Prebiotics from lignocellulosic material

Lignocellulosic Biomass → Prebiotic precursor (Xylan) → Prebiotic (XOS)

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- Lives with nature
- Depends on natural foods
- Successful in charging the body with naturally occurring bioactive molecules

- Increased healthcare expenditure
- Phobia against modern therapeutic agents
- Prefers plant sourced bioactives
In 1907, E. Metchnikoff proposed the concept of longevity that is taking care by beneficial gut microflora from the observation at Village in Bulgaria. Concept of probiotic emerges.

About 460 to 370 BC, Hippocrates, the great ancient Greece physician told: “Let food be thy medicine and medicine be thy food”. Evidently, he emphasized on prevention rather than cure of diseases.
Present Concerns

Human health
Food safety
Animal welfare
Consumer awareness
Restriction on application of antibiotics
Environmental issues
What is prebiotic?

A non-digestible dietary ingredient that beneficially affect the host by selectively stimulating the growth and or activity of a limited number of bacteria in the colon, thus improving host health (Gibson and Roberfroid, 1995).

However, ancient Indian knew about prebiotic since Sindhu civilization and selectively choosing the crops rich in prebiotic (Samanta et al., 2010)
With the growing interests and intense research and development activities on prebiotic, it has been proposed to revisit its definition and suggested to exclude the word ‘non-digestible’.

The updated definition of prebiotic encompasses as “a selectively fermented ingredient that allows specific change, both in the composition and or activity in the GI microflora that confers benefits upon host well being and health” (Gibson et al., 2004)

Prebiotics are: non-viable food components, ingredients or supplements that selectively modulate the microbiota of the digestive ecosystem, thus conferring benefits upon host well being and health” (Roberfroid, 2007)

According to FAO (2007), “a prebiotic is a non-viable food component that confers a health benefit on the host associated with modulation of microbiota”.
Further, attempt has been made to broaden the site of action of prebiotics such as skin, oral cavity and female genital tract in addition to its fundamental action at the GI tract (Pineiro et al., 2008).

Recently, the proposers of prebiotic concept have opposed the philosophy of broadening the its application sites such as vagina and skin and wanted to restrict once again to the gastrointestinal microflora.

Therefore, the accepted definition remains closer to the 1995 concept: “a prebiotic is a selectively fermented ingredient that results in specific changes in the composition and/or activity of the gastrointestinal microbiota thus conferring benefit(s) upon host health” (Gibson et al., 2010).
It seems emphasis was given on prebiotics keeping in view its application in human beings only.

Thus, Samanta et al. (2007, 2013, 2015) suggested following criteria for prebiotics keeping in view the digestive system of both human and animals:

- Selective fermentability
- Desirable changes in gut microflora composition
- Ensures host health and well being
- Originated from plant or synthesized by microbial enzymes
- Remains intact while passing through digestive tract
- No residue problems
- Compatibility with other food/feed/products
- No adverse affects on productivity or product quality
- Should increase nutrient digestibility or reduce enteric methane emission in ruminants
Common prebiotic

- Inulin
- Fructo-oligosaccharides (FOS)
- Glacto-oligosaccharides (GOS)
- Pectic oligosaccharides (POS)
- Soya oligosaccharides
- Mannan-oligosaccharides (MOS)
- Tagatose
- Xylooligosaccharides (XOS)
Prebiotic from Lignocellulosic Biomass

(Samanta et al., 2015)
Lignocellulosic Materials

- **Abundant**
- **Very cheap**
- **Renewable**
- **Processing waste**
- **Civic Problem**
- **Field burning**
- **Tobacco stalks**
- **Cotton stalks**

Disposal problems:
- Invites insects
- Burn at field
Steps in prebiotic production from Lignocellulosic materials

1. Identification raw material/wastes
2. Compositional analysis
3. Xylan extraction
   - Room temperature
   - Steam application
4. Prebiotic production
   - Chemical process
   - Enzymatic process
## Identification of raw materials for XOS production

<table>
<thead>
<tr>
<th>Agricultural biomass</th>
<th>Organic matter</th>
<th>Total ash</th>
<th>NDF</th>
<th>ADF</th>
<th>Hemicellulose</th>
<th>Cellulose</th>
<th>ADL</th>
<th>CP</th>
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</thead>
<tbody>
<tr>
<td>Corn cob</td>
<td>97.71</td>
<td>2.29</td>
<td>75.89</td>
<td>37.11</td>
<td>38.78</td>
<td>27.16</td>
<td>9.40</td>
<td>5.2</td>
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<tr>
<td>Corn husk</td>
<td>97.11</td>
<td>2.89</td>
<td>68.87</td>
<td>31.48</td>
<td>37.39</td>
<td>29.07</td>
<td>2.41</td>
<td>2.68</td>
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<tr>
<td>Wheat bran</td>
<td>92.14</td>
<td>7.86</td>
<td>37.73</td>
<td>16.22</td>
<td>21.51</td>
<td>12.84</td>
<td>3.48</td>
<td>15.05</td>
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<tr>
<td>Rice bran</td>
<td>88.87</td>
<td>11.13</td>
<td>62.90</td>
<td>45.93</td>
<td>16.97</td>
<td>35.84</td>
<td>10.09</td>
<td>8.00</td>
</tr>
<tr>
<td>Cotton seed cake</td>
<td>95.17</td>
<td>4.83</td>
<td>55.26</td>
<td>34.97</td>
<td>20.29</td>
<td>24.89</td>
<td>10.08</td>
<td>28.49</td>
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<tr>
<td>Sugarcane bagasse</td>
<td>99.09</td>
<td>0.91</td>
<td>64.71</td>
<td>41.49</td>
<td>23.22</td>
<td>32.61</td>
<td>8.88</td>
<td>2.31</td>
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<tr>
<td>Paddy straw</td>
<td>82.71</td>
<td>17.29</td>
<td>73.28</td>
<td>48.93</td>
<td>24.35</td>
<td>44.75</td>
<td>4.18</td>
<td>3.15</td>
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<tr>
<td>Gram straw</td>
<td>90.30</td>
<td>9.70</td>
<td>54.32</td>
<td>39.66</td>
<td>14.66</td>
<td>30.47</td>
<td>9.19</td>
<td>6.4</td>
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<tr>
<td>Soyabean hulls</td>
<td>95.25</td>
<td>4.75</td>
<td>70.14</td>
<td>51.91</td>
<td>18.23</td>
<td>33.80</td>
<td>18.11</td>
<td>4.65</td>
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<tr>
<td>Ragi straw</td>
<td>90.31</td>
<td>9.69</td>
<td>72.63</td>
<td>42.93</td>
<td>29.70</td>
<td>36.96</td>
<td>5.97</td>
<td>3.38</td>
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<tr>
<td>Pigeon pea stalk</td>
<td>94.63</td>
<td>5.37</td>
<td>61.30</td>
<td>42.99</td>
<td>18.33</td>
<td>42.71</td>
<td>10.41</td>
<td>4.80</td>
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<td>Green coconut husk</td>
<td>96.27</td>
<td>3.73</td>
<td>76.20</td>
<td>60.96</td>
<td>15.24</td>
<td>57.46</td>
<td>3.50</td>
<td>3.60</td>
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<tr>
<td>Palm press fiber</td>
<td>93.36</td>
<td>6.64</td>
<td>77.19</td>
<td>53.61</td>
<td>23.58</td>
<td>48.98</td>
<td>2.49</td>
<td>--</td>
</tr>
<tr>
<td>Name of raw materials</td>
<td>Extraction with</td>
<td>Yield</td>
<td>Reference</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Cotton stalks</td>
<td>24% KOH+1% NaHBH₄</td>
<td>0.4 g/2 g of cotton stalks</td>
<td>Akpınar, Özlem, Kavas, Bakır, and Yılmaz (2007)</td>
<td></td>
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<td></td>
<td></td>
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</tr>
<tr>
<td>Corn cobs</td>
<td>12% NaOH with steam</td>
<td>83% of original xylan</td>
<td>Samanta et al. (2012a)</td>
<td></td>
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<tr>
<td>Corn stalks</td>
<td>10% NaOH 20 °C</td>
<td>54% of original sugars</td>
<td>Ergues, Sanchez, Mondragon, and Labidi (2012)</td>
<td></td>
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<tr>
<td>Wheat straw</td>
<td>0.5 M NaOH at 55 °C</td>
<td>49.3% of original xylan</td>
<td>Ruzene, Silva, Vicente, Goncalves, and Teixeira (2008)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Poplar wood bagasse</td>
<td>NaOH coupled with sonication</td>
<td>75.5% of original xylan</td>
<td>Yuan, Xu, He, and Sun (2010)</td>
<td></td>
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<td></td>
</tr>
<tr>
<td>Sugarcane bagasse</td>
<td>3% NaOH at 50 °C</td>
<td>74.9% of original xylan</td>
<td>Peng et al. (2009)</td>
<td></td>
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</tr>
<tr>
<td>Tobacco stalks</td>
<td>24% KOH+1% NaHBH₄</td>
<td>21.8% of total dried raw materials</td>
<td>Akpınar et al. (2009)</td>
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</tr>
<tr>
<td>Sunflower stalks</td>
<td>24% KOH+1% NaHBH₄</td>
<td>18.9% of total dried raw materials</td>
<td>Akpınar et al. (2009)</td>
<td></td>
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</tr>
<tr>
<td>Wheat straw</td>
<td>24% KOH+1% NaHBH₄</td>
<td>20.6% of total dried raw materials</td>
<td>Akpınar et al. (2009)</td>
<td></td>
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<td></td>
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</tr>
<tr>
<td>Natural grass</td>
<td>12% NaOH coupled with steam</td>
<td>98% of original xylan</td>
<td>Samanta et al. (2012)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pigeon pea stalks</td>
<td>12% NaOH coupled with steam</td>
<td>96% of original xylan</td>
<td>Samanta et al. (2013)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Green coconut husks</td>
<td>4% KOH coupled with steam</td>
<td>84% of original xylan</td>
<td>Jayapal et al. (2014)</td>
<td></td>
<td></td>
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</tr>
</tbody>
</table>
Quality analysis of xylan

Thermogravimetric analysis

Samanta et al., 2012
FT-IR analysis of xylan

Samanta et al., 2012
Factors influencing the XOS production

- Source of xylan
- Enzyme activity
- pH
- Hydrolysis time
- Temperature
XOS yield from xylan of different lignocellulosic materials

XOS yield from corn cob, wheat bran, peanut shell, oat spelt: Xylobiose: 3.42 to 4.7 mg/ml, Xylotriose: 0.34 to 3.66 mg/ml, Xylotetrose: nil to 1.99 mg/ml (Yang et al., 2007)

XOS yield from Sugarcane Bagasse reached up to 11 g/100g of xylan with pH 5.0, Temperature 45°C, Enzyme dose: 17.4 U/ml, Hydrolysis Time: 10 h (Jayapal et al., 2013)

XOS yield from natural grass reached up to 7g/100g of xylan with pH: 5.0, Temperature: 40 °C, Enzyme dose: 13.2 U/ml, Hydrolysis Time: 16 h (Samanta et al., 2012)
HPLC chromatogram of enzymatic hydrolyzate of sugarcane bagasse xylan following application RSM generated variables

Jayapal et al., 2013
<table>
<thead>
<tr>
<th>Property</th>
<th>Value/Nature</th>
</tr>
</thead>
<tbody>
<tr>
<td>Molecular formula</td>
<td>$C_{5n}H_{8n+2}O_{4n+1}; \ n=2-6$</td>
</tr>
<tr>
<td>DP range</td>
<td>2-6</td>
</tr>
<tr>
<td>Molecular Weight (g)</td>
<td>282 - 810</td>
</tr>
<tr>
<td>Physical form</td>
<td>Crystalline solid, color depends on source of xylan/purification/process of drying</td>
</tr>
<tr>
<td>pH Stability</td>
<td>2-7</td>
</tr>
<tr>
<td>Sweetness</td>
<td>92% of sucrose</td>
</tr>
<tr>
<td>Energy Value</td>
<td>1.5 Kcal/gm</td>
</tr>
<tr>
<td>Absorbability at GI tract</td>
<td>Nil</td>
</tr>
<tr>
<td>Recommended dose</td>
<td>8-12g/day</td>
</tr>
<tr>
<td>Regulatory Status</td>
<td>GRAS</td>
</tr>
</tbody>
</table>
It acts through selective stimulation of growth and multiplication of beneficial gastrointestinal microflora.
Imaginary microflora composition of gastrointestinal tract
### Microbial density at different segments of GI tract

<table>
<thead>
<tr>
<th>Animal Type</th>
<th>Stomach</th>
<th>Small intestine</th>
<th>Cecum</th>
<th>Colon</th>
</tr>
</thead>
<tbody>
<tr>
<td>Human, swine</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Chicken, turkey</td>
<td>Crop</td>
<td>Stomach</td>
<td>Small intestine</td>
<td>Cecum</td>
</tr>
<tr>
<td>Horse, rodents</td>
<td>Stomach</td>
<td>Small intestine</td>
<td>Cecum</td>
<td>Colon</td>
</tr>
<tr>
<td>Cattle, sheep</td>
<td>Rumen</td>
<td>Stomach</td>
<td>Small intestine</td>
<td>Colon</td>
</tr>
<tr>
<td>Salmon</td>
<td>Stomach</td>
<td>Proximal intestine</td>
<td>Pyloric ceca</td>
<td>Distal intestine</td>
</tr>
</tbody>
</table>

- **Stomach**
- **Small intestine**
- **Cecum**
- **Colon**
• The rich GI tract houses approximately $10^{14}$ bacterial cells, representing 30 known genera and 500 known species and many un-described species (Nesih, 2002, Samanta et al., 2015)

• 1-2 kg of bacteria in adult gut, metabolic activity is comparable to liver & tantamount to a virtual organ (Shanahan, 2002)

• Combined no. of genes of gut microbiome can reach 100 fold the size of human genome (Shanahan, 2002)
Beneficial microflora

Harmful microflora

Prebiotic works in the principal of selective stimulation of beneficial microflora
Effect on human

- Increased Lactobacillus and Bifidobacteria
- Makes thinner gut epithelium
- Increased mineral absorption
- Reduced gut pathogens
- Makes acidic pH
- Stimulates immunity
- Reduces cholesterol, triglycerides and glucose in blood
- Posses anti-carcinogenic property
- Posses anti-inflammatory and anti-oxidant activity
Effect of XOS in birds

- Increased Lactobacillus and Bifidobacteria
- Makes thinner gut epithelium
- Reduced gut pathogens
- Stimulates immunity
- Reduces cholesterol, triglycerides and glucose in blood
- Posses anti-inflammatory and anti-oxidant activity
Mechanism of action of prebiotic

- Reached intact at ceacum
- Growth of Beneficial Bacteria
- Production of SCFA
- Pathogens

Ups.. Prebiotic!!!
Lets go out from here (GUT)
Prebiotic beneficial
Global prebiotic market

- Global prebiotics market is expected to reach USD 5.75 billion by 2020, according to a new study by Grand View Research Inc.

- Global prebiotics market was 581.0 kilo tons in 2013 and is expected to reach 1,084.7 kilo tons by 2020, growing at a CAGR of 9.3% from 2014 to 2020.

- Asia Pacific is expected to be the fastest growing market in terms of volume, growing at a CAGR of 9.6% from 2014 to 2020 on account of surging demand.

- Key companies include Friesl and Campina Domo, Cargill inc., Beghin Meiji, Beneo-Orafti SA, Roquette America, Inc., Cosucra Groupe Warcoing SA and Weetabix Ltd.
Sharing My Secrets of Health

Everyday I dose with prebiotic.
Do you?
Future of us will be better and healthy; if we believe & rely on plant sourced bioactive molecules such as prebiotics.
Thank you