Searching for Amylase

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Video II: The Unity of Living Systems

Food chemists utilize a surprising variety of microbes in the manufacture of processed foods. The next time you snack on chips, cookies, ice cream, or a soda, look at the ingredients listed on the packaging. Chances are you'll find maltodextrin (partially hydrolyzed food starch) and either dextrose or high fructose corn syrup among them.

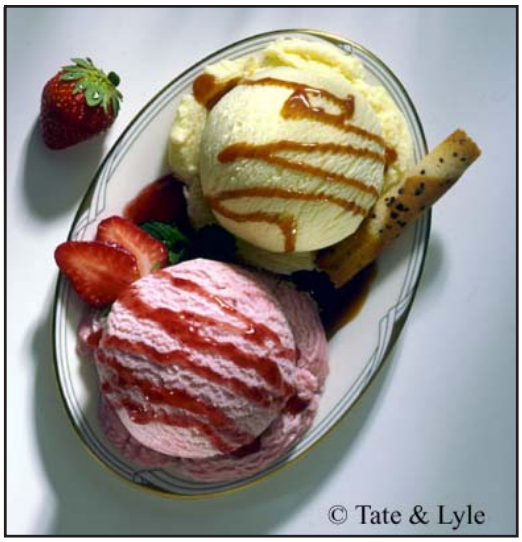

Figure 1. This commercial ice cream product contains both maltodextrin and high fructose corn syrup.

Although corn starch is used to make each of the ingredients above, the specific process differs. The breakdown of starch must be carefully controlled using both physical and chemical means. Enzymes useful in corn starch processing have been isolated from a number of organisms, many of which are microbes. For example, an alpha-amylase found in *Bacillus licheniformis* is commonly used to make maltodextrins.

Alpha-amylase breaks down starch by cleaving alpha 1,4 linkages between glucose monomers randomly within the chain. The partially hydrolyzed starches that result are then removed as maltodextrins. All alpha-amylases will do this, but with different efficiencies under different conditions. Most bacterial amylases, for example, are active at temperatures as high as 50 to 70 degrees Celsius. However, the alpha-amylase from *B. lichenformis* is active at 90 degrees Celsius, which is also the temperature necessary to dissolve corn starch in water. Alpha-amylases found in humans lose reactivity at such high temperatures.

One of the most valuable products from starch in today's market is high fructose corn syrup. This ingredient often replaces sugar to enhance flavors or add

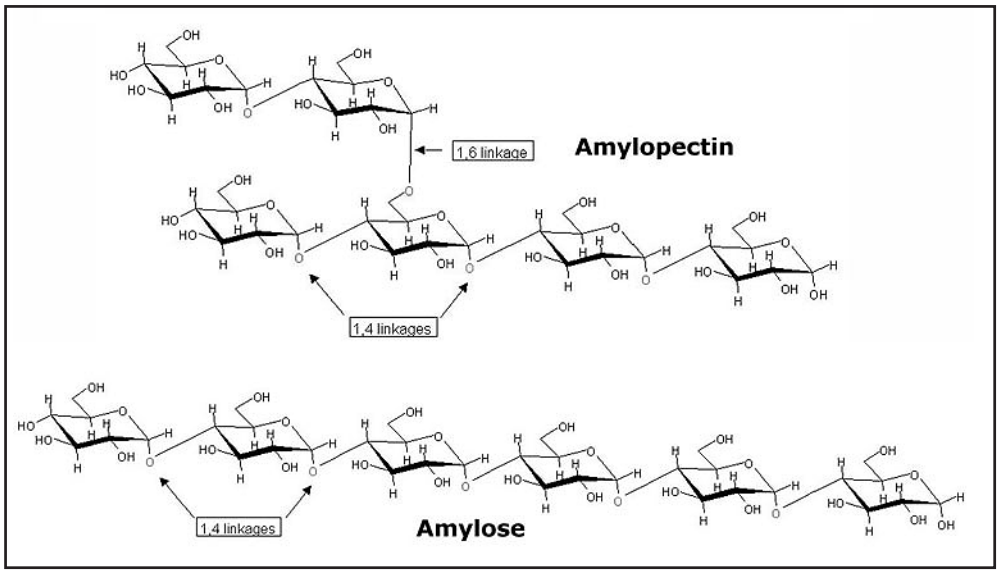

Figure 2. Starch is composed of amylopectin and amylose. The straight chain amylose contains 1,4 linkages between glucose monomers. Amylopectin is branched with 1,6 linkages in addition to 1,4 linkages. (The actual amylose and amylopectin molecules contain thousands of glucose monomers.)

sweetness to products like soda pop. To make high fructose corn syrup, starch is first converted to high dextrose corn syrup by:

1. adding alpha-amylase to the heated starch solution to produce smaller starch fragments,
2. treating with glucoamylase (an amyloglucosidase), which hydrolyzes the starch fragments by cleaving glucose units from the non-reducing end, and
3. in some cases, simultaneously adding a debranching enzyme such as pullulanase that hydrolyzes starch by cleaving alpha 1,6 linkages.

Finally, to produce high fructose corn syrup, a glucose isomerase is used to partially convert the dextrose to fructose.

Microbial enzymes have been examined and found to be effective in other kinds of corn starch processing as well. Table 1 shows some of the microbes that produce enzymes commonly used in industry.

Table 1: Sources for the industrial enzymes used to make high fructose corn syrup.

Microbe	Enzyme
Bacillus licheniformis	Alpha-amylase
Aspergillus niger	Glucoamylase
Klebsiella aerogenes	Pullulanase
Bacillus circulans	Glucose isomerase

- Do any of the organisms in Table 1 make starch themselves?
- Why do you think these organisms produce starch-processing enzymes?

Searching for Industrial Enzymes

What makes one alpha-amylase better than another for specific starch processing tasks? What do chemists look for?

Thermal stability at high temperatures is one desirable characteristic for starch processing. Specificity (how an enzyme interacts with a substrate) is even more important. Enzymes have active sites which include both catalytic sites and binding subsites. While the amino acid sequence of different alpha-amylases may vary, there are specific aspartic acid and glutamic acid units found in the beta-strands of the molecule that are responsible for the catalysis of glycosidic bond cleavage. Other amino acids such as histidine are involved in establishing conformation and binding of the substrate. Binding subsites also affect enzyme efficiency. For example, pig alpha-amylase and barley alpha-amylase have similar catalytic sites, but their binding subsites are different. Pig alpha-amylase has five subsites, while as many as ten subsites are suspected in barley. (MacGregor, 1993)

How do you find new sources of industrial enzymes?

Well, you don't necessarily have to go to the field. Today, accessing data from existing molecular databases and utilizing online bioinformatics and visualization tools are invaluable in tracking down candidates for the next generation of industrial microbes.

You can find sequence information for an enzyme like the TAKA-amylase from *Aspergillus oryzae,* which is used commercially, by searching online molecular databases such as SWISS-PROT. Specifying keywords such as amylase and Aspergillus, an accession number, or a specialized label such as AMYA_ASPOR will allow you to isolate the protein and its molecular information. In the *Biology Workbench*, the search function is called Ndjinn.

(For an overview of the *Biology Workbench* and how it is organized, please see the "Orientation to the *Biology Workbench*" document on the *Microbes Count!* CD. You may also want to take a look at the "Proteins: Historians of Life on Earth" and "Tree of Life: An Introduction to Microbial Phylogeny" activities in Chapter 6 for some examples of using the Biology Workbench.)

In Figure 3, the highlighted sequence GLRIDTVKH is a conserved region that functions as an active site in alpha amylase. See Table 2 for more active site information.

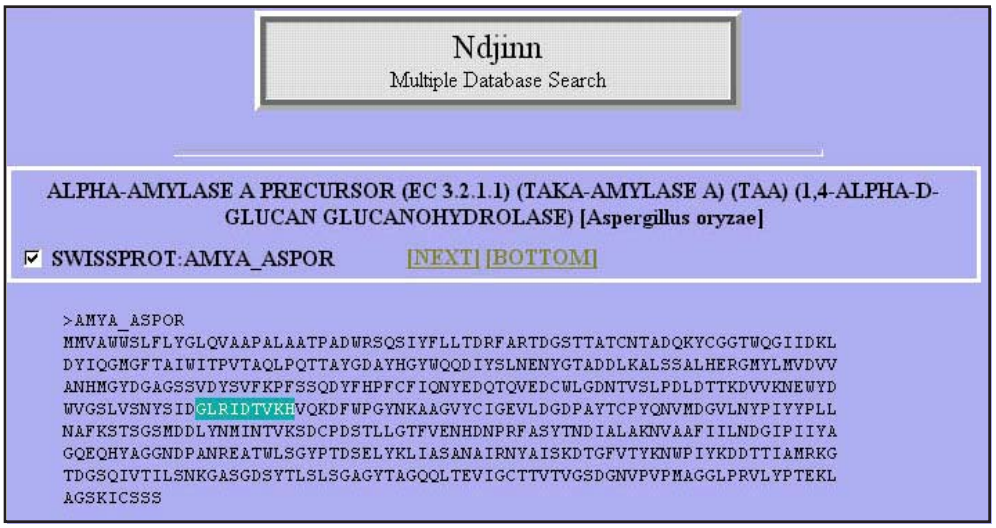

Figure 3. The amino acid sequence for AMYA_ASPOR in the SWISSPROT database was retrieved using Ndjinn on the *Biology Workbench* site.

Starting and ending position of residues	Conserved sequence (Highly conserved in bold)	Some known structure - function relationships	
116 - 122	V**D**VVA**NH**	Subsite 1	H = His 122
202 - 210	GL**RID**TVKH	Catalytic site Subsite 1 Subsite 2	D = Asp 206 H = His 210 K = Lys 209
230 - 233	**E**VLD	Catalytic site	E = Glu 230
293 - 298	VEN**HD**N	Catalytic site Subsite 1	D = Asp 297 H = His 296

Table 2: Well-conserved regions of alpha-amylase from *Aspergillus oryzae* with locations of specific amino acids and known structure-function relationships.

The AMYA_ASPOR sequence can be used with a BLAST procedure to obtain the records for proteins having similar sequences (Figure 4).

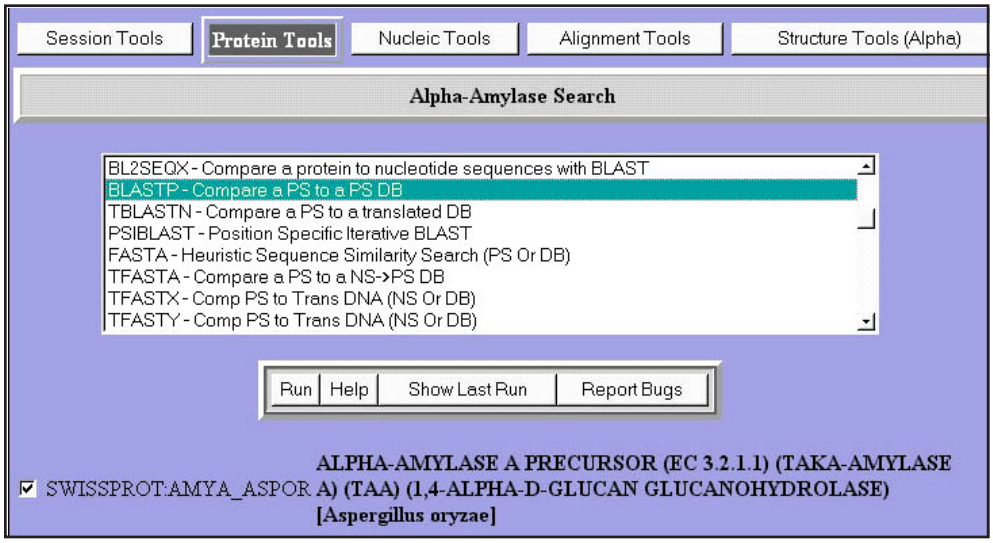

Figure 4. Searching for similar protein sequences using BLASTP on the *Biology Workbench* site.

A subset of the sequences for these similar proteins are chosen for further study. The sequences are extracted and compared systematically with the CLUSTALW procedure which aligns the sequences (Figures 5).

```
AMY_BACCI           NNSTIDTYFKNAIR-LWLDMGIDGIRVDAVKHMPFGWQKNWMSSIYSYKPVFTFGEWFLG
CDGT_THETU          QNSTIDSYLKSAIK-VWLDMGIDGIRLDAVKHMPFGWQKNFMDSILSYRPVFTFGEWFLG
AMYR_BACS8          NNSTSDVYLKDAIK-MWLDLGIDGIRMDAVKHMPFGWQKSFMAAVNNYKPVFTFGEWFLG
AMY3_WHEAT          LNPRVQRELSAWLNWLKTDLGFDGWRLDFAKGYSAAMAKIYVD---NSKPAFVVGELY--
AM3C_ORYSA          LNTRVQTELSDWLNWLKSDVGFDGWRLDFAKGYSATVAKTYVD---NTDPSFVVAEIWSN
6056337_6056338     EKDYVRSMIADYLN-KLIDIGVAGFRIDASKHMWPGDIKAVLDKLHNLNTNW-FPAGSRP
397984_397985       GNQWVRDRIVDLMN-KCVGYGVAGFRVDAVKHMWPGDLEHIYSRLNNLNTDHGFPHGAKP
AMYA_ASPOR          TKDVVKNEWYDWVGSLVSNYSIDGLRIDTVKHVQKDFWPGYN----KAAGVYCIGEVLDG
```

Figure 5. Sample output showing a portion of the aligned sequences using CLUSTALW on the *Biology Workbench* site.

This subset of aligned sequences can be used to generate trees that group the proteins according to their degree of similarity (Figure 6). Note: this procedure very broadly defines the relationships since not all sequence data carry the same weight.

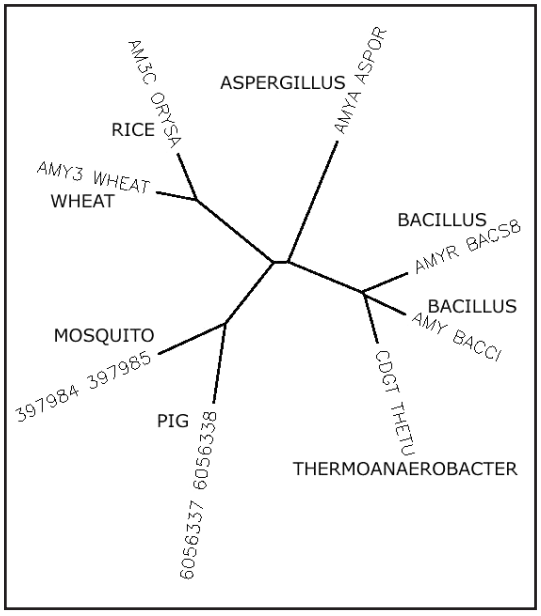

Figure 6. Unrooted tree for selected proteins generated by a ClustalW search on the *Biology Workbench* site.

Label and Name of Organism		Conserved Sequence
AMY_BACCI	*Bacillus*	GIRVDAVKH
CDGT_THETU	*Thermanoaerobacter*	GIRLDAVKH
AMYR_BACS8	*Bacillus*	GIRMDAVKH
AMY3_WHEAT	Wheat	GWRLDFAKG
AM3C_ORYSA	Rice	GWRLDFAKG
6506337_605633	Pig	GFRIDASKH
397984_397985	Mosquito	GFRVDAVKH
AMYA_ASPOR	A*spergillus*	GLRIDTVKH

Table 3. Amino acid sequences that correspond to positions 202-210 in the Aspergillus amylase AMYA_ASPOR are compared for similar proteins found in a selected group of diverse organisms.

Looking at an example with beta-amylases

Beta-amylases are used in starch processing to make high maltose corn syrup which is useful in the brewing industry. Let's use a general bioinformatics approach to look at beta-amylases in several different organisms and make some recommendations for potential use in industry.

Beta-amylases, like all proteins, are made up of sequences of amino acids. The sequences that make up the active sites in the protein tend to be more highly conserved; that is, they do not show as much variation as sequences in the molecule that are not directly involved in enzymatic activity.

Active Site	Conserved Amino Acid Sequence
Site 1	HxCGGNVGD
Site 2	Gx<SA>GE<LIVM>RYPSY

Table 4. Conserved sequences for two of the catalytic sites in beta-amylase.

In Table 4, the amino acid sequence is HxCGGNVGD for active site 1. These one-letter codes represent a different amino acid. For example, H refers to the amino acid histidine. The lowercase x indicates that any one of several different amino acids could be found in that specific position. (See your text book to interpret codes.)

The sequence for active site 2 is Gx<SA>GE<LIVM>RYPSY. Here the letters in brackets, such as <SA>, indicate that any one of the amino acids shown is found in this position.

- Do all of the following sequences fit the sequence algorithm for active site 2?

  GPSGELRYPSY

  GAAGELRYPSY

  GPAGEMRYPSY

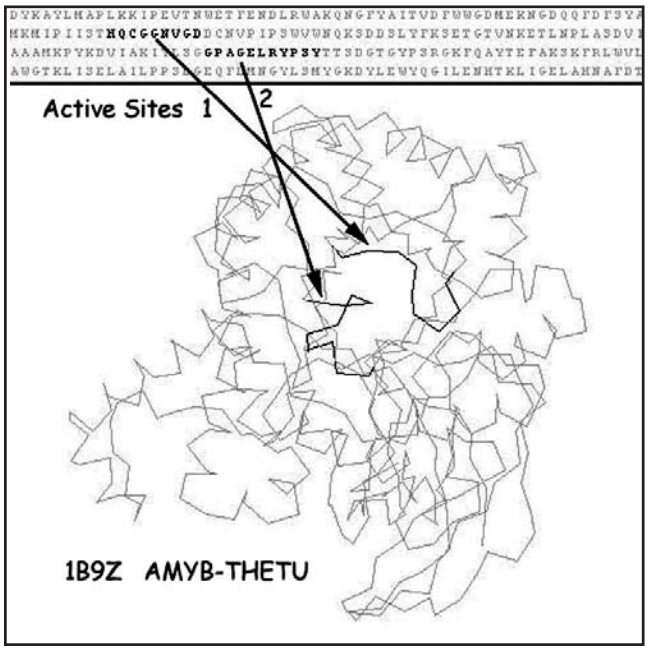

Figure 7: Sequences are matched to the regions of active sites in a wire frame model of the beta-amylase produced by the bacterium *Thermanoerobacter thermosulfurogenes*.

In Figure 7 above, the arrows link two amino acid sequences to their sites in the model of the beta-amylase enzyme. The two catalytic sites appear closer to each other in this model of the enzyme than the amino acid sequences are.

- Suggest a reason for their proximity?

Note: The open area in the center is the substrate binding site.

Scientists can infer something about the expected activity of similar proteins produced in different organisms based on similarities and differences in the active sites. The table below shows beta-amylase sequences from the SWISS-PROT database for two active sites in soybean and the bacterium, *Bacillus circulans*.

- Fill in the sequences for the other organisms by searching SWISS-PROT using the label provided and then aligning sequences with ClustalW.

Label and Name of Organism		Active Site 1	Active Site 2
AMYB_SOYBN	Soybean	HQCGGNVGD	GPAGELRYPSY
AMYB_ARATH	Mouse-Ear Cress		
AMYB_IPOBA	Sweet Potato		
AMYB_HORVU	Barley		
AMYB_PAEPO	*Bacillus polymyxa*		
AMYB_BACCI	*Bacillus circulans*	HRCGGNVGD	GPSGELRYPSY
AMYB_THETU	*Clostridium thermosulfurogenes*		
AMYB_BACCE	*Bacillus cereus*		

- Besides the active sites, what other kinds of information would you like to know about a beta-amylase enzyme before recommending it for use in the starch processing industry?
- AMYB_THETU is a commonly used beta-amylase in corn starch processing. This enzyme is produced by *Thermanoaerobacter thermosulfurogenes (also known as Clostridium thermosulfurogenes)*. Recommend a different beta-amylase for chemists at a corn starch processing plant to work with. Explain why you think it would be a good choice for commercial use.
- Find a beta-amylase you would *not* recommend. Explain.

Web Resources Used in this Activity

Biology Workbench

The *Biology Workbench* was originally developed by the Computational Biology Group at the National Center for Supercomputing Applications at the University of Illinois at Urbana-Champaign. Ongoing development of version 3.2 is occurring at the San Diego Supercomputer Center, at the University of California, San Diego. The development was and is directed by Professor Shankar Subramaniam.

Platform Compatibility: Requires an internet connection and a current web browser.

Additional Resources

Available on the *Microbes Count!* CD

Text

A PDF copy of this activity, formatted for printing

"Orientation to the *Biology Workbench* "

Related *Microbes Count!* Activities

Chapter 2: Sourdough Symbiosis

Chapter 4: Molecular Forensics

Chapter 4: Exploring HIV Evolution: An Opportunity for Research

Chapter 6: Proteins: Historians of Life on Earth

Chapter 6: Tree of Life: Introduction to Microbial Phylogeny

Chapter 6: Tracking the West Nile Virus

Chapter 6: One Cell, Three Genomes

Chapter 7: Visualizing Microbial Proteins

Unseen Life on Earth Telecourse

Coordinates with Video II : The Unity of Living Systems

Relevant Textbook Keywords

Amylase, Enzymes, Monomers, Specificity, Substrate

Related Web Sites (accessed on 3/4/03)

Biology Workbench
http://workbench.sdsc.edu

Industrial Starch Processing
http://home3.inet.tele.dk/starch/

Microbes Count! Website
http://bioquest.org/microbescount

Unseen Life on Earth: A Telecourse
http://www.microbeworld.org/htm/mam/is_telecourse.htm

References

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MacGregor, E. A. (1993). Relationships between structure and activity in the alpha-amylase family of starch-metabolising enzymes. *Starke* 45:232–237.

MacGregor, E. A. (1996). Structure and activity of some starch metabolizing enzymes in *Enzymes for Carbohydrate Engineering*. Park, K-H., J. F. Robyt, and Y. D. Choi, Editors. Elsevier: Amsterdam.

Stanley, E. and K. Stanley (2001). Looking into the Glycosidases: A Bioinformatics Resource for Biology Students. In *The BioQUEST Library Volume VI,* Jungck, J. R. and V. G. Vaughan, Editors. Academic Press: San Diego, CA.

Figure and Table References

Figure 1. Photo courtesy of Tate and Lyle (http://www.tateandlyle.com/)

Figure 2. Courtesy of Keith D. Stanley

Figure 3. Modified from *Biology Workbench* (http://workbench.sdsc.edu)

Figure 4. Modified from *Biology Workbench* (http://workbench.sdsc.edu)

Figure 5. Modified from *Biology Workbench* (http://workbench.sdsc.edu)

Figure 6. Modified from *Biology Workbench* (http://workbench.sdsc.edu)

Figure 7. Modified from a CHIME image of 1B9Z generated by QuickPDB.
http://www.rcsb.org/pdb/cgi/explore.cgi?job=graphics&pdbId=1B9Z&page=&pid=235141041544236
Click on QUICKPDB

Table 1. Guzman-Maldonado & Paredes-Lopez, 1995

Table 2. Adapted from MacGregor, 1996

Table 3. *Biology Workbench* (http://workbench.sdsc.edu/)

Table 4. From the Prosite website: Accession number PS00506
http://ca.expasy.org/cgi-bin/prosite-search-ac?ps00506

Table 5: *Biology Workbench* (http://workbench.sdsc.edu/)