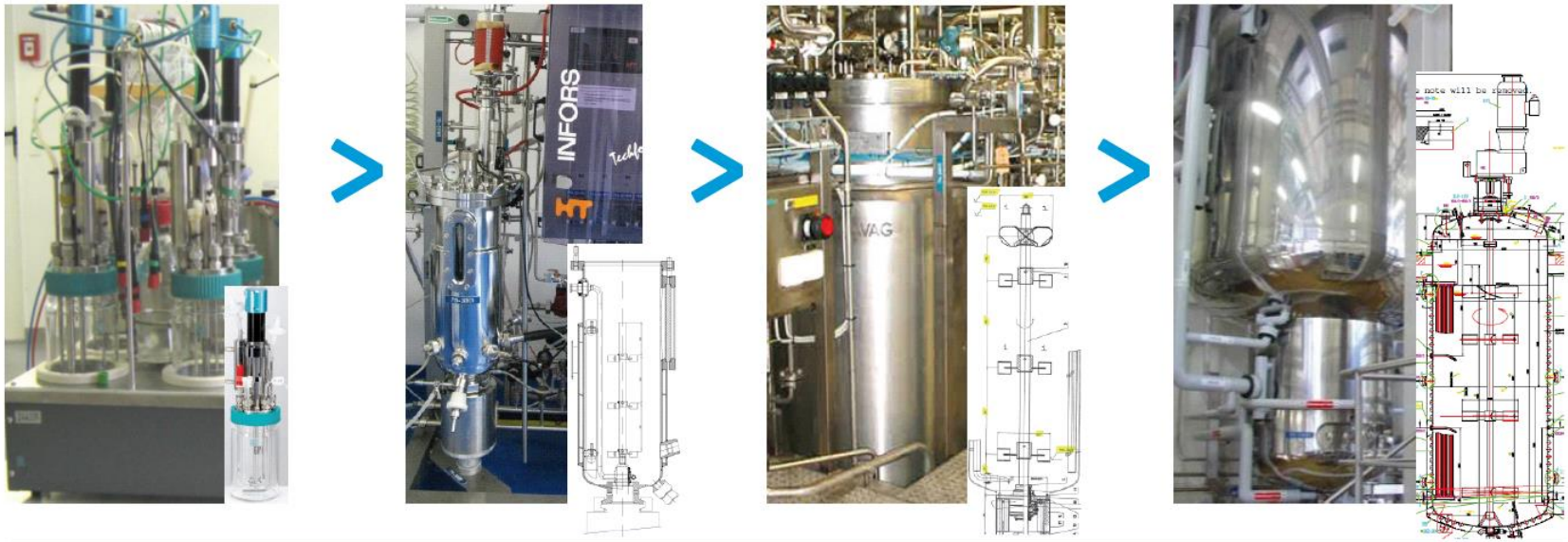
3. Improved strain testing @ bioreactor scale

Selection of potential strain candidates

Process Optimization

Process Technology Transfer
Scale-up of an *E. coli* fermentation

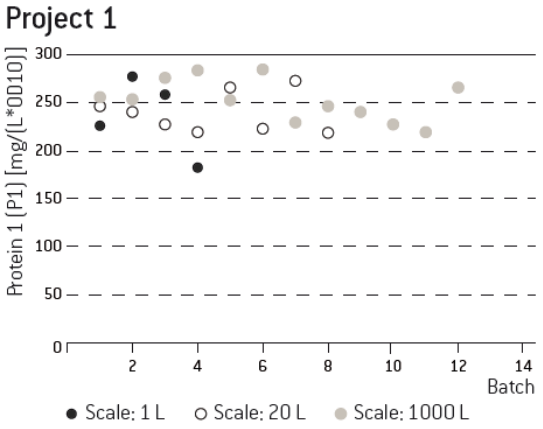
Bioreactors at Different Scales

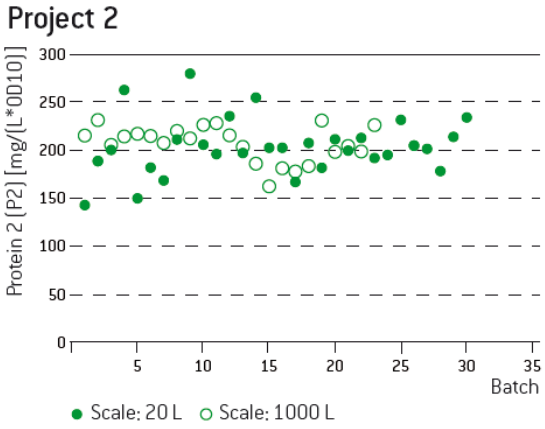


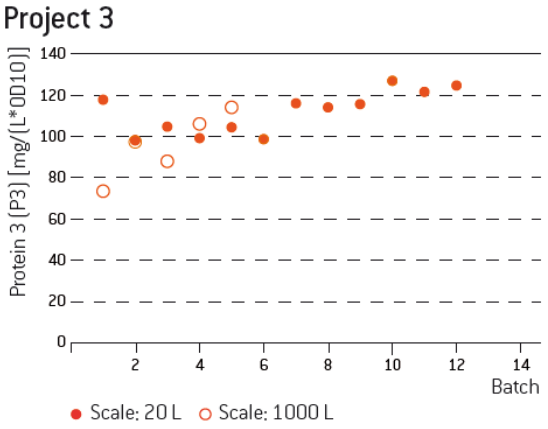
Parameters		Reactor volume 1L	20L	1000L	15 000L (1)	15 000L (2)
Stirrer speed	[rpm]	1200	1100	275	145	145
Liquid volume	[L]	0.5	15	800	10 000	10 000
Gas flow	[L/min]	0.5	12	480	8000	8000
$P_{sg}1, 2, 3 / V$	[W/m³]	7310, 12 190, 14 620	3790, 6320, 7580	2710, 4520, 5420	2580, 4290, 5150	1010, 1680, 2020
Kla ($P_{sg}1, 2, 3$)	[1/s]	0.27, 0.38, 0.44	0.23, 0.33, 0.38	0.22, 0.31, 0.35	0.25, 0.36, 0.41	0.13, 0.19, 0.21
Mixing time	[s]	1.4	3	7	12	23
Hold up 1 (2)	[%]	6 (4)	8 (4)	12 (6)	20 (11)	16 (7)

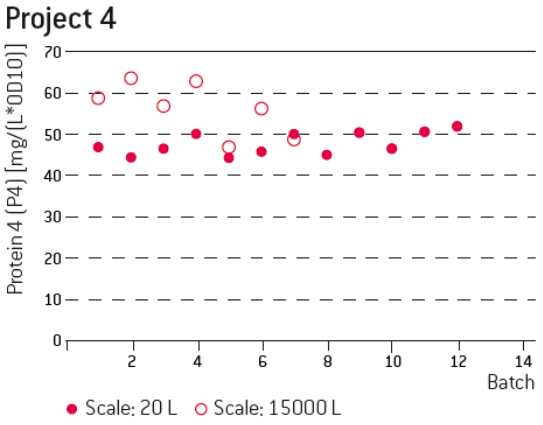
Process Technology Transfer
Scale-up of an *E. coli* fermentation

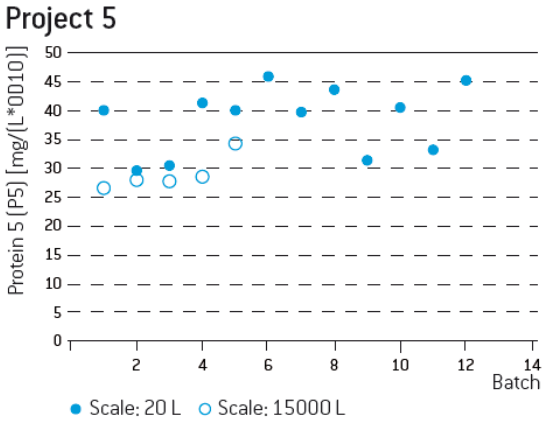
Lonza

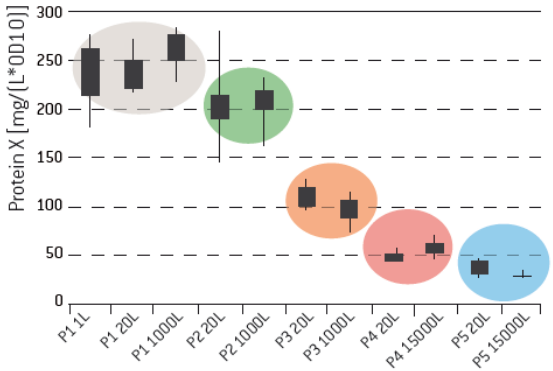








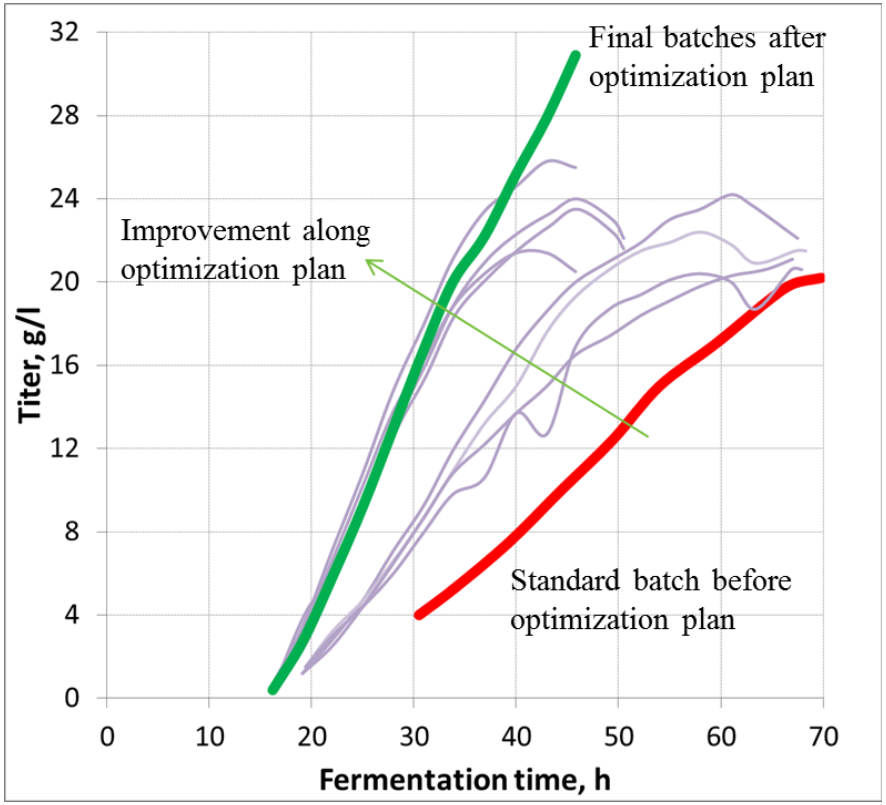




J. Wenger, B. Sommer, S. Núñez, H. Engelking, T. Bartek, M. Funke, M. Eiting, T. Schmidt, Lonza Ltd, Visp, Switzerland

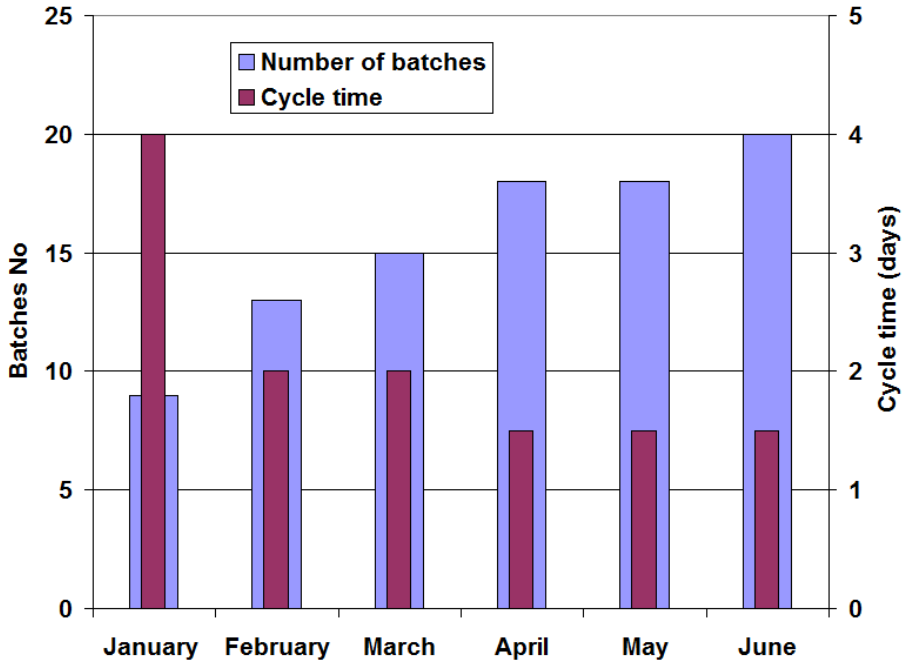
Manufacturing Optimization
Focus: Yield and Fermentation Time

- **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**



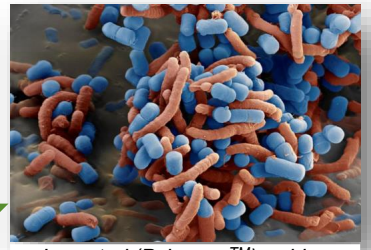
More than 50% reduction in cycle time achieved within an optimization project of 6 months

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

Development time @ Lonza : 1 year

Fermentation
Maturation
Harvesting
Standardization in Matrix
Spray Drying
Packaging
Pylopass™



L. reuteri (Pylopass™) = blue
H. pylori = red
SEM magnification = 13'000 x

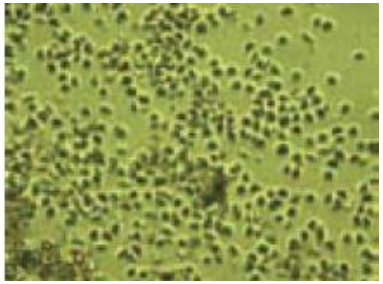
Exclusive Biotechnological Manufacturing

Clariva™ Complete Beans

Clariva™ pn

- *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

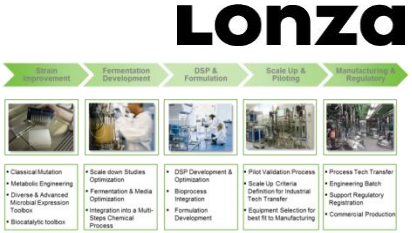
Soybean cyst nematode and egg



Pasteuria nishizawae spores

With permission of 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 then 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


Fermenter Line


Down Stream Process Plant

Thank You !


Ultra Filtration Unit


Centrifugation Unit


Spray Dryer (Upper part)


Control room

Michael Helwig
Head Global Product Application Development
BU Agro Ingredients
Münchensteiner Strasse 38
CH-4002 Basel
michael.helwig@lonza.com
+ 41 61 316 8705 (office)
+ 41 79 818 5249 (mobile)

http://www.lonza.com/products-services/agro-ingredients.aspx

Lonza

Agro Ingredients

BACK-UP Slides Full Version

Large Scale Fermentation Manufacturing Services for the Biocontrol Industry

Business Unit (BU) Agro Ingredients

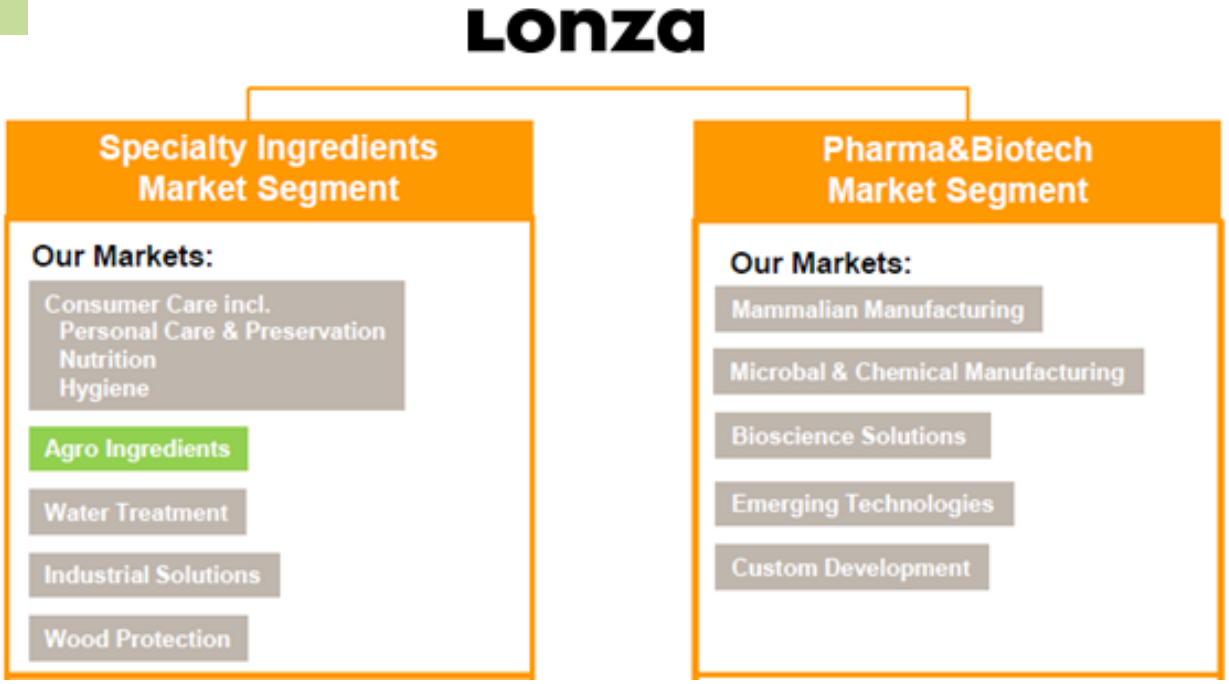
October 2015

Lonza

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:
  - 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

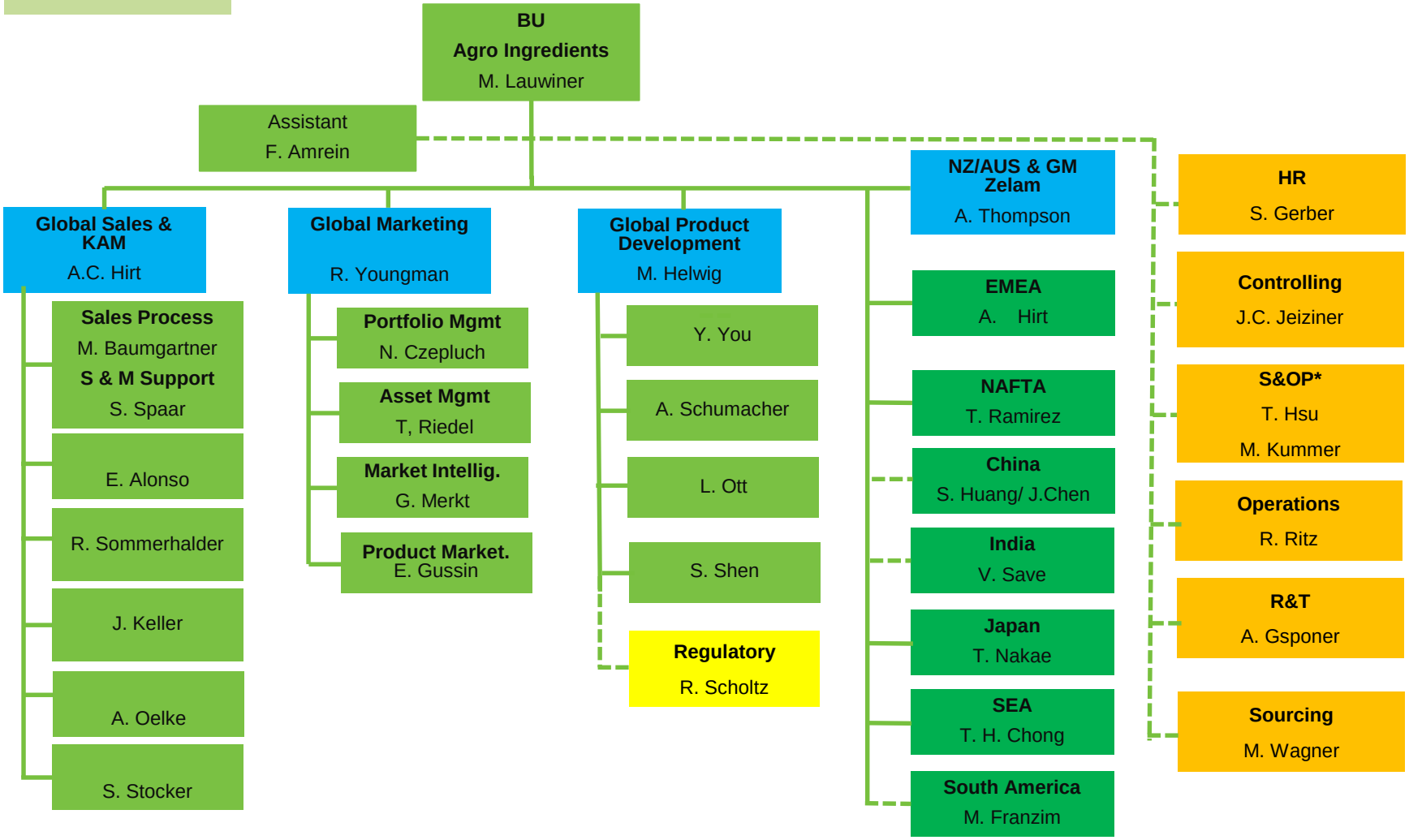
Lonza Segments



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"

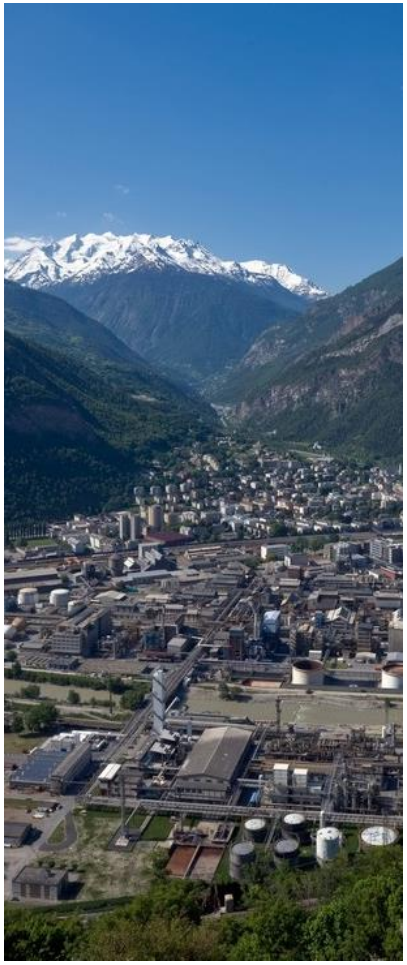
BU AI - Global Business Organization



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

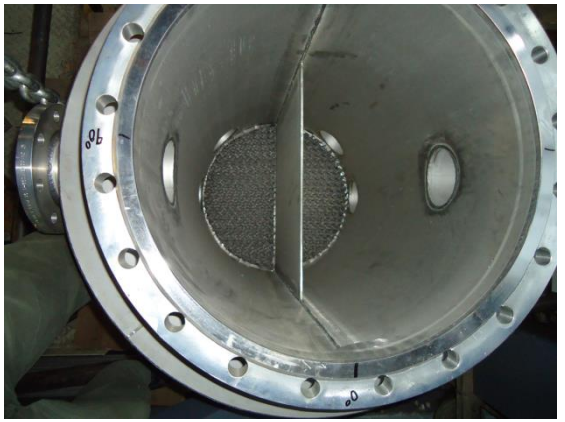
Backward integrated site in Visp, Switzerland, with complex multi-purpose plants, broad technology portfolio, and integrated waste management facilities



Continuous expansion of Hastelloy capacity to account for F-chemistry

Inside Lonza multi purpose plant

16 m^3 Hastelloy batch reactor, up to 16 bar,



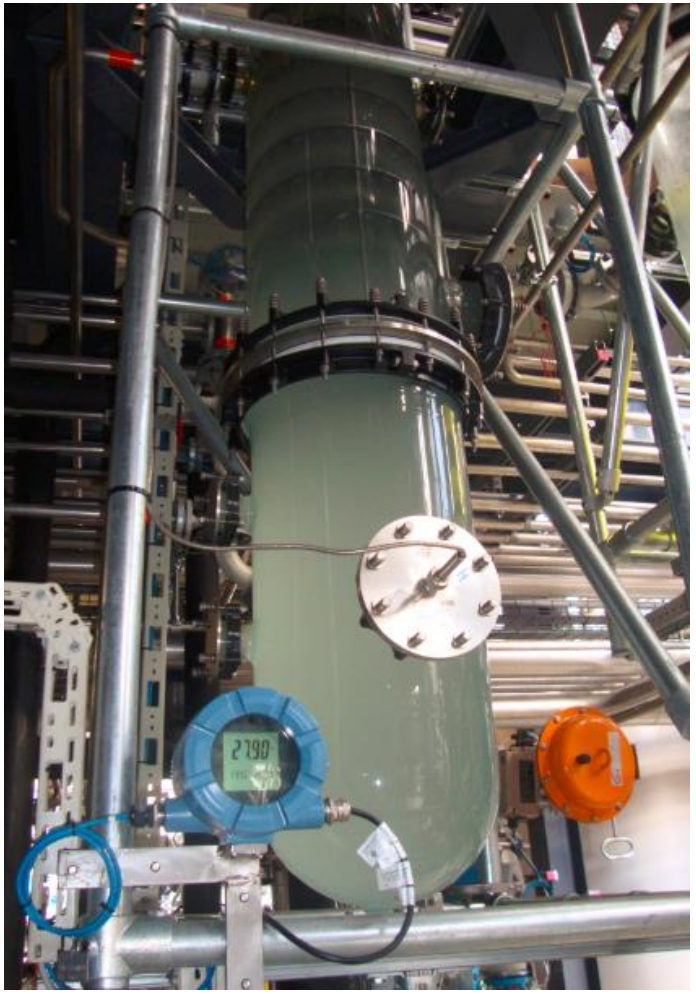
Inside multi-product Hastelloy divided wall column for highest purity products

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



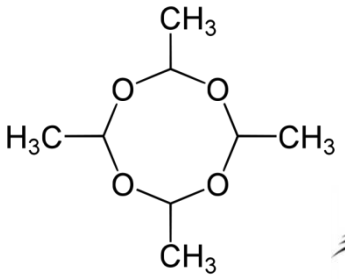
N-Fertilizer Production in Visp

Sales of N-based fertilizer in Switzerland via Agroline

Molluscicide - Global Market Player for 60 Years

Lonza

axCela®



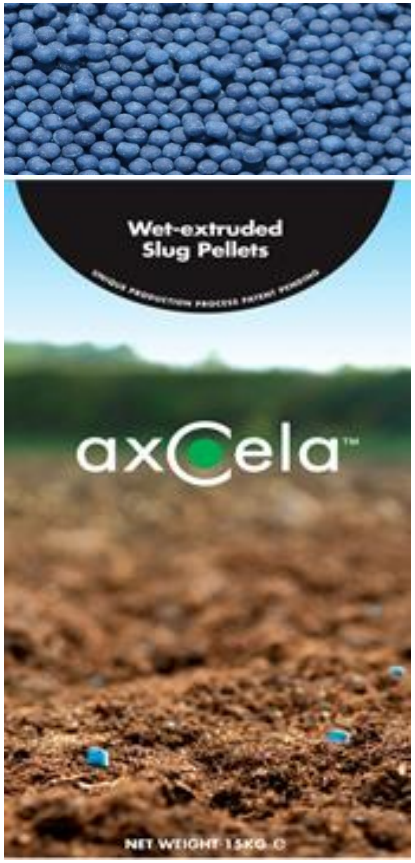
meta® the molluscicide

Market leader for supply of Meta® metaldehyde as active ingredient

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

Global launch of Lonza's formulated products

Manufacturing plant for Lonza Slug Pellets in Visp



Frexus® - Application Brand

Pre- and post-harvest treatment

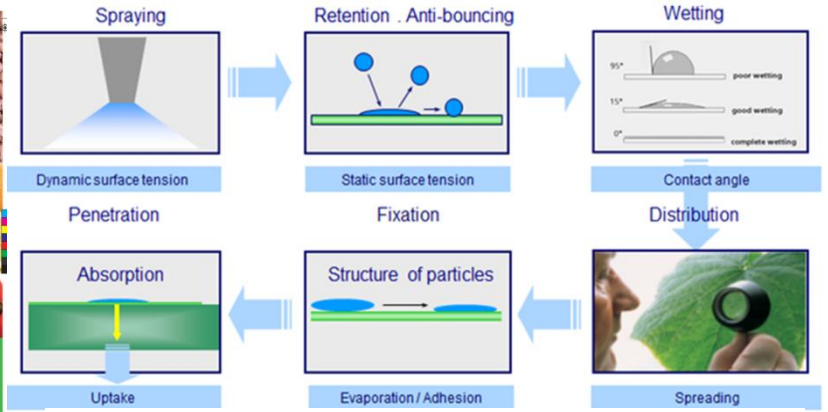
- Coffee beans
- Fruit & vegetables

Adjuvants for tank mix

- pH-reducer
- Surfactant

Biocides for fighting plant diseases

White Spot on Corn Leaves



Improvements through application of Frexus® pH Agro

Frexus® product line is not registered in USA

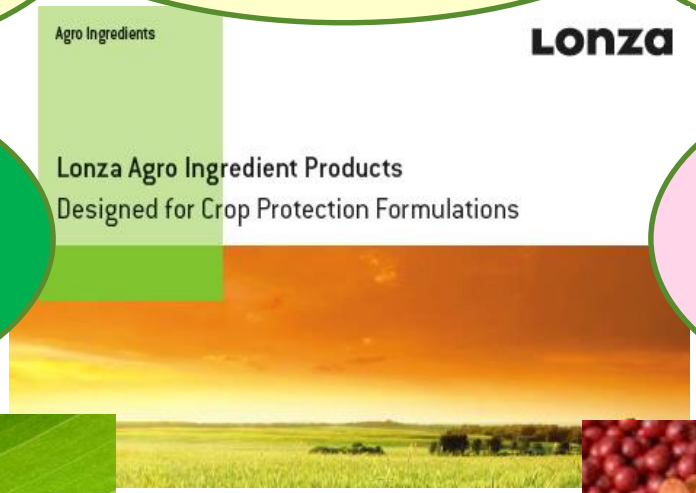
Agro Formulation Ingredients

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

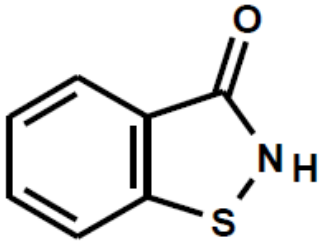
Naturally Based Surfactants
Sustainability
Environmentally Friendlier



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

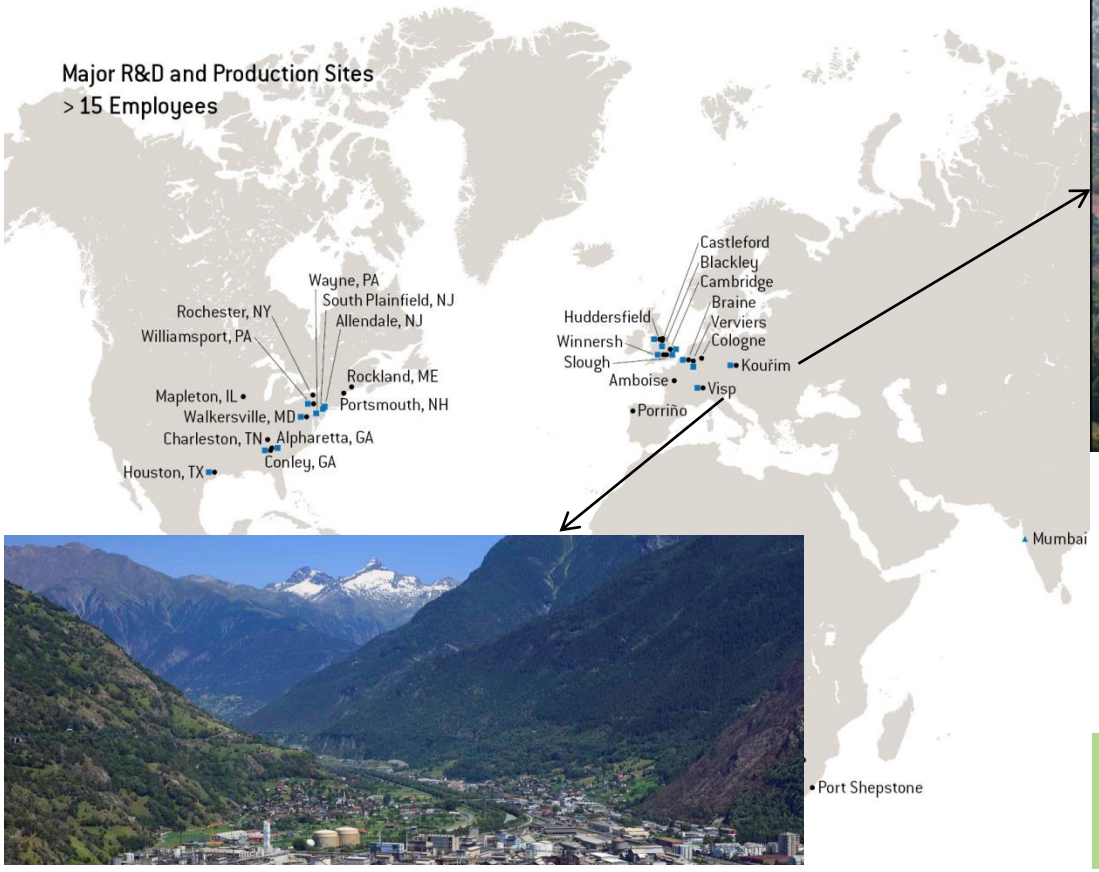
Proxel® – Wet State Preservation

- 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



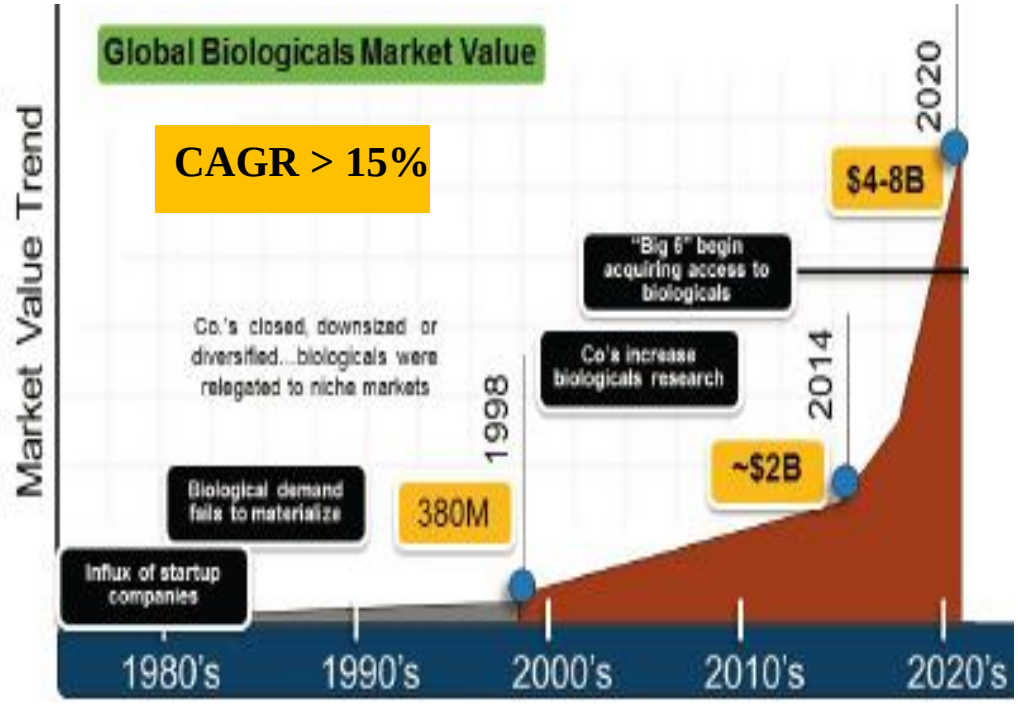
Fermentation scale-up and manufacturing in Kouřim, Czech Republic

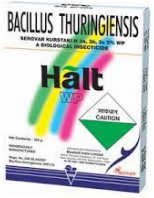
Fermentation development and optimization in Visp, Switzerland

~ 200 people in all different functions at Kouřim and Visp

Lonza

What drives increasing interest in biologicals





KOPPERT BIOLOGICAL SYSTEMS

VALENT BIOSCIENCES CORPORATION

CERTIS USA

Marrone Bio Innovations
Natural Products for Pest Management

GLOBAL BIO PESTICIDES MARKET

syngenta
PASTEURIA bioscience

BASF
The Chemical Company
BECKER UNDERWOOD

Bayer CropScience
AGRAQUEST
better food. better world

novozymes
Rethink Tomorrow

MONSANTO

Lonza

What drives increasing interest in biologicals

- Public perception
- Reduced chemical residues
- Sustainability
- Worker safety / Short re-entry
- Registration advantage
- Resistance management
- Performance
- Less high development costs
- Fast growing segment
- Short-Cut for chemical pesticides

Lonza

What drives increasing interest in biologicals

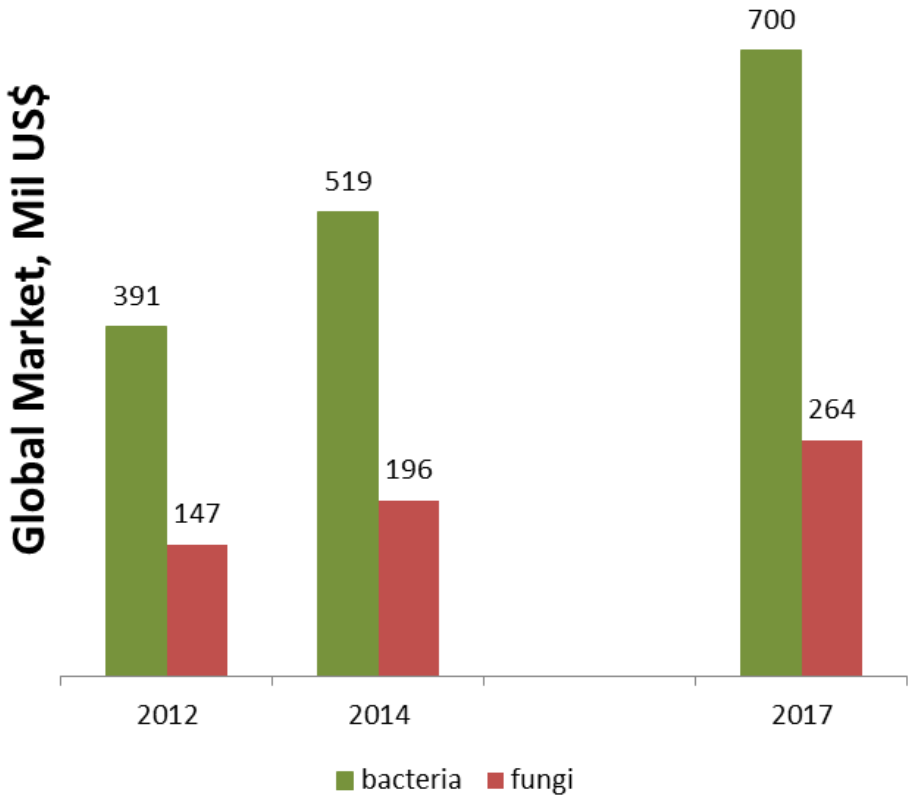
Public Perception	People believe that bio-based products are of advanatge in terms of health, wellness and sustainability if compared to chemical-based ones
Reduced Chemical Residues	Retailers demand lower residue levels than regulatory requires, setting secondary standards for marketing purposes - MRL;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

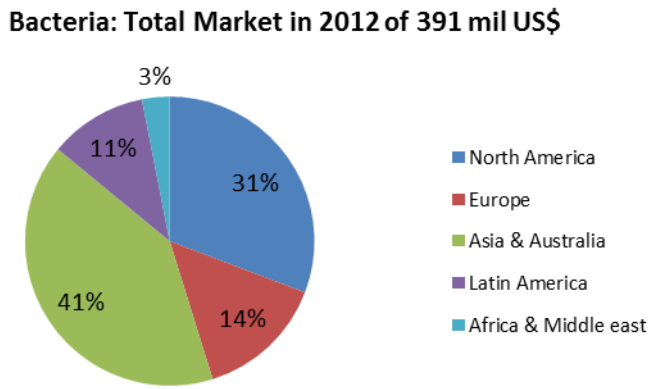
Lonza

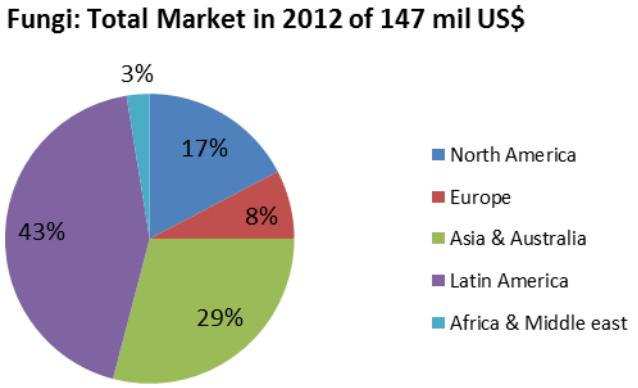
What drives increasing interest in biologicals

Resistance Management	Alternative mode of actions to control pests, diseases and to over-come resistance build-up. Fit in where only few/no chemicals exist. Use in Integrated Pest Management, and Resistance Management
Performance	New solutions for growers/farmers. No general replacement of chemicals, but in combination with chemicals for high performance programs.
Less High Development Costs	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)
Fast Growing Segment	Bio-based products are one of the fastest growing segments with CAGR of 10-16%, however, still having a share of << 10% on the over-all pesticide market
Short cut for Chemical Pesticides	Biologically derived intermediates, and biotransformation catalysts can help to replace complex chemical route to same end-molecule. Large market for biologcally derived products like Spinosad, which are no biopesticides, but experience certain registration advantages.

Global Market of fermentation based bacterial and fungal products (“new biopesticides”)







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.

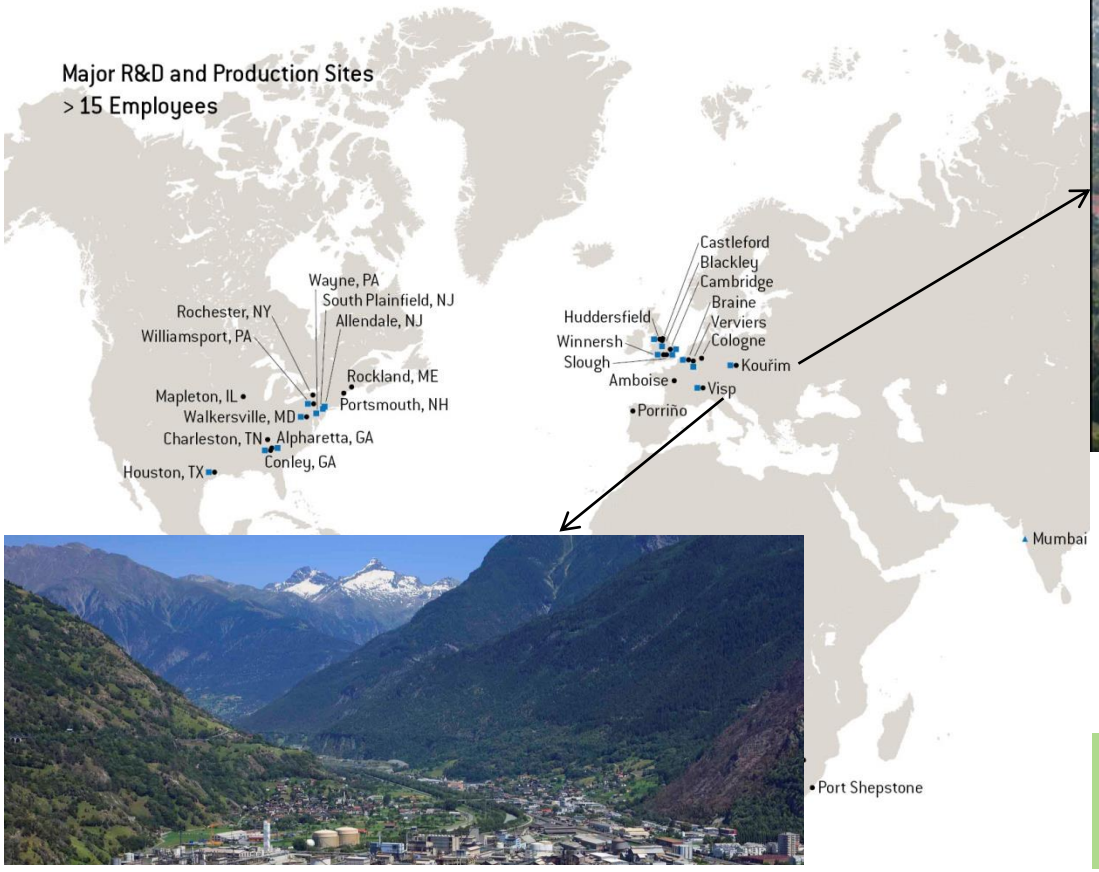
Source: CPL 2013

Recent Acquisitions

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

Biotechnology @ Lonza



Fermentation scale-up and manufacturing in Kouřim, Czech Republic

Fermentation development and optimization in Visp, Switzerland

~ 200 people in all different functions at Kouřim and Visp

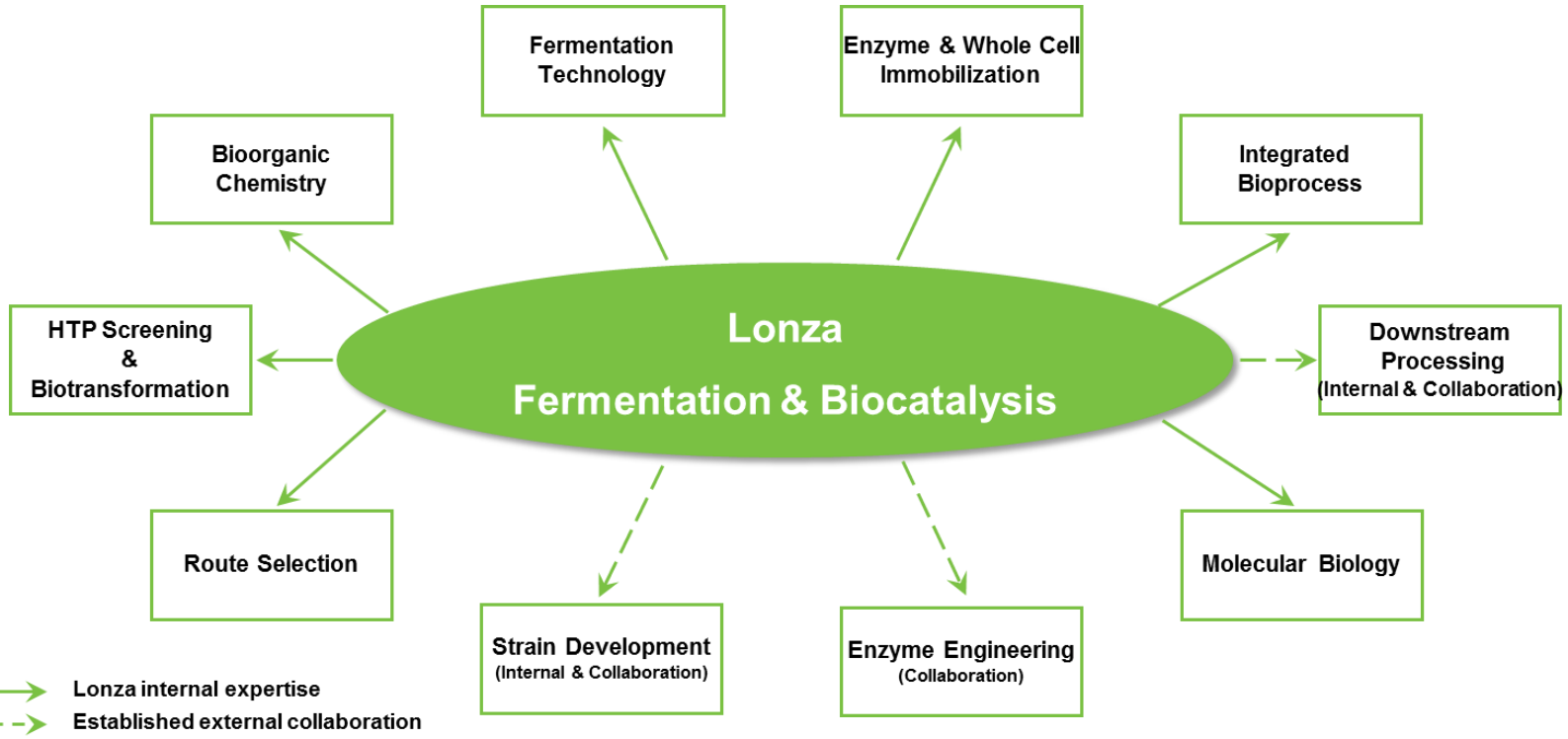
From Feasibility to Manufacturing

Strain Improvement	Fermentation Development	DSP & Formulation	Scale Up & Piloting	Manufacturing & Regulatory
				
▪ 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

- A one-stop-shop at every stage of your project along the value-chain
- 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 m^3 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**

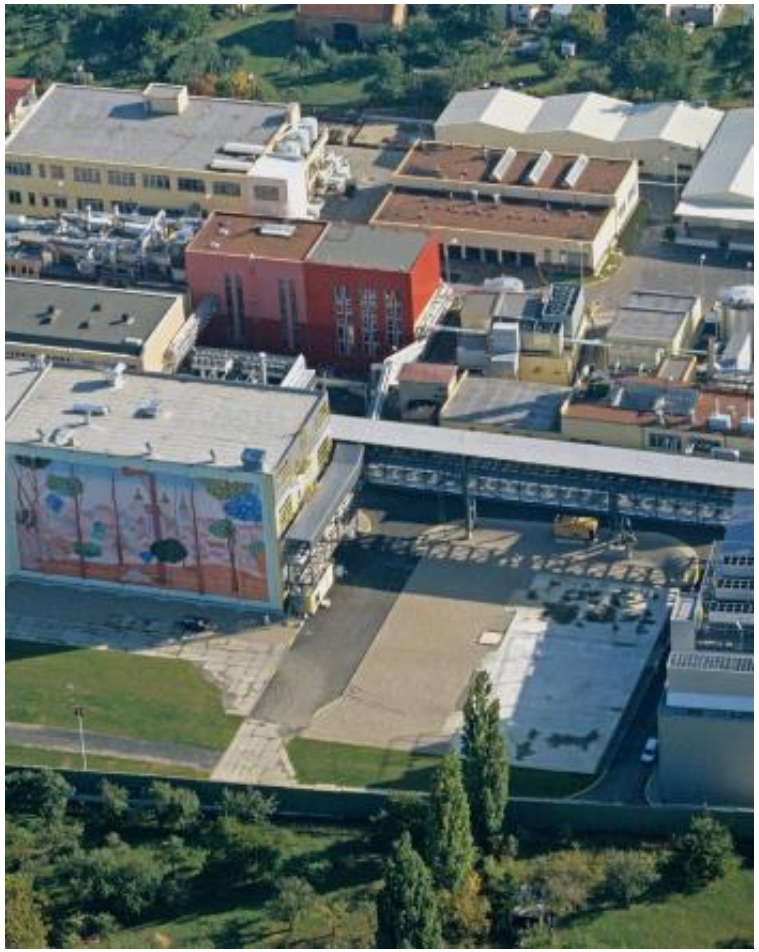
MSAT Kouřim
Optimization of Fermentation Processes

- Dedicated team of MSAT, QC, and production with support from any other organization unit required, e.g. engineering, R&D, 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
- Achieving the most reliable and economical solution




State-of-the-Art Manufacturing Assets in Kouřim

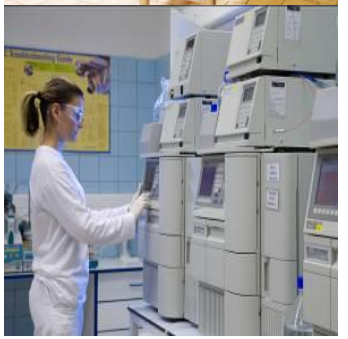
- 80’000 m^2 site including infrastructure
- 5 individually operated lines for commercial scale production with total capacity of 475 m^3
  - 2 x 15 m^3 (Bio Safety Level 2)
  - 3 x 15 m^3
  - 2 x 50 m^3
  - 3 x 50 m^3
  - 2 x 75 m^3
- Ex-Proof DSP facilities for solvent handling
- On-site waste water treatment plant
- 3’400 m^2 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
- **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)
- Titration (Karl-Fischer, …)
- ELISA
- Bioprofile – IPC for fermentation
- Testing of microbial contaminants
- SDS-Page
- Enzyme activity assays
- Particle size distribution

Fermentation Processes and Microorganisms

Bacteria

Bacillus (lentus, subtilis) (GMO)

Gluconobacter

Rhizobium

Pseudomonas

Streptomyces sp.

Burkholderia sp

Acetobacter sp. (mutated)

Nonomuraea 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 then 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

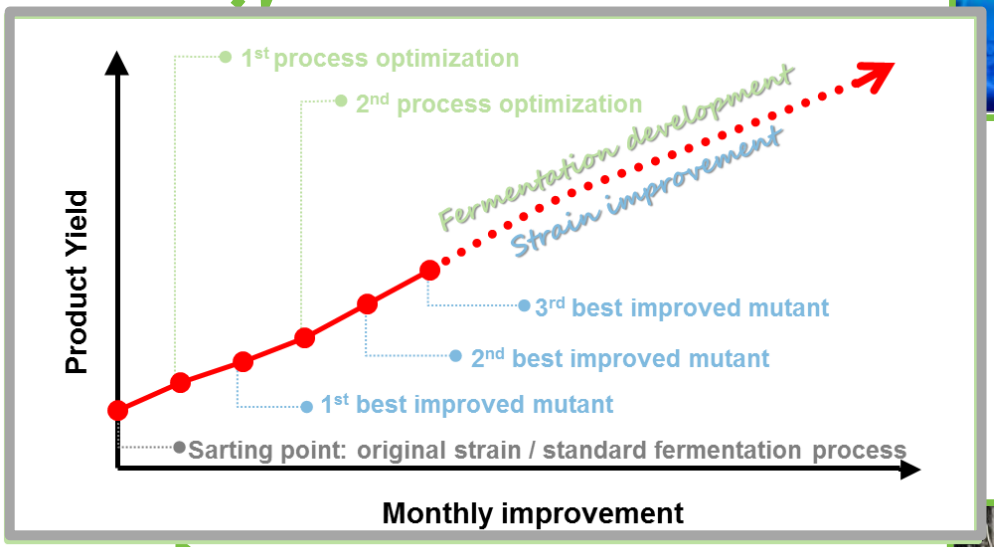
Spray Dryer (Upper part)

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

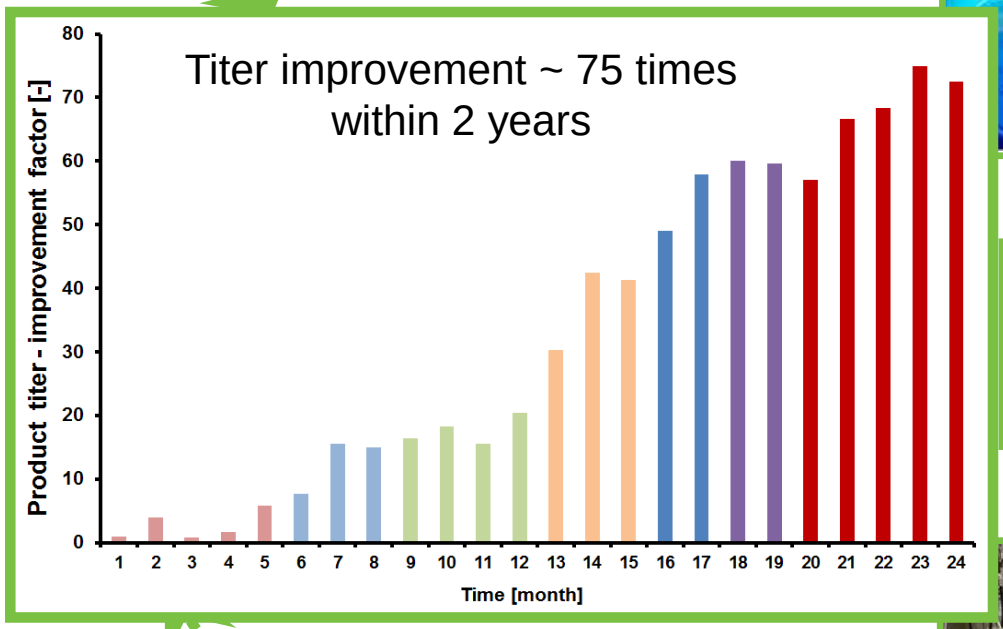
3. Improved strain testing @ bioreactor scale

Selection of potential strain candidates

Process Optimization

Focus Strain/Fermentation Improvement
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

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

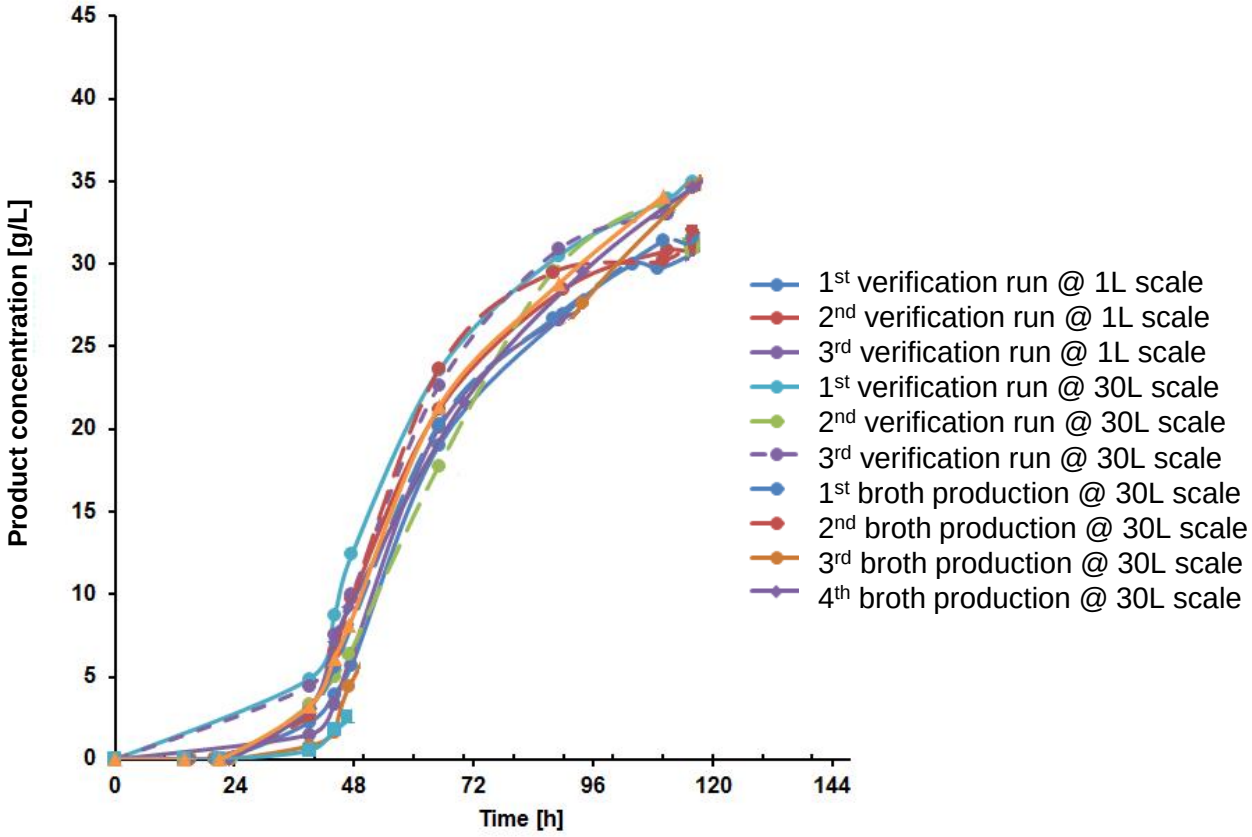
4 x 1L Dasgip fermentation system

Process Scale Up

30L Bioreactor

Fermentation Verification and Scale-up runs

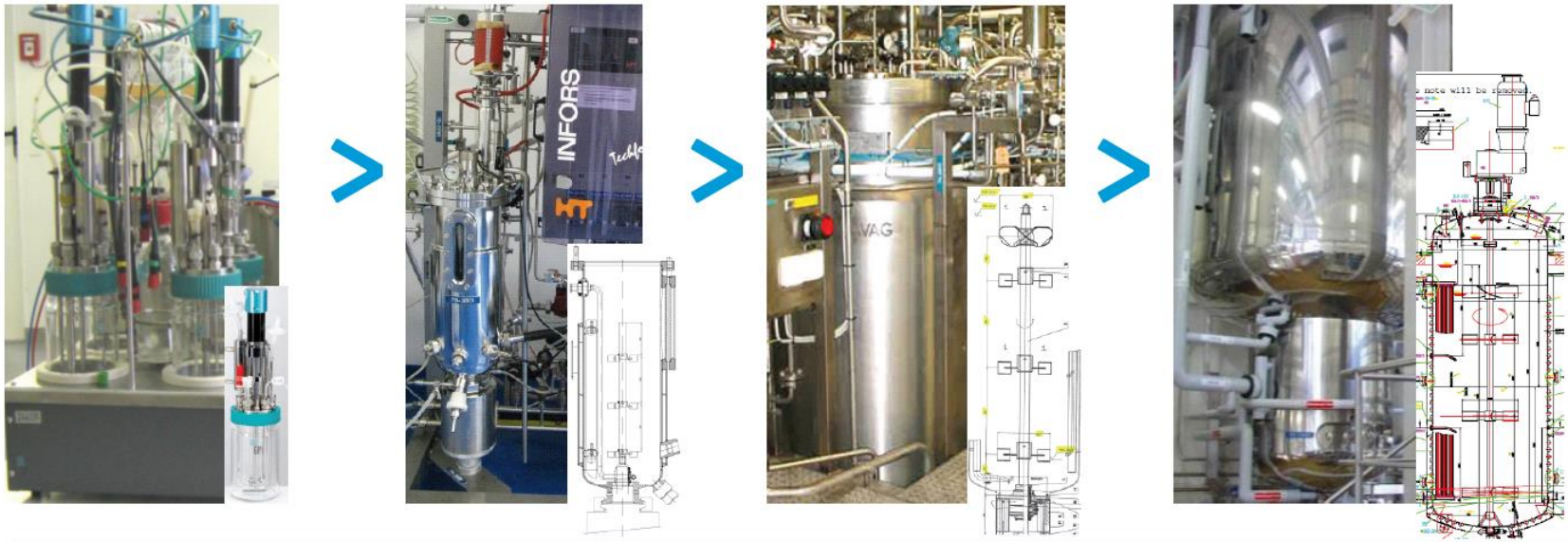
- ***Validation runs at 1L and 30L scale to test the process robustness***
- ***Material production for primary recovery optimization and validation runs***



Scale-up of an *E. coli* fermentation

Bioreactors at Different Scales

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.



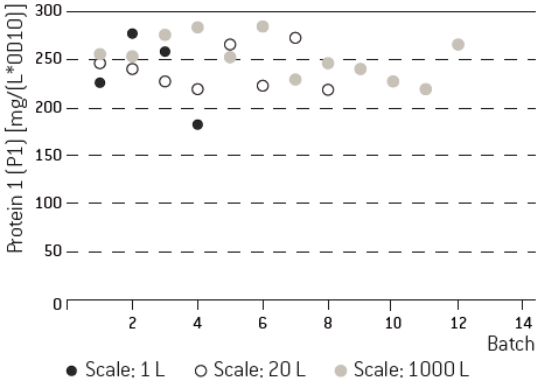
Parameters		Reactor volume 1L	20L	1000L	15 000L (1)	15 000L (2)
Stirrer speed	[rpm]	1200	1100	275	145	145
Liquid volume	[L]	0.5	15	800	10 000	10 000
Gas flow	[L/min]	0.5	12	480	8000	8000
$P_{sg}1, 2, 3 / V$	[W/m³]	7310, 12 190, 14 620	3790, 6320, 7580	2710, 4520, 5420	2580, 4290, 5150	1010, 1680, 2020
Kla ($P_{sg}1, 2, 3$)	[1/s]	0.27, 0.38, 0.44	0.23, 0.33, 0.38	0.22, 0.31, 0.35	0.25, 0.36, 0.41	0.13, 0.19, 0.21
Mixing time	[s]	1.4	3	7	12	23
Hold up 1 (2)	[%]	6 (4)	8 (4)	12 (6)	20 (11)	16 (7)

J. Wenger, B. Sommer, S. Núñez, H. Engelking, T. Bartek, M. Funke, M. Eiting, T. Schmidt, Lonza Ltd, Visp, Switzerland

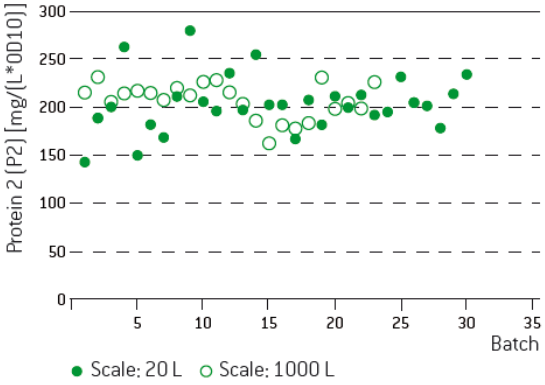
Scale-up of an *E. coli* fermentation

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.

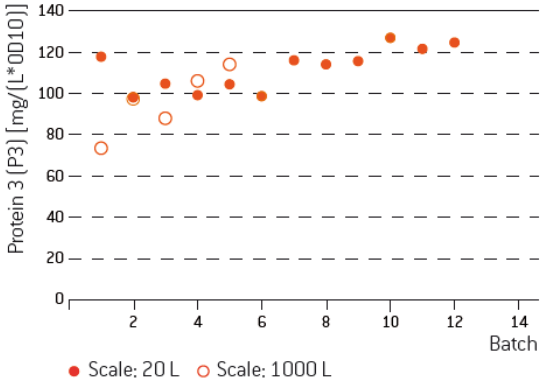
Project 1



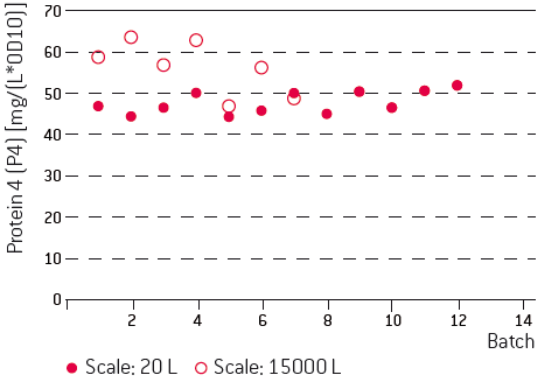
Project 2



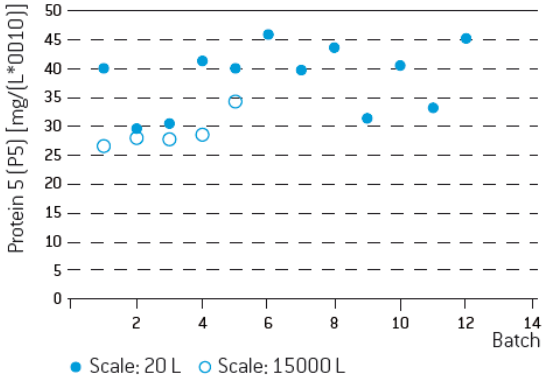
Project 3

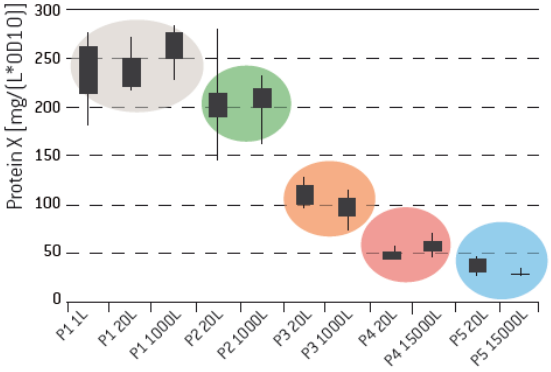


Project 4



Project 5





J. Wenger, B. Sommer, S. Núñez, H. Engelking, T. Bartek, M. Funke, M. Eiting, T. Schmidt, Lonza Ltd, Visp, Switzerland

Spray drying of biologic material - Development program (High level)

Lab scale, Büchi spray dryer

Pilot scale Anhydro Spray dryer

Industrial scale Spray dryer Lonza CZ

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 m^3 / 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

Parameter / Dependance	Aspirator	Humidity drying gas	Inlet temperture	Spray gas flow	Feed rate	Solid concentration	Organic solvent instead of water
Outlet temperature	↑	↑	↑	↓	↓	↑	↑
Particle size	—	—	—	↓	↑	↑	↓
Humidity in final product	↓	↑	↓	—	↑	↓	↓
Yield	↑	↓	↑	—	↓↑	↑	↑

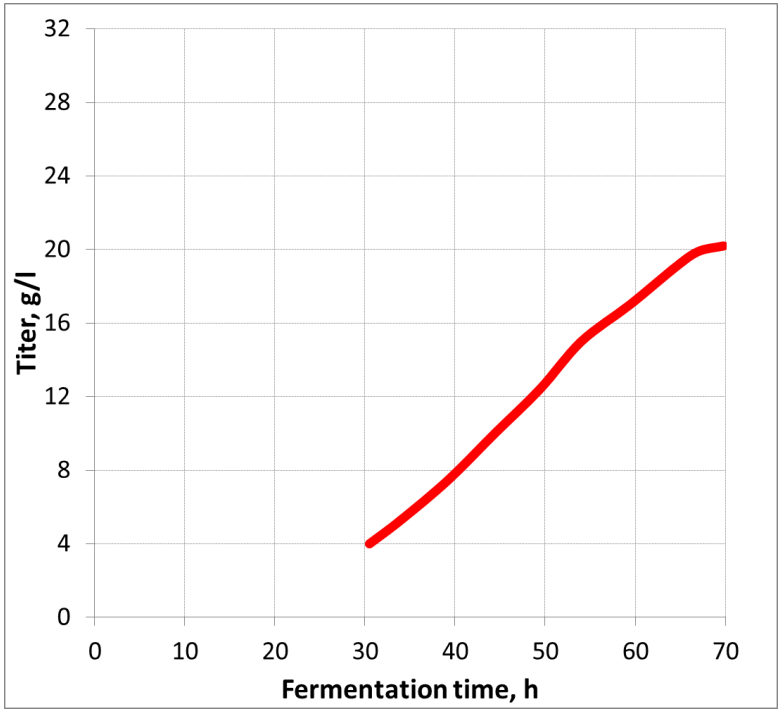
Legend:		
	High influence	Increasing parameter
	Moderate influence	Increasing variable
	Minor influence	Decreasing variable
	No influence	

Lab Spray dryer

- 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)

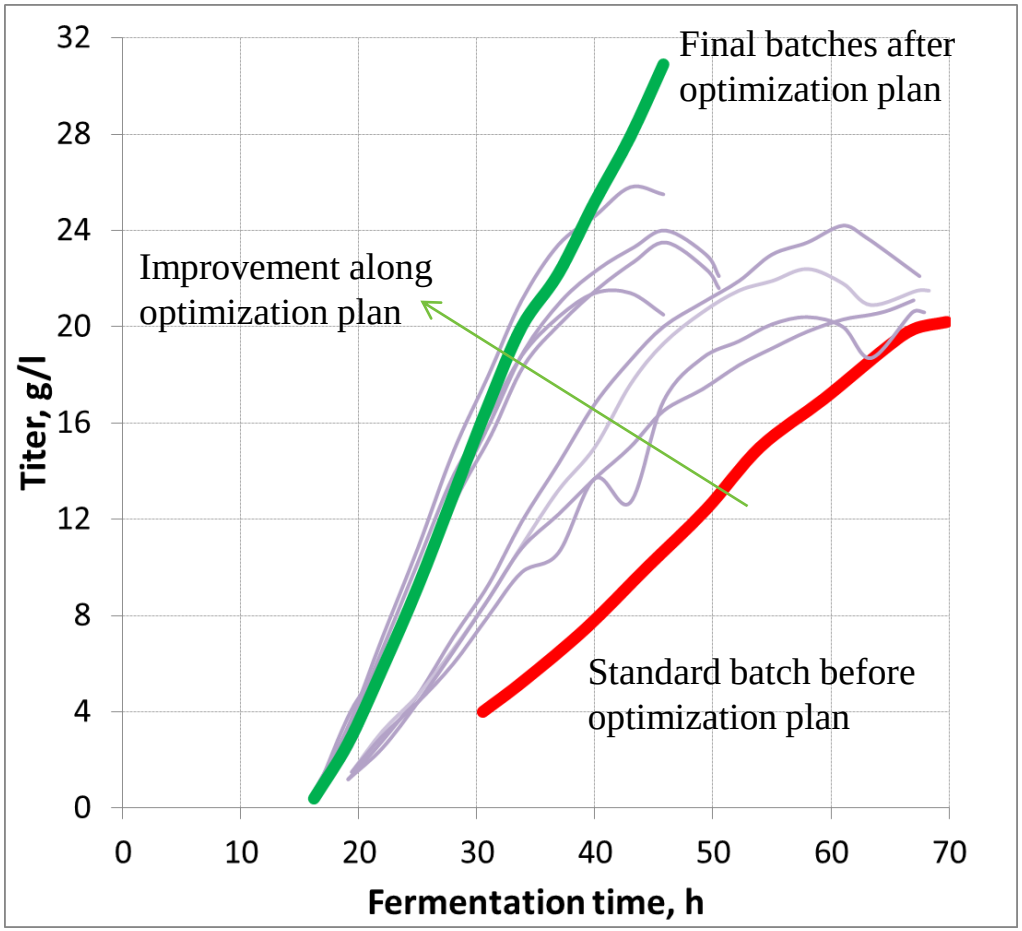
Manufacturing Optimization
Focus: Yield and Fermentation Time

- **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

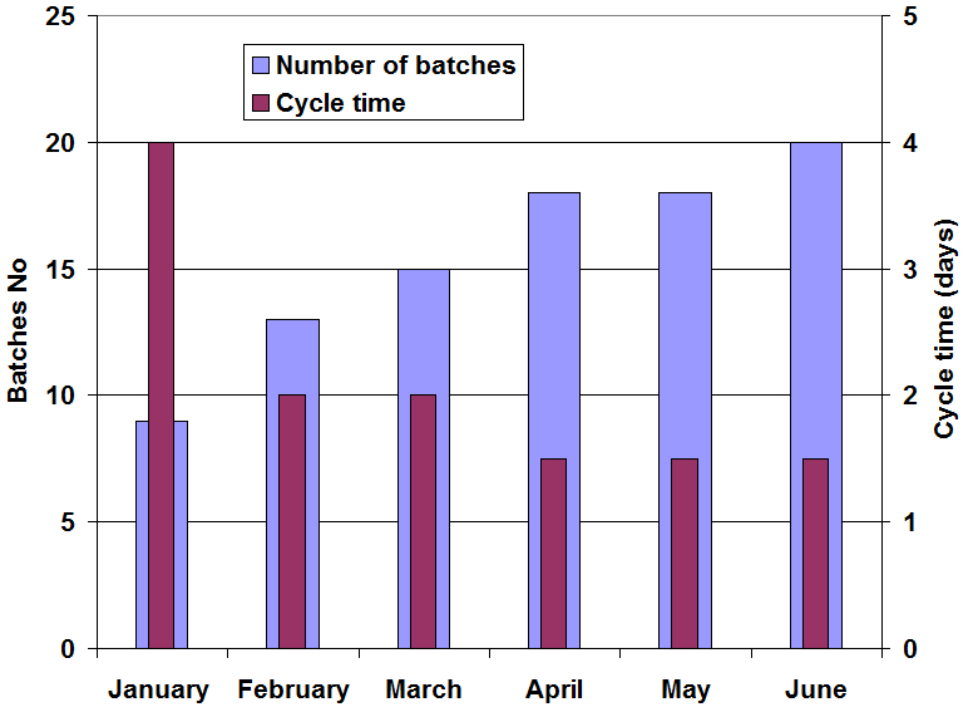
Manufacturing Optimization
Focus: Yield and Fermentation time II



- 50% reduction of fermentation time to achieve same titer
- 3-times higher titer after 2/3 of fermentation time
- Significant cost reduction within 6 month 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

Lonza Development of Commercial Product

Lonza

Pylopass™

- **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)

ORGANO BALANCE

- 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

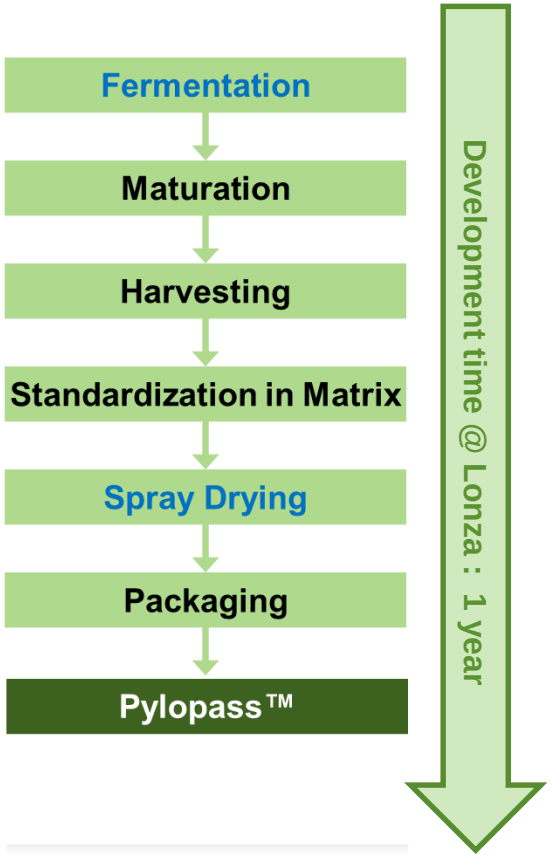
L. reuteri (Pylopass™) = blue | H. pylori = red | SEM magnification = 13'000 x

From Shake-Flask to Formulated Product within One Year

Lonza

Pylopass™

- 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



Spray Drying Carrier Screening

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

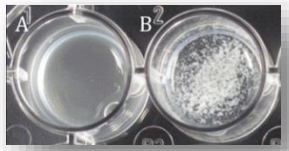
- **Problem to overcome**
  - Problem with auto-aggregation
  - Activity loss after spray drying
  - Formulation issues without carrier
- **Carrier selection and Screening**
  - High water soluble
  - Cheap
  - Food approved
  - Activity conservation
  - Compliance
- **Spray drying optimization**
  - Temperature profile
  - Feed concentration
  - Residual moisture

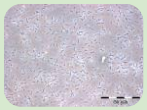
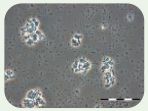
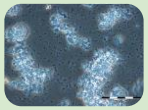
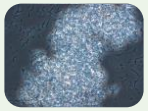
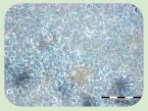
Spray drying @ lab scale

Product testing

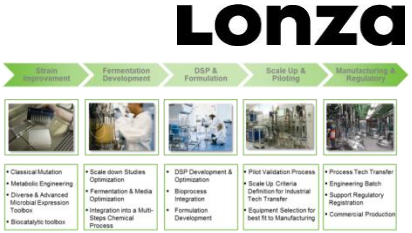
Co-Aggregation Activity level

Negative 0% Positive 100%



Suitable carrier ?	Carrier Type	Inlet Air Temperature Spray dryer	Outlet Air temperature Spray dryer	Feed concentration	Auto-Aggregation level <25%	Co-Aggregation Activity > 50%	Microscopic picture of co-aggregation
- NO	**Carrier 1**	High	Low	High	**Negative - / 0%**	**Negative - / 0%**	
- NO	**Carrier 2**	High	High	Low	**Negative - / 0%**	**Neutral +/- / 25%**	
- / + NO	**Carrier 2**	High	Low	High	**Negative - / 0%**	**Positive + / 50%**	
++ YES	**Carrier 3**	High	High	Low	**Negative - / 0%**	**Positive ++ / 75%**	
+++ YES	**Carrier 3**	High	Low	High	**Negative - / 0%**	**Positive +++ / 100%**	

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 then 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.