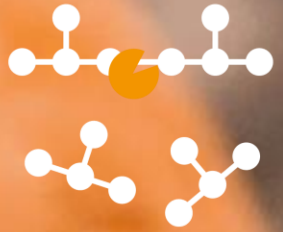




More than 35 years of pioneering expertise

BASF Enzymes

The science of making more from less



BASF White Biotechnology & Feed Enzyme Innovation

Arne Solbak

AF&GA Nutrition Seminar
Huntsville, Alabama











The science of sustainable feed that succeeds

BASF's feed enzymes ►

Support breakdown of antinutritional substances feed stuffs

Antinutritional substances in main feed ingredients and corresponding enzyme product:

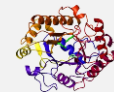
| Rye | Barley | Wheat | Maize | Soybean Meal | Rapeseed Meal | Sunflower Meal | Palm Kernel Meal |
|---|---|---|--|---|---|---|---|
|  |  |  |  |  |  |  |  |
| Phytate | Phytate | Phytate | Phytate | Phytate | Phytate | Phytate | Phytate |
| Arabinoxylans | Arabinoxylans | Arabinoxylans | Arabinoxylans | Arabinoxylans | Arabinoxylans | Arabinoxylans | Arabinoxylans |
| β-Glucans | β-Glucans | β-Glucans | | | | | |
| | | | | β-mannans | β-mannans | β-mannans | β-mannans |

Corresponding products:

Natuphos® E



Natugrain® TS



Natupulse® TS

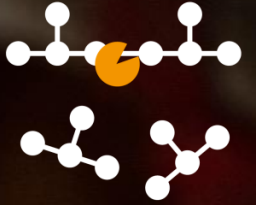


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BASF's feed enzymes

- Helps drive more sustainable animal protein production
- Supports Mitigation of high feed costs and reduce inorganic phosphate requirements
 - Enhance nutrient digestibility of feed ingredients
 - Reduce raw material resource dependencies
- Phytase-driven reduction in phosphorus excretion to limit eutrophication
- Supports animal vitality and gut health

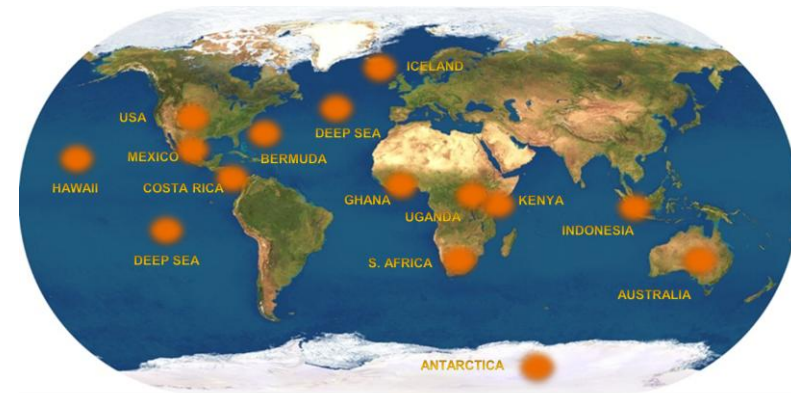
BASF – „The science of making more from less“

Leveraging nature's toolbox to enhance digestibility

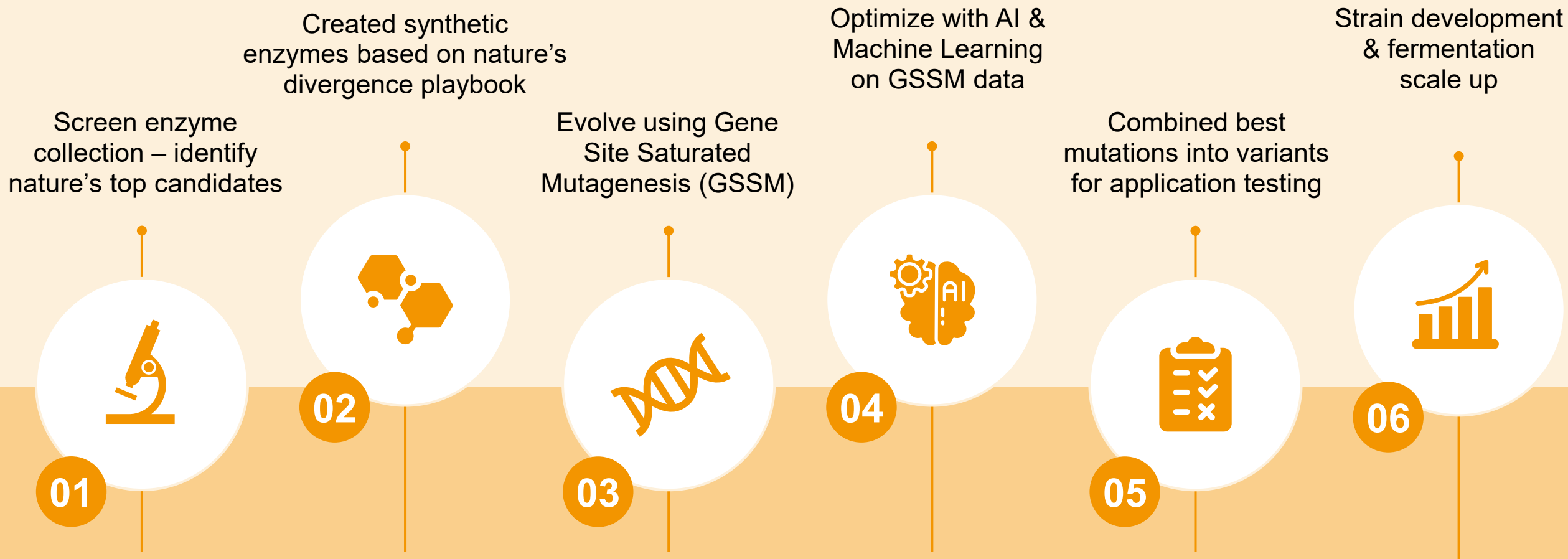
- 2000+ Environmental Libraries
 - ▶ Diverse global sampling from microbial hotspots to routine environments
 - ▶ Fully compliant with the Nagoya Protocol / Convention on Biological Diversity
- Deep proprietary genome collection
 - ▶ >130,000 strains of which ~100,000 functionally annotated

Nutrition and White Biotechnology Competencies

- Integrated platform from discovery through commercialization
 - ▶ High-throughput screening & protein evolution
 - ▶ Advanced strain engineering & fermentation technologies
 - ▶ Predictive *In vitro* digestive models
 - ▶ Real-world validation across network of research farms



BASF Biotechnology Innovation Platform



BASF Biotechnology Innovation Platform

01



Screened enzyme collection – identified top candidates

02



Created synthetic diversity based on nature's divergence playbook

03



Evolve using Gene Site Saturated Mutagenesis (GSSM)

04



Optimize with AI & Machine Learning on GSSM data

05



Combined best mutations into variants for application testing

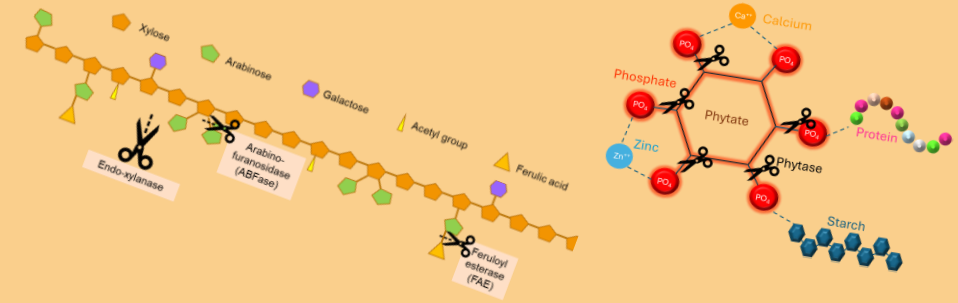
06



Strain development & fermentation scale up

Unparalleled Access to Nature's Biodiversity

Identify anti-nutritional mitigating & beneficial biochemistry

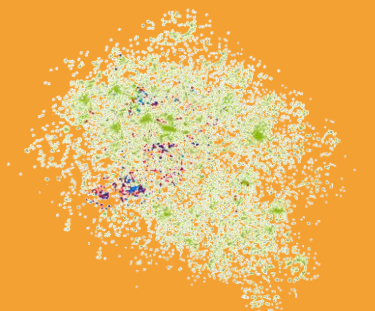


Screen for enzyme activities & ID sequences → explore metagenomic databases



Identify best starting point for optimization
Nature vs. Synthetic

- Natural Sequence
- Generative Model 1
- Generative Model 2
- Generative Model 3



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Strain development & fermentation scale up

Screening & Evolution Parameters

Application
Performance

Manufacturing
Performance

Temperature pelleting stability
pH performance
Substrate promiscuity
Inhibition Relief
Catalytic Efficiency
Gastric Stability
Substrate → product profile
Binding affinity (Km)
Substrate specificity
Solvent tolerance

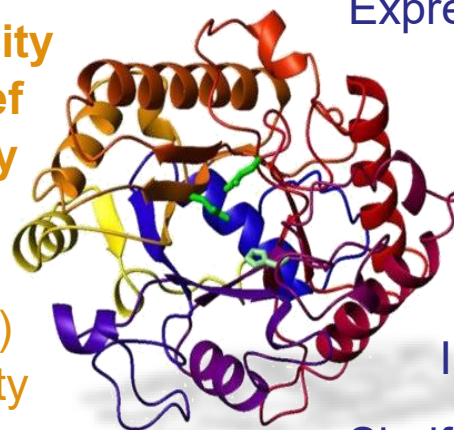
Expression levels

Protein quality

folding, resistance to degradation, control of glycosylation







Improved recovery

Shelf-life

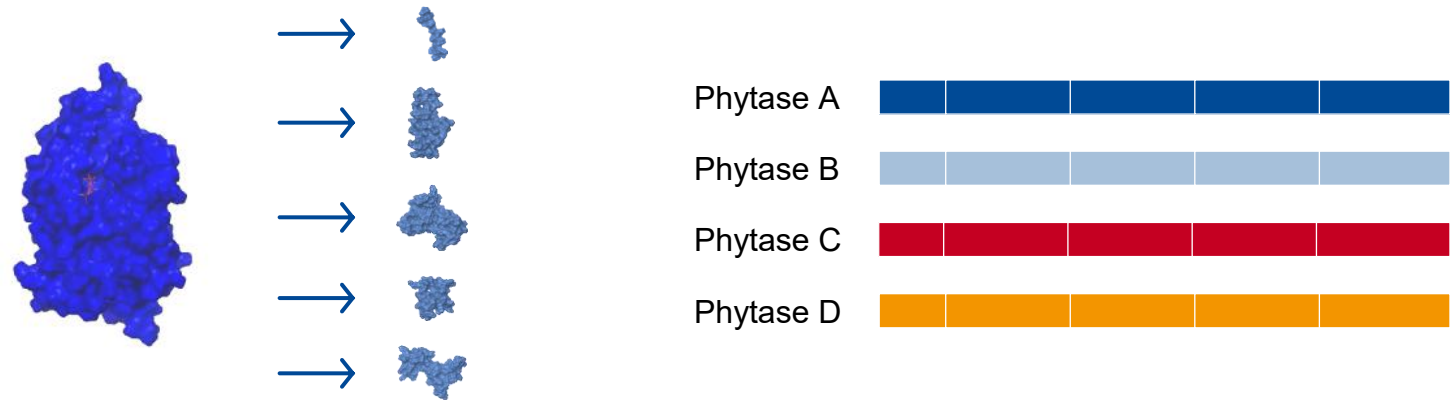


- High-throughput Enzyme Challenge Assays Designed to Identify Mutation Up Mutants
- Multi-plex Evolution Assays to target several evolution targets

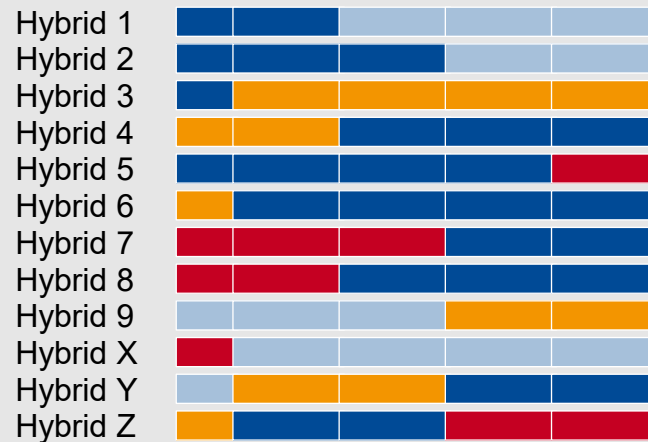
BASF Biotechnology Innovation Platform

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Creation of a Novel Hybrid 6-Phytase



Construction of hybrids

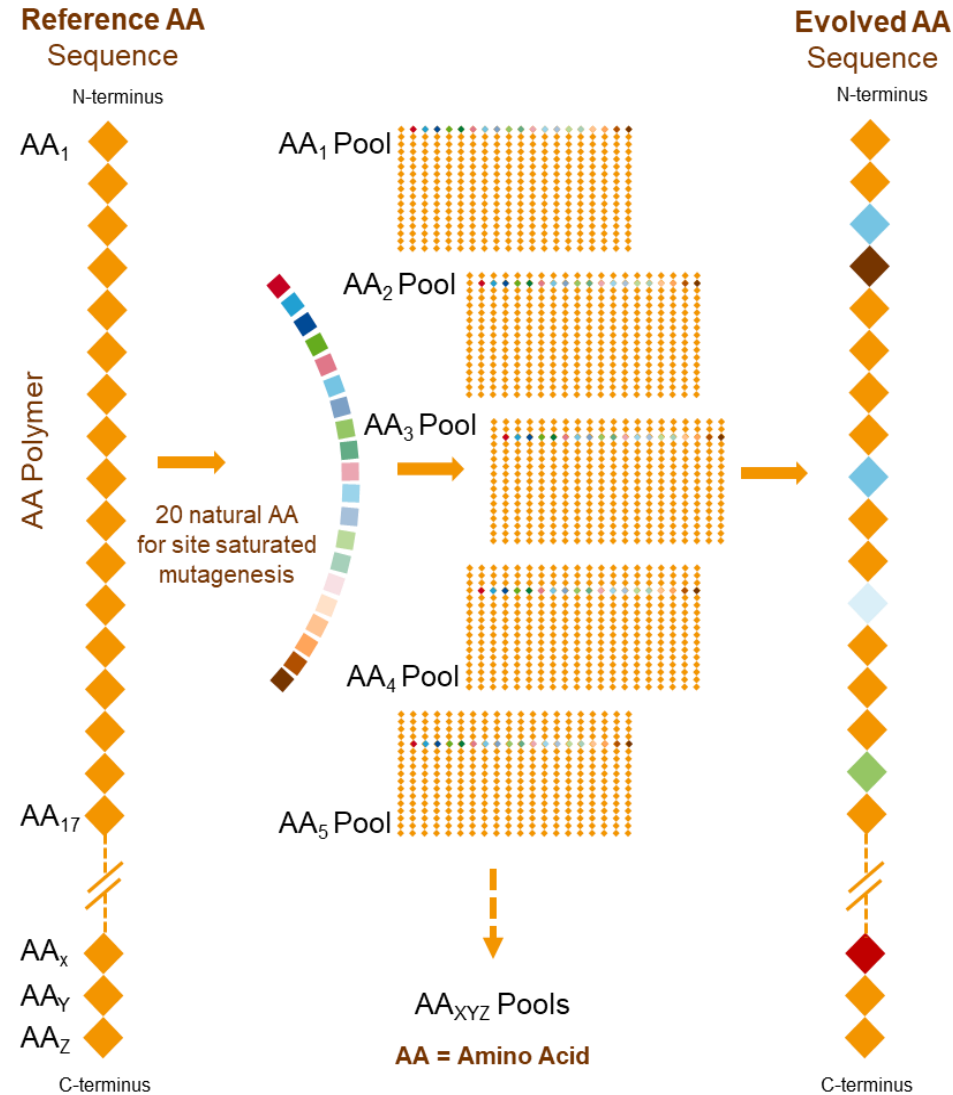


BASF Biotechnology Innovation Platform

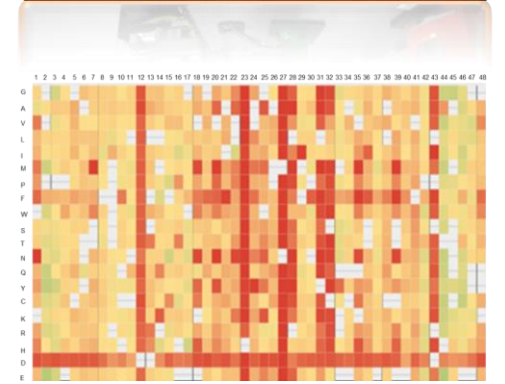
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Directed Evolution Strategy

GSSM Library Overview






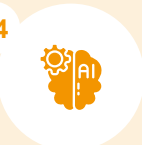


High-throughput activity screening enables...

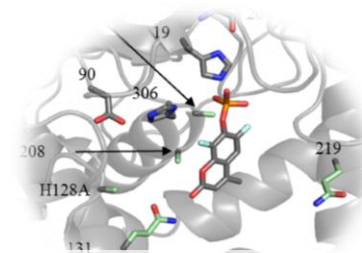
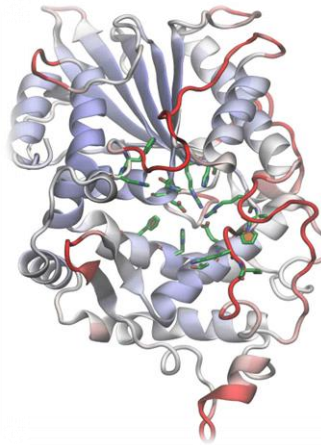


Down mutant Up mutant

...comprehensive data for Machine Learning

BASF Biotechnology Innovation Platform

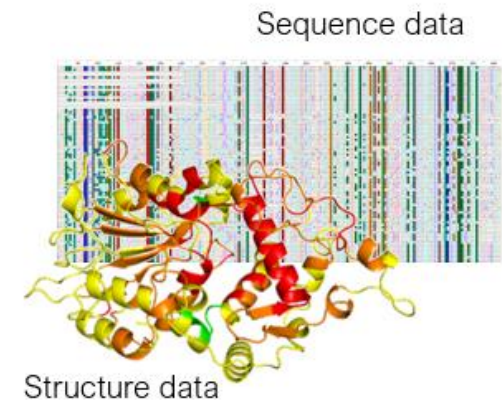
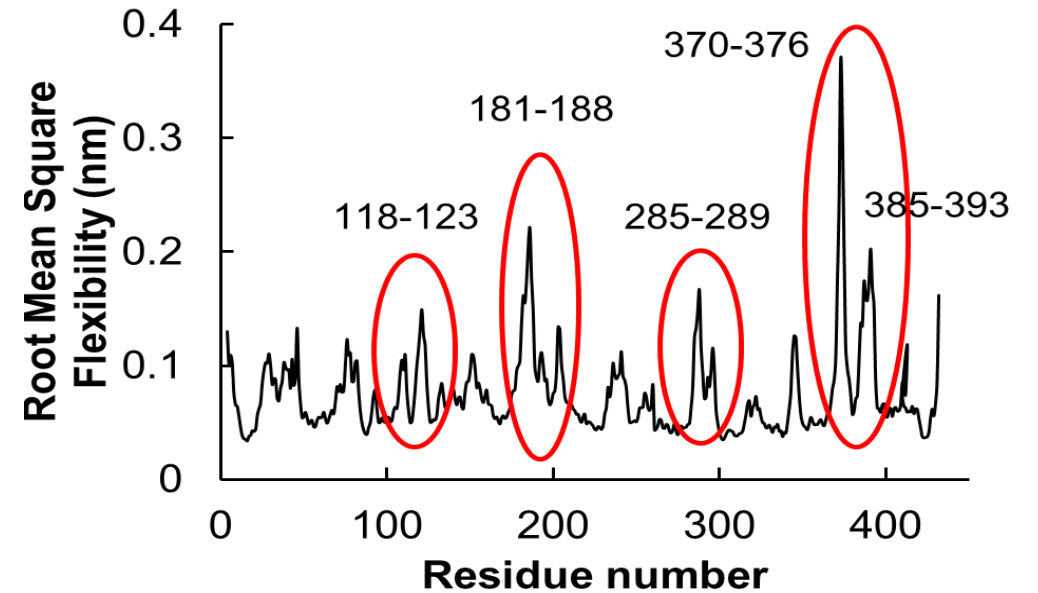
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Substrate docking modelling

Computational Biology AI, Machine Learning, & Structural Insights

Protein Molecular Dynamics Simulation



BASF Biotechnology Innovation Platform



01 Screened enzyme collection – identified top candidates



02 Created synthetic diversity based on nature's divergence playbook



03 Evolve using Gene Site Saturated Mutagenesis (GSSM)



04 Optimize with AI & Machine Learning on GSSM data

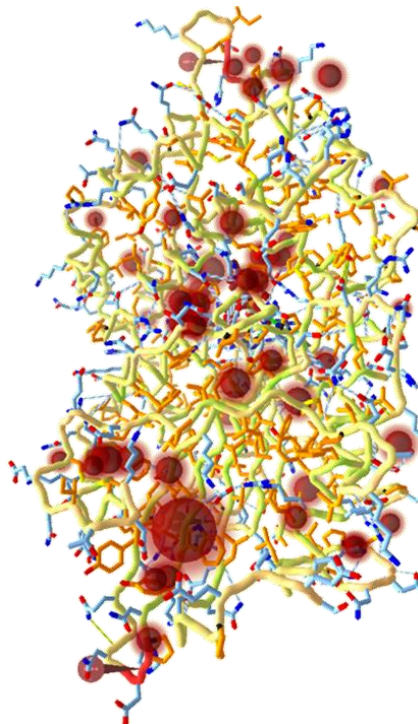


05 Combined best mutations into variants for application testing

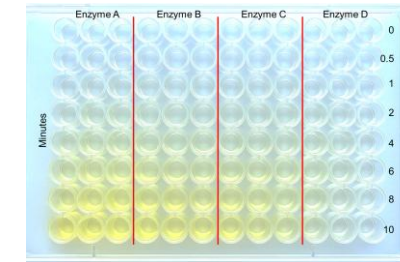


06 Strain development & fermentation scale up

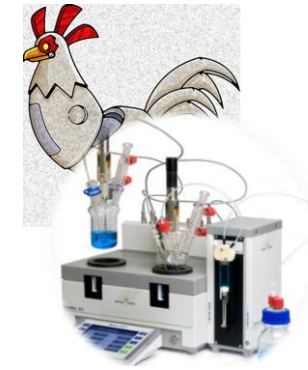
- ***In vitro*** studies
- **Pelleting Trials**
- ***In vivo*** Trials



Wet Lab → Application Studies



Colorimetric Phytase Assay



Mechanical Chicken



Biochemistry
R&D

Application
R&D

Field
Research

BASF Biotechnology Innovation Platform

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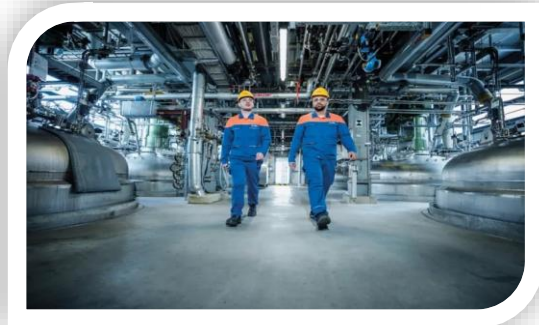
06



Strain development & fermentation scale up

Microbial Strain & Process Development

- Engineered fungal strains play a central role in our biotech platform
- Strains designed for safe deployment and high productivity to meet regulatory and commercial targets
- Process development starts at research scale (0.2 L – 1500L) and then advances to production scale (80,000 L)



BASF Biotechnology Innovation Platform

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Strain development & fermentation scale up

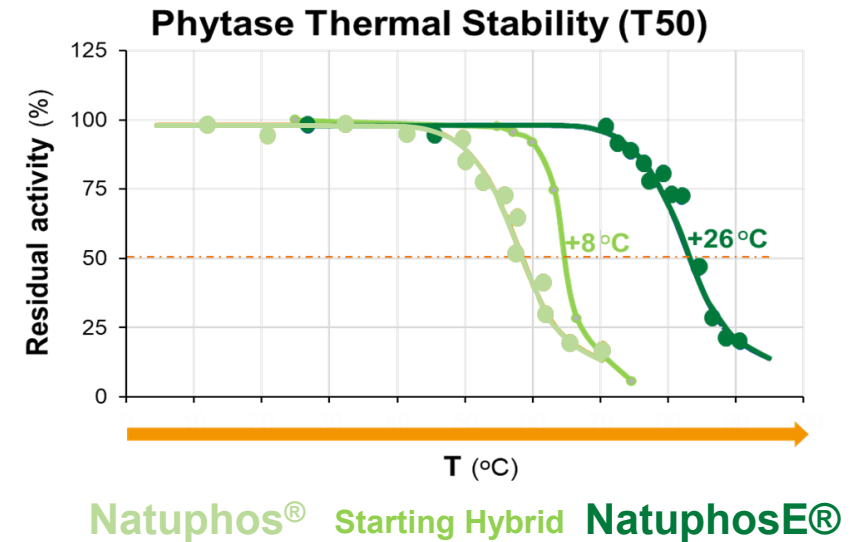
14

Evolution Proof Points

natuphos[®] E

6-Phytase

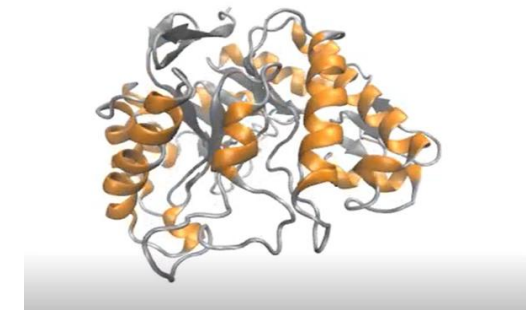
- Improved Thermal Stability (+26°C)
- Improved phosphorous release
- Improved Gastric Stability (w/pepsin)
- Improved Specific Activity



Natupulse[®]

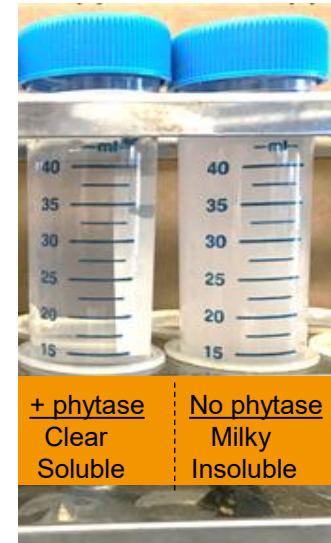
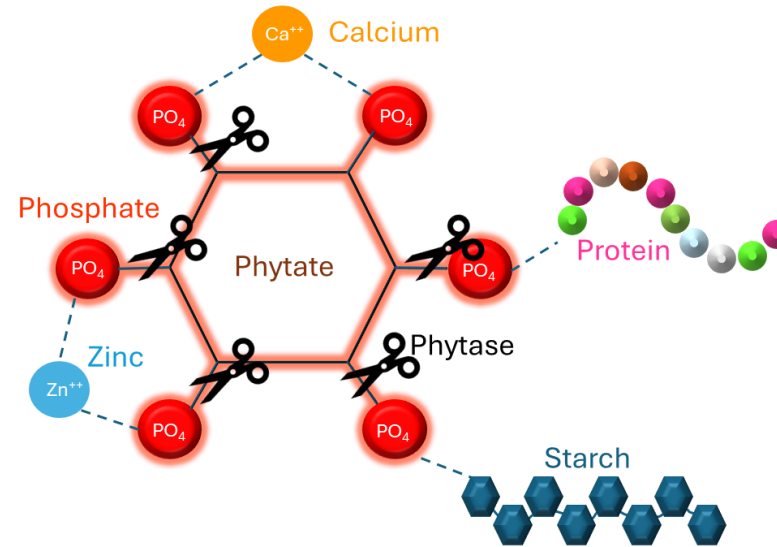
Mannanase (GH5)

- Improved Thermal Stability (+10°C)
- Improved Gastric Stability (w/pepsin)



Natuphos® E

Phytase Mechanistic Studies

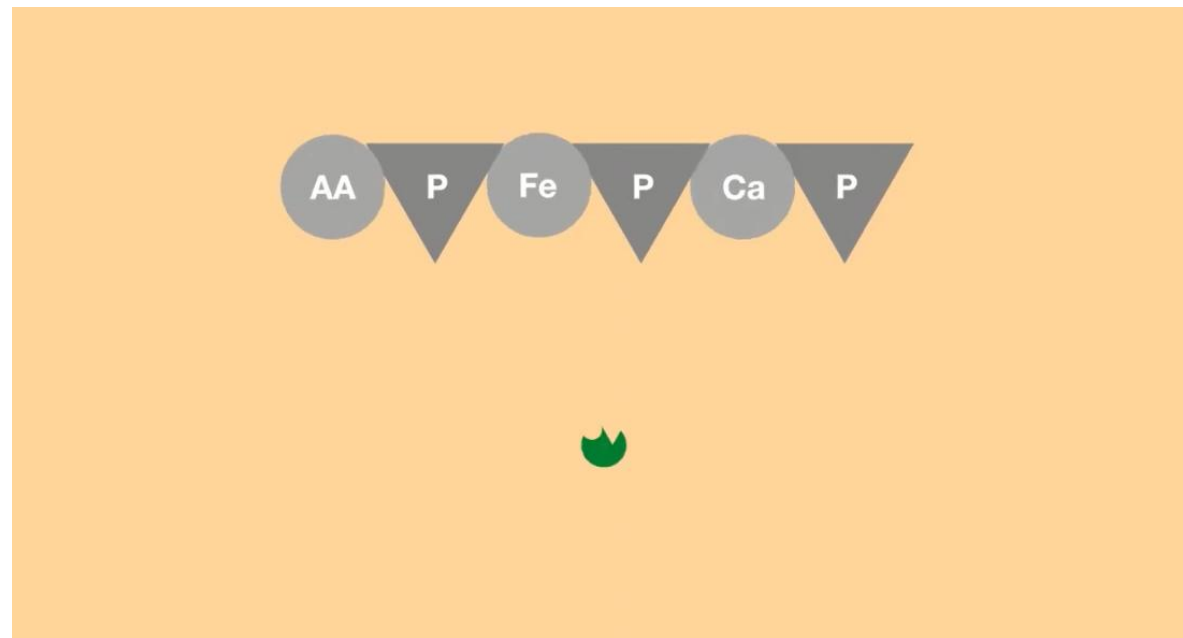


Phytate mineral complex at pH 5.5

- Copper and zinc – strongest phytate binding → reduced bioavailability.
- Iron forms insoluble complexes in intestine (pH 6–8) → reduced bioavailability.
- Calcium has weaker affinity for phytate, still produces precipitates at neutral–alkaline pH
- Seed storage protein (cereals & legumes) form protein-phytate complexes

Toda and Hajika, 2018, Food Science and Technology Research

Benefits of Our Feed Enzymes



Natuphos® E (hybrid 6-Phytase)

Main effect –

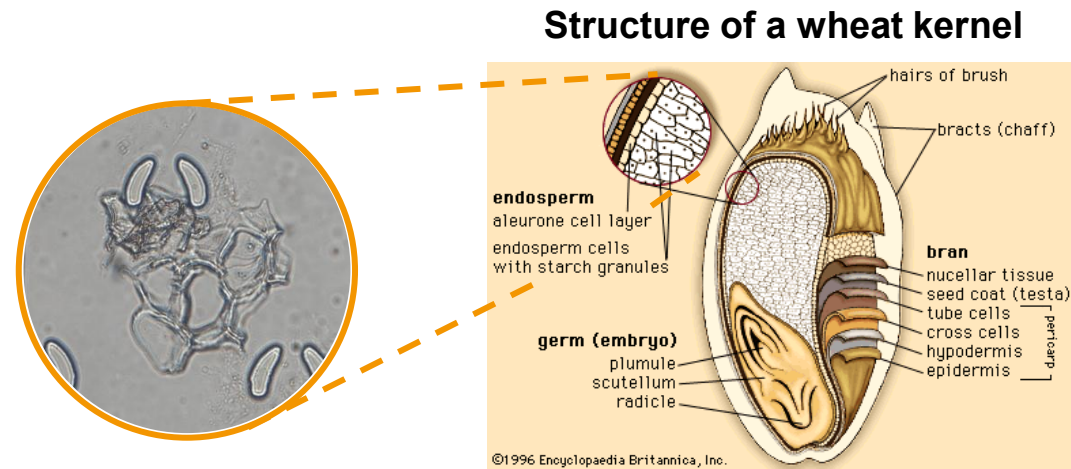
Release Phytate Bound Phosphorus

Secondary effect –

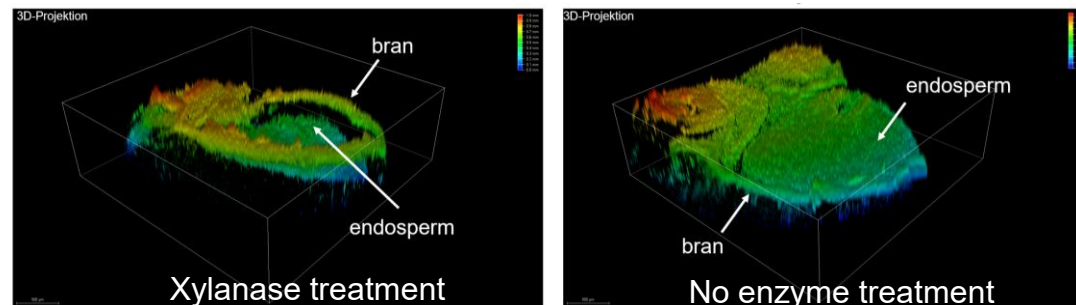
Improve Ca, Zn, starch, & amino acid digestibility

Natugrain® TS

Xylanase Mechanistic Studies



Microscopy Studies
Breakdown of insoluble xylan
(aleurone layer)

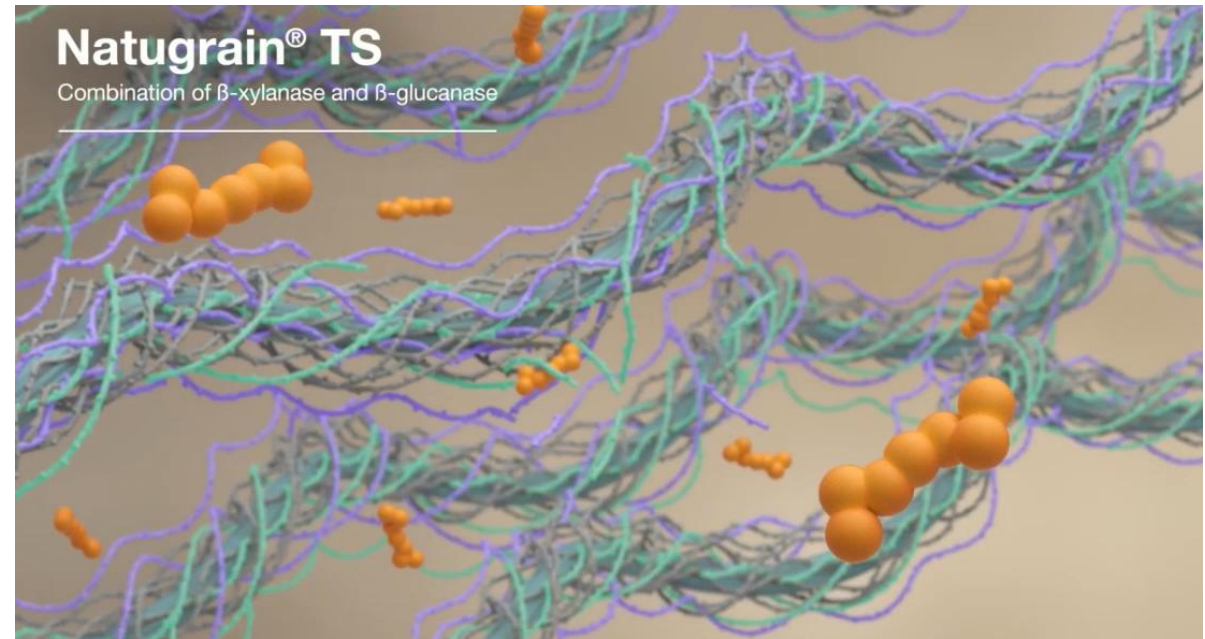


**Confocal Fluorescence
Microscopy Studies**
Breakdown of insoluble xylan
(aleurone layer)

Wheat kernel treatment – significant amount of the endosperm released with xylanase treatment compared to no enzyme treatment

BASF
Biotechnology
Innovation Platform

Benefits of Our Feed Enzymes



Natugrain® TS (GH10 Xylanase and Glucanase)

Main effect –

Reduce gut viscosity by hydrolyzing xylan polymers

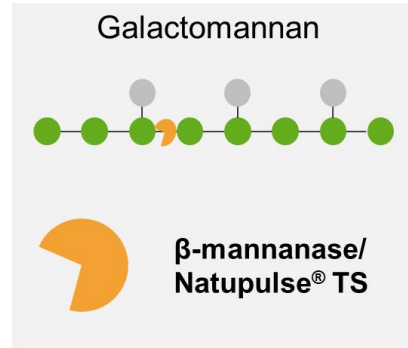
Secondary effect –

Release trapped nutrients

Generate smaller prebiotic xylan oligosaccharides (XOS)

Natupulse® TS

Mannanase Mechanistic Studies

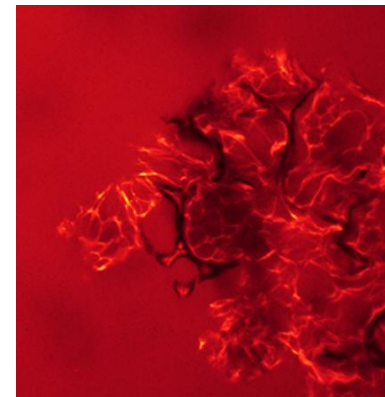
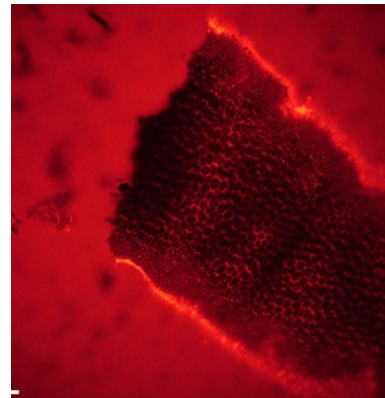


Natupulse® TS hydrolyzes
β-mannans

Adding the
endo-1,4-β-D mannanase
Natupulse® TS
to the diet, decreases
the viscosity



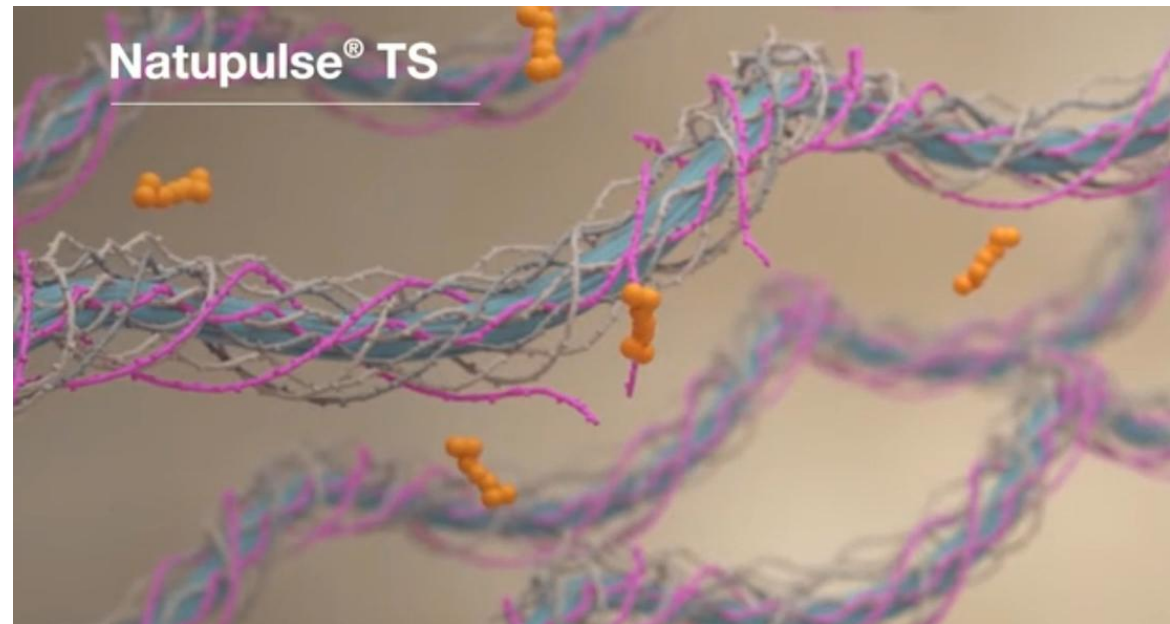
Decreases the viscosity (left)



Natupulse® TS was active (highlighted areas show activity of mannanase) at the cell wall structure of the soy hull (left) and intracellularly and at the middle lamellar structure (right).

Rueckel et al., 2024, Nature scientific reports

Benefits of Our Feed Enzymes



Natupulse® TS (GH5 Mannanase)

Main effect –

Reduce gut viscosity by hydrolyzing mannan polymers

Secondary effect –

Increase soybean cell wall permeability
Generate smaller prebiotic mannan oligosaccharides (MOS)

Shaping Tomorrow Through Continuous Innovation

- Innovation is Our Ongoing Journey
- Next generation feed enzymes are just around the corner

„The science of making more from less“ continues

- ▶ Expand Flexibility in Feed Formulation
 - Enhancing enzyme performance → digestibility & gut health
 - Introducing greater pellet stabilities
- ▶ Increase value for the industry
 - Additional reductions in overall feed costs
 - Further reduction of nitrogen and phosphorus excretion



We're dedicated to providing the right BASF enzyme product offerings for you and your customers



BASF Enzymes

- Natuphos® E
(Phytase)
- Natugrain® TS
(Xylanase, Glucanase)
- Natupulse® TS
(Mannanase)

Enhance nutrient
digestibility

More than 35 years of pioneering expertise

BASF Enzymes

The science of making more from less





We create chemistry