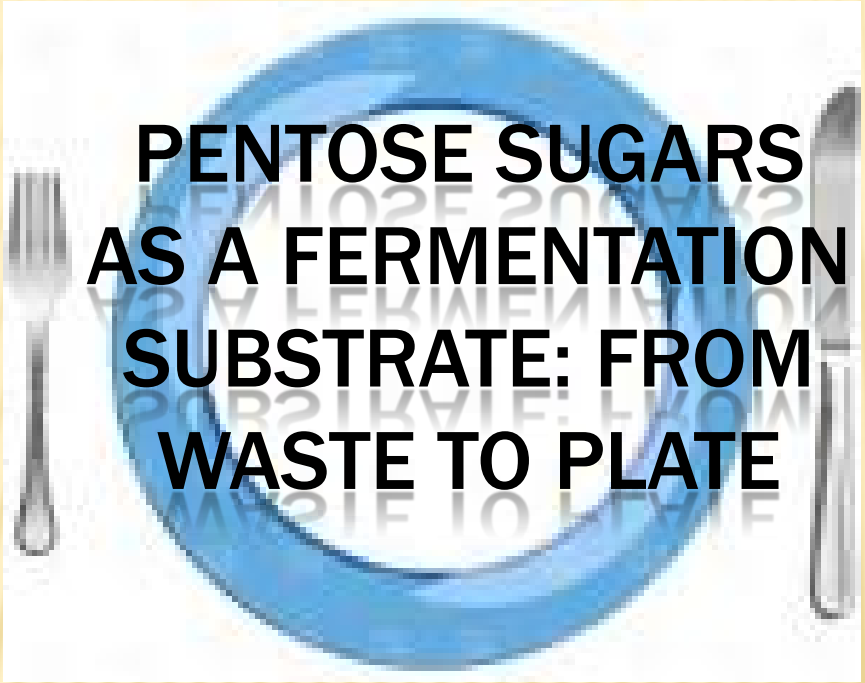
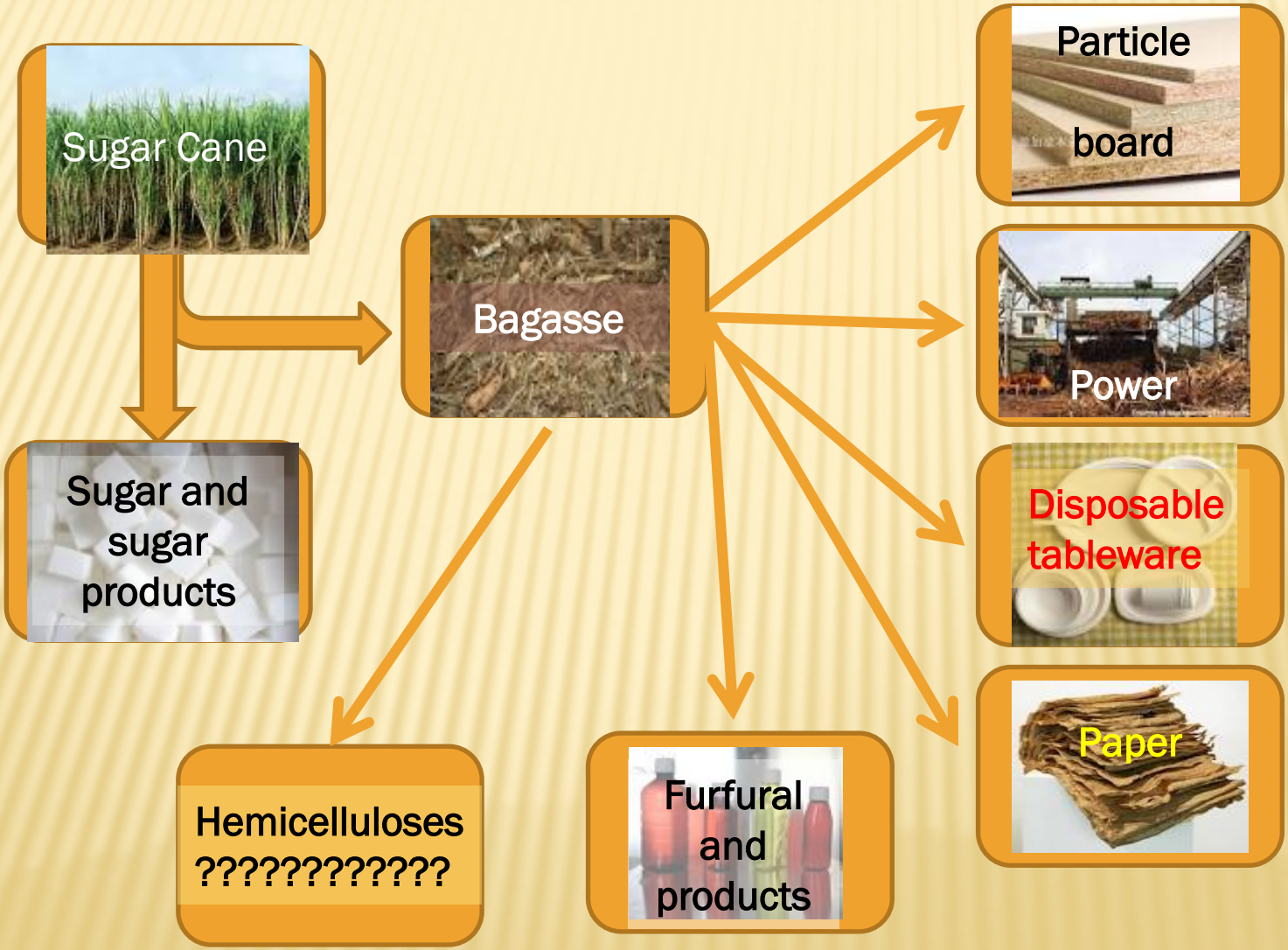


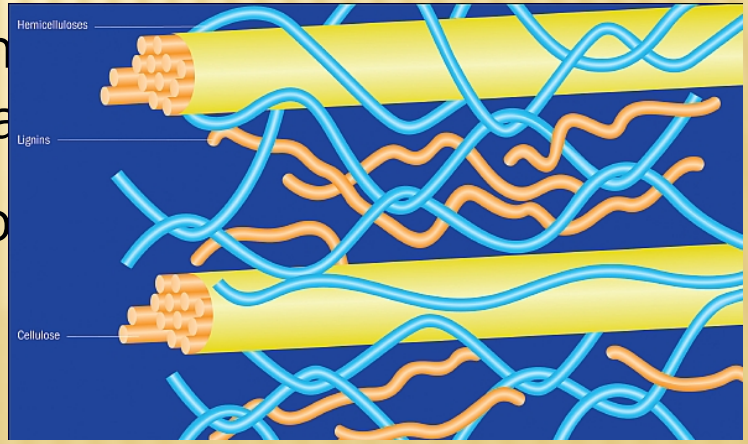
Megan Hargreaves and Farhana Sharmin

SOURCE AND USES OF BAGASSE



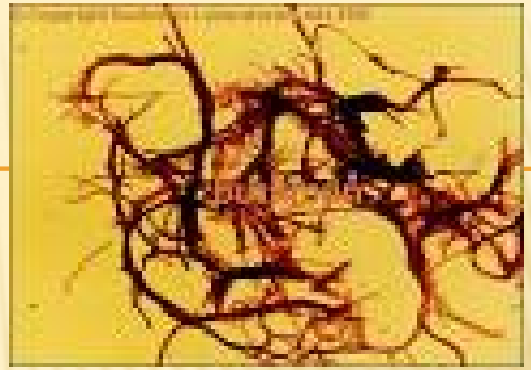
HEMICELLULOSES

- Plant cell walls are composed of cellulose and hemicellulose, pectin and in many cases lignin
- Hemicelluloses include xylan, glucuronoxylan, arabinoxylan, glucomannan and xyloglucan. These polysaccharides contain many different sugar monomers.
  - Besides glucose, sugar monomers in hemicellulose can include xylose, mannose, galactose, rhamnose and arabinose
  - Hemicelluloses contain mostly D-pen
    occasionally small amounts of L-suga
  - Xylose is in most cases the sugar mo
    amount

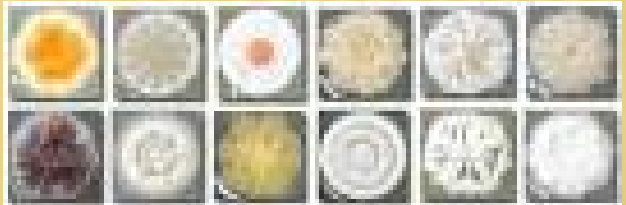


http://www.scidacreview.org/0905/html/biofuel.html

USING PENTOSE SUGARS



- Search for microbes that can
  - Metabolise the pentoses in the presence of glucose, preferably without being subject to catabolite repression OR
  - Carry out an efficient diauxie process using two or more sugars sequentially OR
  - Form a sequential process involving a number of microbial processes to enhance the selective fermentation of hemicellulose pentoses
  - Resist inhibition by other end-products such as hydroxymethylfurfural



TEST AREA - BACKGROUND

- More than 6 300 sugar growing families own and operate farms along Queensland's east coast.
- Farms range in size from 20 to 250 hectares, average size is 65 hectares.
- Queensland's east coast has the right conditions for growing sugar cane which needs:
  - At least 1 500mm of rain each year or access to irrigation
  - Temperatures over 21 degrees Celsius while growing
  - Flat to gently sloping land
  - Fertile and well drained soil.

http://www.rochedalss.eq.edu.au/sugar.htm

EXPERIMENTAL DESIGN

- Search for test cultures
  - Isolate pentose sugar utilizing bacteria from soil samples in sugar mill areas
  - Identify isolates using DNA technology
- Testing of Isolates for growth with pentose and hexose sugars
- Analysis of end-products following utilization of single and dual sugar carbon sources

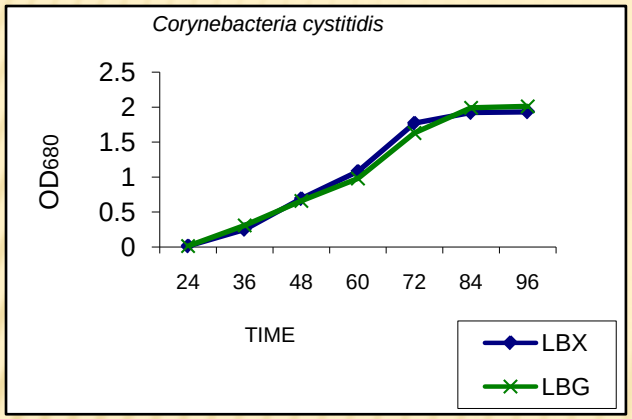
THE SEARCH

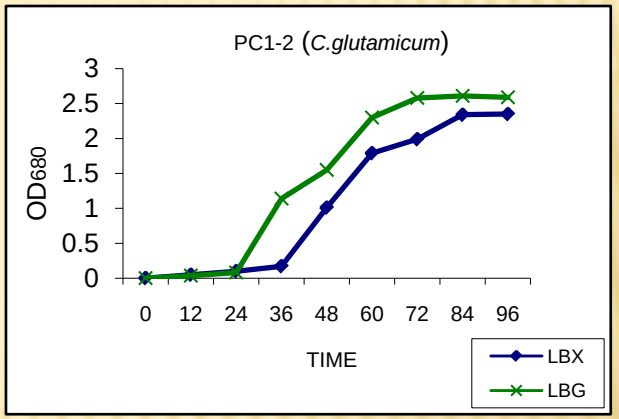
- Soils from areas surrounding sugar mill waste ponds were collected from the Maryborough and Proserpine sugar mills
- Bacterial strains were isolated from soil samples by means of a series of enrichment steps - broths containing one of 0.5% xylose or arabinose or ribose
- Six cultures of interest from 191 isolates, from the two different sites

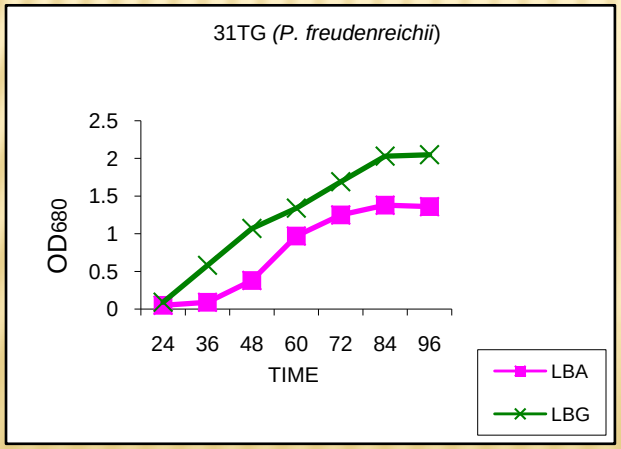
IDENTITY OF TARGET ISOLATES

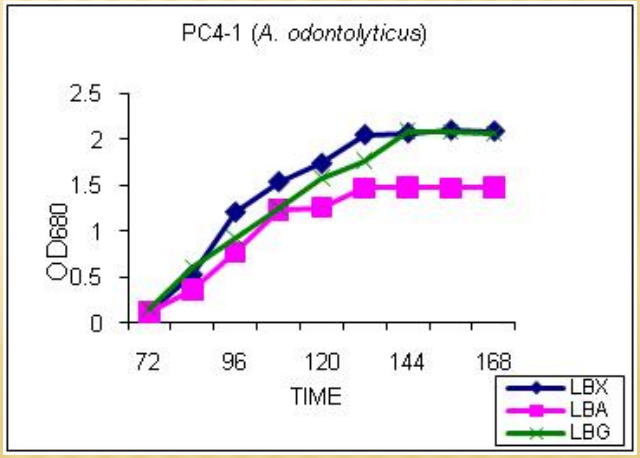
- DNA analysis was performed in order to confirm the identity of the isolated species
- Isolates were identified as
  - *Corynebacterium glutamicum (x2)*
  - *Actinomyces odontolyticus (x2)*
  - *Nocardia elegans*
  - *Propionibacterium freudenreichii*
- All are known soil organisms and all members of the Order *Actinomycetales*

GROWTH WITH PENTOSE SUGAR CARBON SOURCES









SUMMARY OF GROWTH FIGURES

- Results showed that the six indigenous isolates, PC4-1 and NC1-3 (*A. odontolyticus*), PC1-2 and NC1-2 (*C. glutamicum*), NC4-1 (*N. elegans*) and 31TG (*P. freudenreichii),* could utilize various pentoses and also glucose
- The specific growth rates (μ) of all organisms using pentoses and glucose were calculated
- There was very little significant difference between specific growth rates using the three pentose sugar carbon sources
- A significant difference was found between utilization of xylose and glucose by all of the environmental isolates and the ATCC control

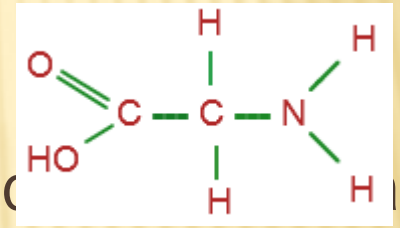
END PRODUCT ANALYSIS

The analysis was performed using two identical Agilent 1100 HPLC (Heracles, Japan) systems. Each system consisted of a binary pump, a UV detector, a fluorescence detector and an auto sampler. A reverse phase Agilent Zorbax Eclipse C18 column AAA (4.6150 mm, 3.5 micron) was used for the chromatographic separation.

Amino acid end-products using single and dual carbon sources as substrates

Isolate	Amino acid from single sugar substrate	Concentration mg/L	Amino acid from dual sugar substrate	Concentration mg/L
N. elegans (NC4-1)	Threonine	36	Glycine	22
A. odontolyticus (PC4-1, NC1-3)	Arginine	45	Glycine	22
	Cysteine	6		
C. glutamicum (NC1-2, PC1-2))	Arginine	46		
	Cysteine	3		
	Glycine	5	Glycine	22
P. freudenreichii (31TG)	Arginine	47		
	Cysteine	10		
	Glycine	5	Glycine	22
	Alanine	6		

SIGNIFICANCE OF END-PRODUCTS

- Major product of dual-sugar fermentation was amino acid – glycine
  - Simplest amino acid
  - Is becoming known for many medic 

l uses
    - protects against shock caused either by blood loss or endotoxin
    - reduces alcohol levels in the stomach
    - improves recovery from alcoholic hepatitis
    - diminishes liver injury caused by hepatotoxic drugs
    - blocks programmed cell death
    - reduces the nephrotoxicity caused by the drug cyclosporin A in the kidney, preventing hypoxia and free radical formation.
    - could be also useful in other inflammatory diseases since it diminishes cytokine production.

CONCLUSIONS

- Six indigenous bacteria were isolated and identified from the environment, and were able to use pentose sugars without any genetic modification
- The isolates were able to utilize pentoses in the presence of glucose
- The fermentation process resulted in a valuable commercial product, namely the amino acid glycine
- Optimization of growth media and conditions will be necessary to increase the efficiency of the process and the size of the yield

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