Using Starch Binding Domain (SBD) technology to modify starch granule morphology and starch composition in potato

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Various levels of polymer organization within the starch granules
Starch granules from various plant species can have different appearance, composition, structure and properties related to the varying levels of starch biosynthetic enzymes among plant species and (slightly) different substrate specificity of the enzymes of different species.
Possible starch modifications

- starch yield
- reduction of contaminating substances such as lipids
- granule size distribution
- ratio amylose/amylopectin
- chain-length distribution
- degree of phosphorylation

- endogenous enzymes
- heterologous enzymes
  - “glycogen enzymes”
  - granule boundness
  - linkage types
The “easy” modifications have all been performed

- **Antisense GBSS**: in many crops including potato and cassava. ~100% amylopectin, starch with different physicochemical properties

- **Antisense BE**: in potato. Increased apparent amylose content to ca 60%; starch with different physicochemical properties

- **Overexpressing different other genes encoding BE, AGPase, R1, etc. etc.**
Partitioning of enzymes during starch biosynthesis

- Exclusively soluble enzymes
- Partially granule-bound enzymes
- Exclusively granule-bound enzymes
Granule-bound enzymes often have a larger impact on the structure of the starch granule.

- GBSSI: Amylose yes or no
- SBE: A or B-type crystalline
Can we make “artificial” granule-bound enzymes to generate different starches with new or improved functionalities?
Targeting of proteins to the starch granule in the amyloplast in the potato tuber
Principle of SBD technology

SBD

favorite protein

bioynthesis

catalytic activity

degradation
• Modifying starch granule size and granule size distribution
  - smaller granules for printing, baby food, fat replacers
  - bigger granules for mouth feel
Schematic depiction of pBIN19 base expression vectors used for potato transformation
Antibodies against SBD

- KDS
- KDS4
- KDSS
- KDS5
- KDS3
- amfSS

Frequency (%)

SBD accumulation class

0+ 1+ 2+ 3+ 4+ 5+ 6+SBD accumulation class
Antibodies against SBD

Frequency (%)

SBD accumulation class

0+ 1+ 2+ 3+ 4+ 5+ 6+

KDS KDS4
KSS KDS5
KDS3 amfSS
Light micrographs of the Starch granules
SEM analysis of transgenic starch granules in amf
Alpha amylase treatment - +

Particle diameter (μm)

Number (%)
Granule size distributions for starches of the various SBD2 accumulation classes
Expression of an amylosucrase from *Neisseria polysaccharea* fused to a starch binding domain in potato
Amylosucrase retains activity in fusion proteins

Expression of protein in *E. coli* and demonstration of activity on agar plates

- **AS**
  - Large diffuse halo

- **AS-SBD**
  - Small intense halo

- **SBD-AS**
  - Small intense halo
Summary of these results

- Smaller granules
  - <6 μM mean
  - 1. Double SBD transformants
  - 2. Wider granule size distribution
  - 3. Sometimes even bimodal
  - 4. No physico chemical changes of starch

- Bigger granule sizes
  - ~ 50 μM mean
  - 1. Amylosucrase linked to SBD
  - 2. Narrower distribution
  - 3. Physico chemical changes:
    - Lower peak viscosity;
    - higher end viscosity (up to ~ 35 μM)

Nazarian et al Planta 2007

Nazarian et al to be submitted
Production of ‘different’ polymers

- Activities which can change starch characteristics during biosynthesis or postharvest
- New carbohydrate polymers
- In planta derivatisation
Expression of an engineered granule-bound *mutan sucrase* in wild-type and *amf* potato plants

Nazarian et al Transgenic Res 2007
mutansucrase SBD constructs

A) NPTII NOS promoter
Patatin pro. FD SBD GTFICAT
Xhol Smal Smal EcoRI/EcoRV Xhol

B) NPTII NOS promoter
Patatin pro. FD GTFICAT SBD NruI EcoRV Xhol
Xhol

1,000 bp

- NOS promoter
- NOS terminator
RNA expression levels

Figure 2

RNA expression levels

A

B
Staining with erythrocine
SEM pictures wt background
amf background
Expression of an engineered granule-bound *E. coli* maltose acetyl transferase in wild-type and *amf* potato plants

Nazarian et al Plant Biotech J 2007
Starch acetate

- Increase in hydrophobicity of starch
- Good freeze-thaw stability
- Lower gelatinization temperature
- Clear pastes
SEM images of different transgenic starches in *amf*
SEM images of different transgenic starches in Kardal
Post harvest MAT activity
In summary

- Starch was found acetylated, however starch properties did not change dramatically.

- MAT was found active inside the starch granules.

- As a result of fusion proteins accumulation, starch granule morphology was changed in both backgrounds (only about 10%)
In conclusion

- Potato starches with different granule sizes and granule size distribution can be made
  - Relatively little changes in physico chemical properties
  - Background depended

- New polymers can be made in starch granules
  - Mutan, but also alternan, dextran and fructan
  - Very low amounts in all backgrounds
  - Changes in granule shape (only percentage of population)
  - Sometimes (severe) effects on physico chemical characteristics

- Enzyme activities can be introduced which change starch properties
  - MAT was found active inside the starch granules
  - Other activities are underway
  - Changes in granule shape (from low to very high percentage of population)
  - Sometimes (severe) effects on physico chemical characteristics
Plant Breeding

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Starch uses

- Starch is used in many different daily products
  - Food
  - Feed
  - Technical applications: Paper, textile, oil drilling, pharmacies, coating etc
- 80% of all starch is derivatised (chemically modified); 20% is used in native form
- Largest production by maize (20 million tonnes per year, 90% converted in HFS for softdrinks) followed by potato, cassava and wheat