Joint Graduate Presentation
Department of Microbiology

Gut Microbiota and you

Presenter: Henry Chow
MPhil Student
Supervisor: Prof. Margaret Ip

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  - Stomach distress in foreign countries
- 2. Digestion of Porphyran, a sulphated polysaccharide found in seaweeds
  - Digestion by *Z. galactanivorans* (marine bacteria)
  - Digestion by *B. plebeius* (gut bacteria)
  - Digestion of Porphyran between Japanese and Westerners
- 3. Relation of Food and gut microbiota

Gut microbiota

Introduction:

Stomach distress when eating foreign food?

Stomach distress in Foreign places

- 50% of people travel to foreign places suffer from stomach distress [1]
  - Contaminated food and beverages
  - Allergy from exotic foodstuff

- Other reasons?

- 1. Travelers' Diarrhea,Centers for Disease Control and Prevention, http://www.cdc.gov/ncidod/dbmd/diseaseinfo/travelersdiarrhea_g.htm Accessed on 1 December 2010

Diet and digestion

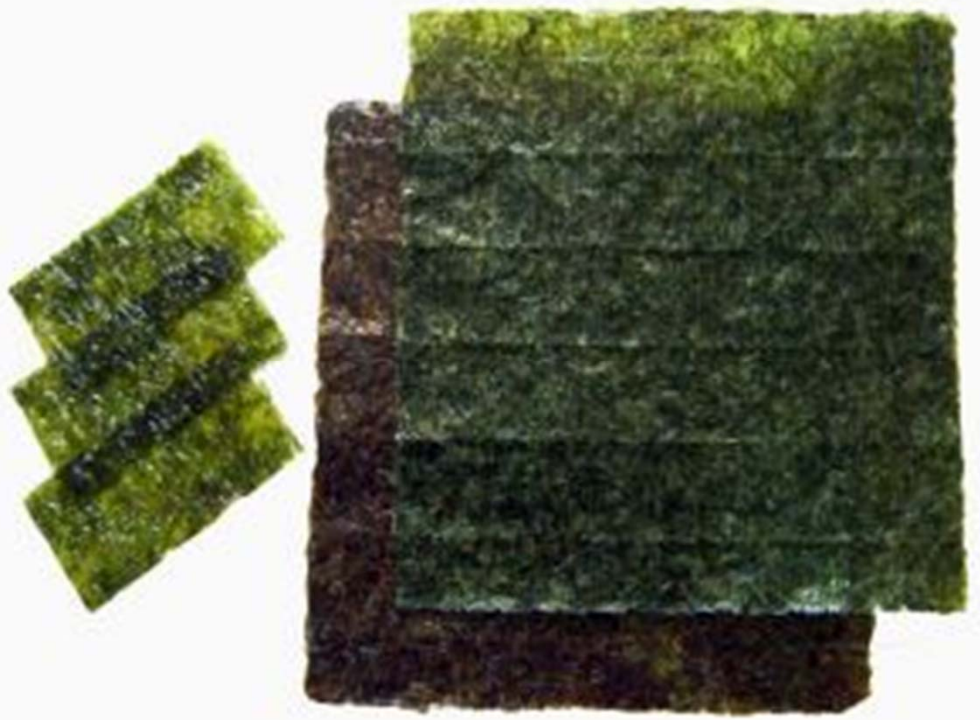
- 2. Digestion of Porphyran

Did you suffer indigestion in Japan?

Japanese diet

Seaweeds (Nori)

- Comprises mainly Sulphated polysaccharides porphyran
- Indigestible by human and our gut microbiota
- Digestible by Japanese AND…
- A marine bacteria which cannot survive in human gut



- 2. Cynthia L. Sears "A dynamic partnership: Celebrating our gut flora" Anaerobe, Volume 12, Issue 2, April 2006, Page 114

Zobellia galactanivorans

Digestion of porphyran by *Z. galactanivorans* (marine bacteria)

Zobellia galactanivorans

- Whole genome sequencing of *Z. galactanivorans,* a marine Bacteroidetes isolated from *Delesseria sanguinea* (marine red algae)
- Identify sequences with catalytic signature EXDXXE motif typical of glycoside hydrolase family 16, which digest agarose or κ-carrageenan (polysaccharides from algae) but not homologous to existing β-agarases and κ-carrageenases
- *Looking for targets which may digest other polysaccharides produced from algae*[6]

- 6. Jan-Hendrik Hehemann, Gaëlle Correc, Tristan Barbeyron, William Helbert, Mirjam Czjzek & Gurvan Michel, Transfer of carbohydrate-active enzymes from marine bacteria to Japanese gut microbiota, Nature 464, 908-912

GH16 homologues

- Five distant homologues were found which may relate glycoside hydrolase family 16
- Two out of five proteins were cloned and expressed successfully in *E.Coli*
- Expressed proteins have no effect on agarose or κ-carrageenan (Function of known GH16 enzymes)[6]

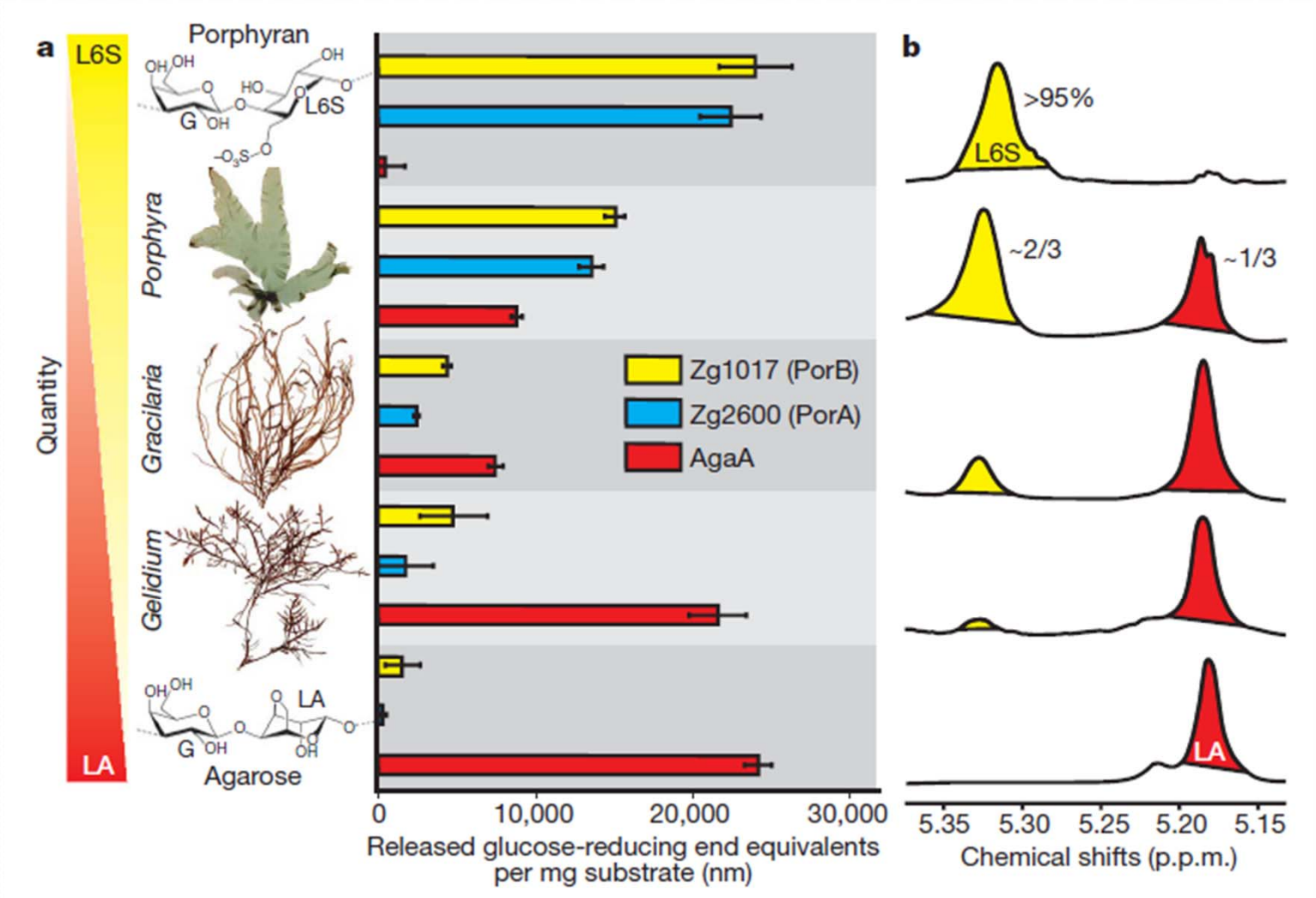
- 6. Jan-Hendrik Hehemann, Gaëlle Correc, Tristan Barbeyron, William Helbert, Mirjam Czjzek & Gurvan Michel, Transfer of carbohydrate-active enzymes from marine bacteria to Japanese gut microbiota, Nature 464, 908-912

GH16 homologues

- Screened the hydrolytic activities of the expressed proteins against natural polysaccharides extracted from marine macrophytes (seaweeds)
- The proteins digested sulphated polysaccharides porphyran from seaweeds and released their monomers, L6S [6]

- 6. Jan-Hendrik Hehemann, Gaëlle Correc, Tristan Barbeyron, William Helbert, Mirjam Czjzek & Gurvan Michel, Transfer of carbohydrate-active enzymes from marine bacteria to Japanese gut microbiota, Nature 464, 908-912

GH16 homologues



GH16 homologues

- Follow ups:
  - Name the two proteins (PorA, PorB)
  - X ray crystallography
  - Predict the structure of the three proteins which cannot be expressed
  - Characterized their mechanism in sulphated polysaccharide degradation

GH16 homologues

- Summary: They find a class of enzyme exist in a marine bacteria which can digest seaweeds

- Question: Why Japanese can digest seaweeds?

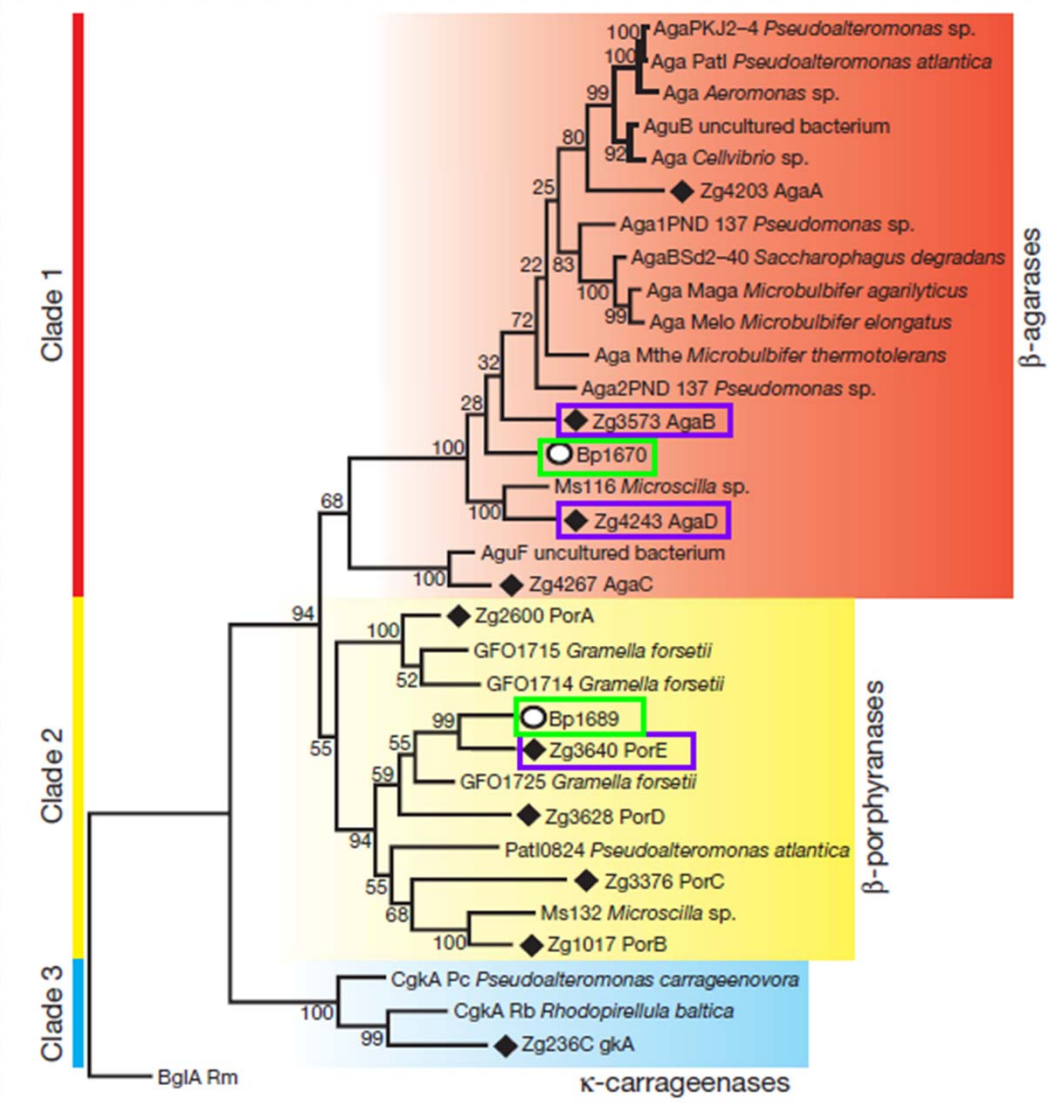
Bacteroides plebeius

Digestion of porphyran by *B. plebeius* (gut bacteria)

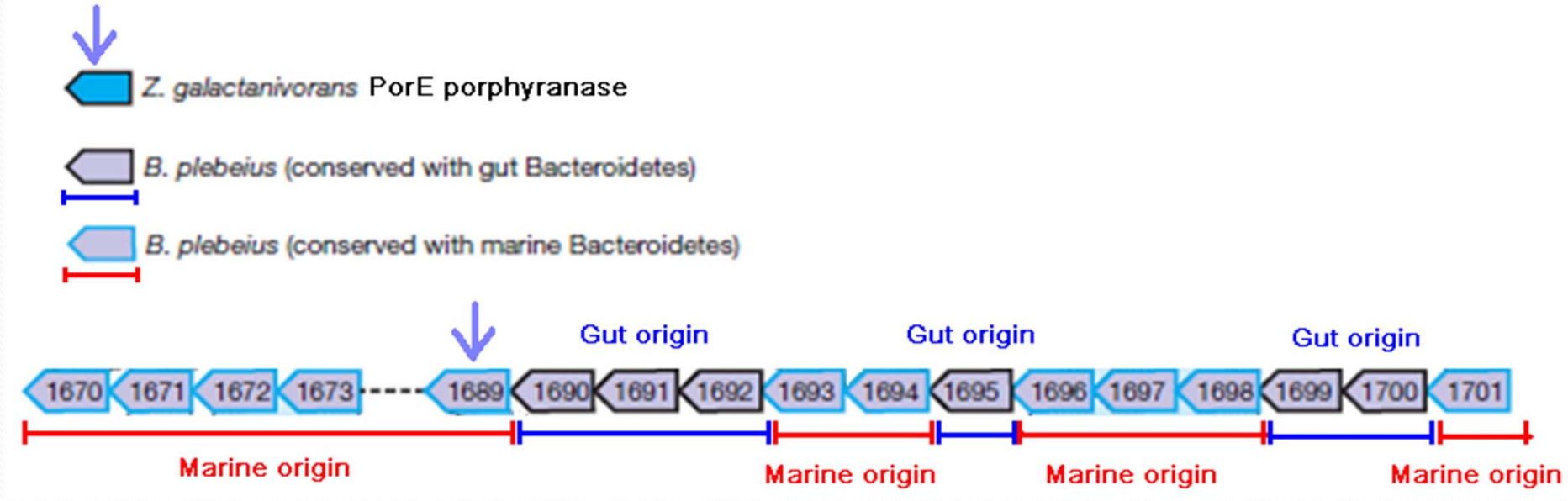
Bacteroides plebeius

- Sequences of ß-porphyranases were blasted in NCBI
- Exclusive in marine bacteria
- Except *Bacteroides plebeius* in human gut

Phylogenetic Tree of GH16 enzymes



Porphyranase gene in B. Plebeius



B. plebeius in humans

- Summary: *B. plebeius (human gut bacteria) acquired a gene from Z. galactanivorans (marine bacteria) by horizontial gene transfer*

- *Question: Why only Japanese can digest seaweeds, when B. plebeius exists in all human gut?*

B. plebeius in humans

Digestion of Porphyran between Japanese and Westerners

B. plebeius in humans

- Analyzed gut metagenomes in Japanese and western people:
- In 13 healthy Japanese:
  - Seven individuals have porphyranase sequences in their gut metagenome
- In 18 healthy western Americans:
  - None possess any porphyranase genes

B. plebeius in humans

- Summary: Only *B. plebeius* in Japanese possess porphyranases

- Question: Why this happens?

Food culture and gut microbiota

1. Japanese use raw seaweeds to make sushi since 8th century[7]
2. *Z. galactanivorans* transfer the porphyranase genes *to B. plebeius* in human gut by conjugation
3. "Transfromed" *B. plebeius* colonize in human gut of Japanese
4. Transformed gut bacteria pass to the next generation of Japanese

- 7.http://ja.wikipedia.org/wiki/%E6%B5%B7%E8%8B%94 海苔 accessed on 2 Dec 2010

Food culture and gut microbiota

- Conclusion: Japanese can digest some seaweeds so that they don't get a diarrhea.

- After ~10 centuries of co-evolution of human diet and their gut microbes…
- To digest 1 food component

- What about other food?

Food culture and gut microbiota

Food culture and gut microbiota

Food culture and gut microbiota

References

1. Travelers' Diarrhea,Centers for Disease Control and Prevention, http://www.cdc.gov/ncidod/dbmd/diseaseinfo/travelersdiarrhea_g.htm Accessed on 1 December 2010
2. Cynthia L. Sears "A dynamic partnership: Celebrating our gut flora" Anaerobe, Volume 12, Issue 2, April 2006, Page 114
3. MB Roberfroid, F Bornet, C Bouley and JH Cummings, Colonic microflora: nutrition and health: summary and conclusions of an International Life Sciences Institute (ILSI) (Europe)] workshop held in Barcelona, Spain, Nutr Rev 53 (1995), pp. 127–130
4. JH Cummings, EW Pomare, WJ Branch, CP Naylor and GT Macfarlane, Short chain fatty acids in human large intestine, portal, hepatic and venous blood, Gut 28 (1987), pp. 1221–1227
5. JH Cummings, ER Beatty, SM Kingman, SA Bingham and HN Englyst, Digestion and physiological properties of resistant starch in the human large bowel, Br J Nutr 75 (1996), pp. 733–747
6. Jan-Hendrik Hehemann, Gaëlle Correc, Tristan Barbeyron, William Helbert, Mirjam Czjzek & Gurvan Michel, Transfer of carbohydrate-active enzymes from marine bacteria to Japanese gut microbiota, Nature 464, 908-912
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End

Sulphated monosaccharides

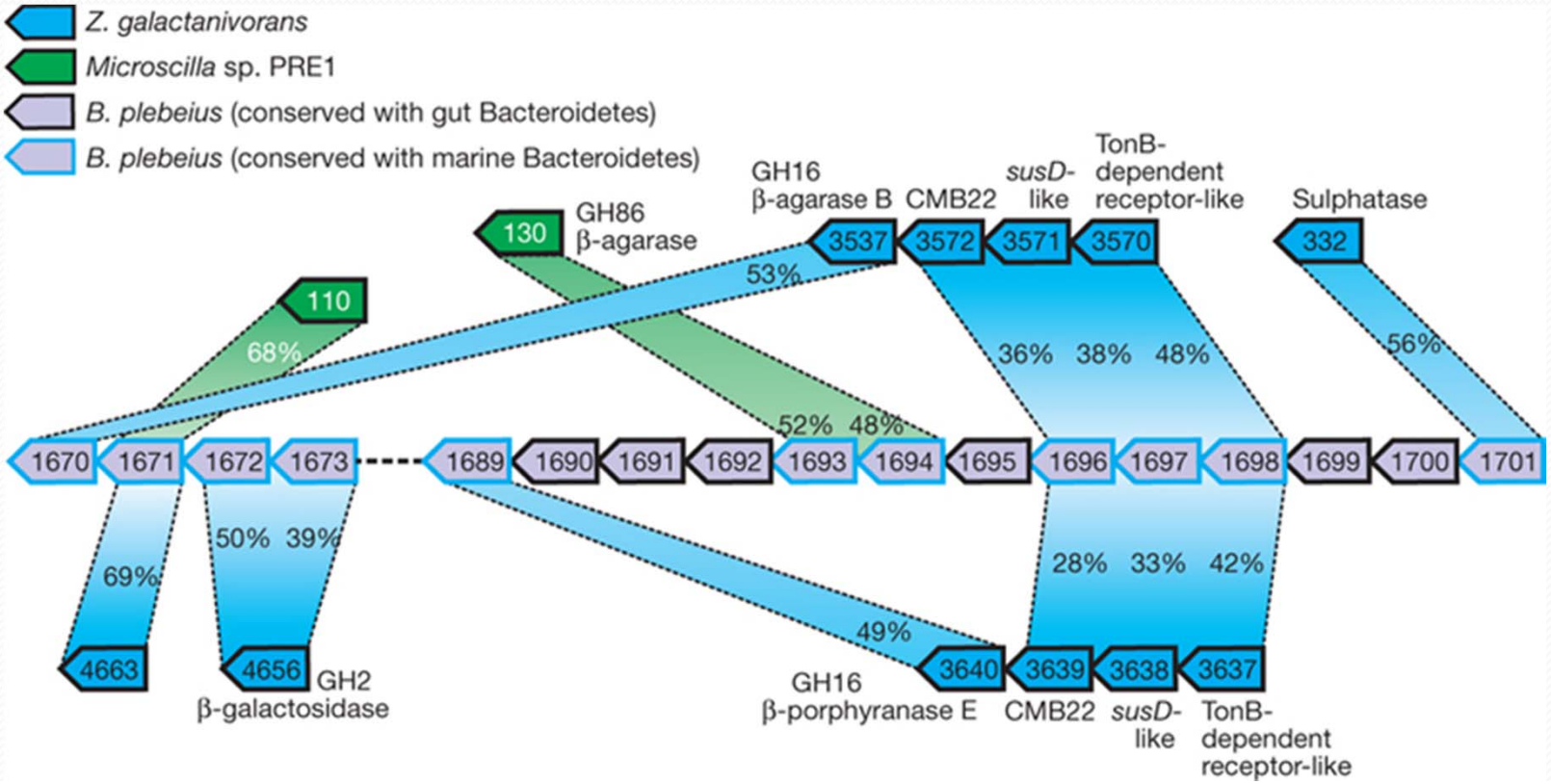
- 4-linked α-l-galactopyranose-6-sulphate
- 4-linked 3,6-anhydro-α-l-galactopyranose

Genomic islands

- Sequence based methods depend on the naturally occurring variation that exists between the genome sequence composition of different species. Genomic regions that show abnormal sequence composition (such as nucleotide bias or codon bias) suggests that these regions may have been horizontally transferred. Two major problems with these methods are that false predictions can occur due to natural variation in the genome (sometimes due to highly expressed genes) and that horizontally transferred DNA will ameliorate (change to the host genome) over time; therefore, limiting predictions to only recently acquired GIs.

Conjugation process

- Contains relaxase/mobilization proteins in Bp1662 and Bp1663/Bp1665, upstream of the porphyranase



Traveler's diarrhea

- One out of two travelers suffers from traveler's diarrhea.
  - Primary source of infection is ingestion of fecally contaminated food or water. [1]
  - Yet we cannot isolate the causative agent most of the time. [1]
- Question:
  - Why travelers can get diarrhea in highly advanced countries like Japan, Hong Kong and Germany?
  - Why local residents are rarely affected?

1. Travelers' Diarrhea, Centers for Disease Control and Prevention, http://www.cdc.gov/ncidod/dbmd/diseaseinfo/travelersdiarrhea_g.htm Accessed on 1 December 2010

Microbes in digestion

- In animals:
  - Cellulose fermentation in herbivores
  - *Bacteroides thetaiotaomicron* metabolize 30% of calories in the food into usable form for mice and humans[2]
- In humans:
  - Production of Vitamin K by *E.coli*
    - What else?

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Microbes in digestion

- Endogeneous mucus from gut linings[3]
- Resistant starches[4,5]
- Cellulose and hemicelluloses[4,5]
- Pectins[4,5]
- Gums[4,5]
- Unabsorbed oligosaccharides and sugars[4,5]
- Alcohols[4,5]

AND

- Substrate dependent microbes…

- 3. MB Roberfroid, F Bornet, C Bouley and JH Cummings, Colonic microflora: nutrition and health: summary and conclusions of an International Life Sciences Institute (ILSI) (Europe)] workshop held in Barcelona, Spain, Nutr Rev 53 (1995), pp. 127–130
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