Growth of Beneficial Bifidobacterium on Unique Oligosaccharides

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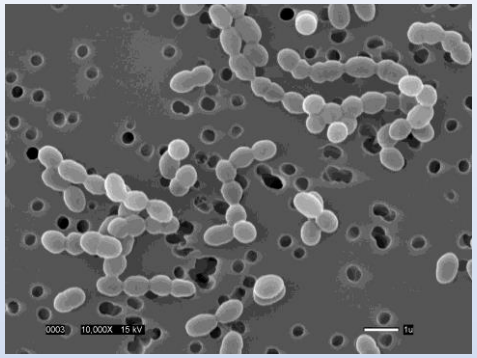
Characteristics of Leuconostoc



Leuconostoc (bacterial genus)

- gram positive
- non-pathogenic
- lives on vegetation

http://genome.jgi-psf.org/finished_microbes/images/leume.jpg

Transferring sauerkraut to the canning room at the old Frank Pure Food Co. in Franksville, WI – Oct., 1926

Leuconostoc-Significance

- food fermentations (sauerkraut, dairy)
- interferes w/ sugar processing (reduce yields)
- cause off-flavors & odors minimal package meats

- make exopolymers & derivatives
  sucrose-derived, glucan polymers
  applications (prebiotic, pharmaceutical, foods)

Leuconostoc B-1355 exopolymer formation

Synthesis of Sucrose Derived-Oligosaccharides Using Glucansucrase from Leuconostoc

Native Polymer Formation

sucrose G-F G-F G-F

glucansucrase

F + F

$[G\text{-}G\text{-}G\text{-}G\text{-}G\text{-}G\text{-}G\text{-}G\text{-}]_n$

dextran or alternan native polymers

Oligosaccharide Synthesis

sucrose G-F G-F G-F

maltose "acceptor", chain-ending G-G G-G G-G

glucansucrase

F + F

oligosaccharides 2-10 sugar units

G-G-G

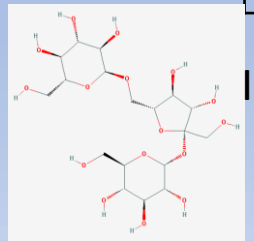
G-G-G-G

G-G-G-G-G

G-G-G-G-G-G

Oligosaccharides Prepared via Alternansucrase Acceptor Reactions

Acceptor	Acceptor product Composition and Structure
Gentiobiose GEN	84% DP3, α-D-Glc-(1→6)ß-D-Glc-(1→6)-D-Glc 9% DP4, [α-D-Glc-(1→6)]2 ß-D-Glc-(1→6)-D-Glc 7% DP4, α-D-Glc-(1→3) α-D-Glc-(1-(1→6)-6)β-D-Glc-(1→6)-D-Glc
Maltitol MAL	50% DP3, α-D-Glc-(1→6) α-D-Glc-(1→4)) α-D-Glucitol (panitol) 25% DP2, α-D-Glc-(1→4) α-D-Glucitol (maltitol) 13% DP4, α-D-Glc-(1→6) panitol 12% DP4, α-D-Glc-(1→3) panitol
Maltose SM	67% DP3, 6²-O-α-D-Glucosylmaltose (panose) 20% DP4, α-D-Glc-(1→3)- α-D-Glc-(1→6)-α D-Glc-(1→4)-D-Glc α-D-Glc-(1→6)- α-D-Glc-(1→6)-α D-Glc-(1→4)-D-Glc 13% DP>4, structures not determined
Melibiose MEL	90% DP3, α-D-Glc-(1→3)-α-D-Gal-(1→6)-D-Glc <5% DP2, α-D-Gal-(1→6)-D-Glc (melibiose) <5% DP3, α-D-Glc-(1→4)-α-D-Gal-(1→6)-D-Glc <5% DP4, structure not determined
Raffinose RAF	85% DP4, α-D-Glc-(1→4)-α-D-Gal-(1→6)-α-D-Glc-(1↔2)-β-D-Fru 10% DP4, α-D-Glc-(1→3)-α-D-Gal-(1→6)-α-D-Glc-(1↔2)-β-D-Fru <5% DP3, α-D-Gal-(1→6)-α-D-Glc-(1↔2)-β-D-Fri (raffinose) <5% DP5 and higher, structures not determined



Isomelizitose
IMZ

a trisaccharide with the structure
α-D-glucopyranosyl (1 → 6) β-D-fructofuranosyl
(2 ↔ 1) α-D-glucopyranoside.

Cote, Holt, and Miller-Fosmore, 2003
Cote and Holt, 2007, Holt et al, 2018

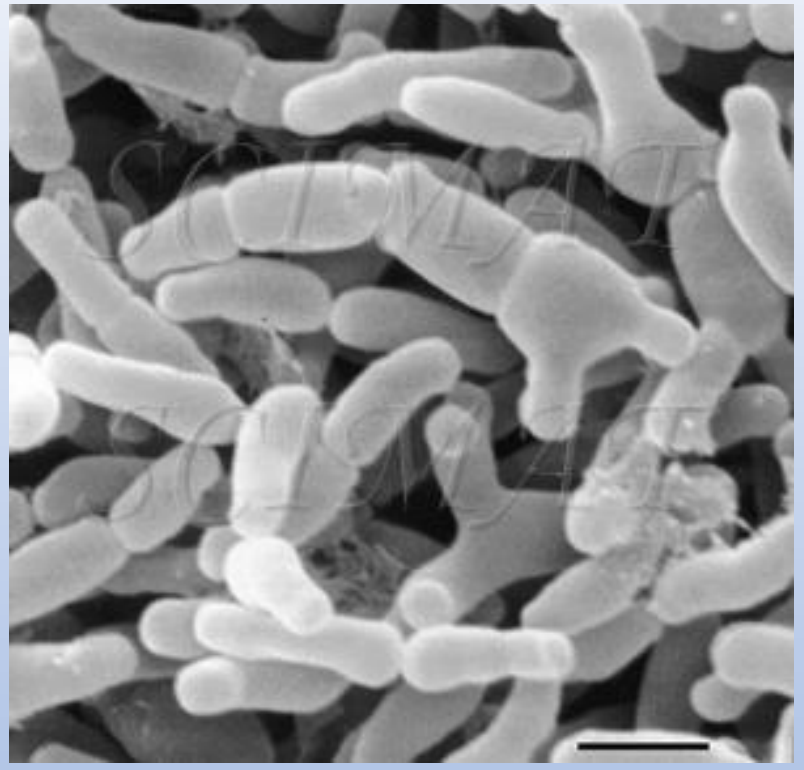
Do Oligosaccharides Support Growth of Beneficial Bacteria?

Growth of various colonic bacteria on five different alternansucrase-derived oligosaccharides (AOS)

Organism Tested	Glucose	Gent. AOS	Maltitol AOS	Malt. AOS	Melibio. AOS	Raffinose AOS
Bifidobacterium adolescentis 15703	.99±.09	.85±.03	.90	.92±.04	.85±.16	1.09± .04
Bifidobacterium pseudocatenulatum 27919	.94±.07	.91±.02	.53±.03	.81±.04	.78±.02	1.12±07
Bacteroides thetaiotaomicron 29148	.68±.07	.16±.02	.18±.01	.2 ±.01	0	.09 ±.01
Clostridium perfringens 13124	.74±.05	0	.03	.2±03	0	0
Enterobacter aerogenes 35028	.57±.06	.02	.25±02	0	.14	0
Escherichia coli 8739	.61±.02	0	0	0	0	0
Salmonella typhimurium 14028	.69	0	0	0	0	0

A_{600nm} values were determined in triplicate and reported as mean ± SD.

Cote, Holt, and Miller-Fosmore, 2003, Cote and Holt, 2007

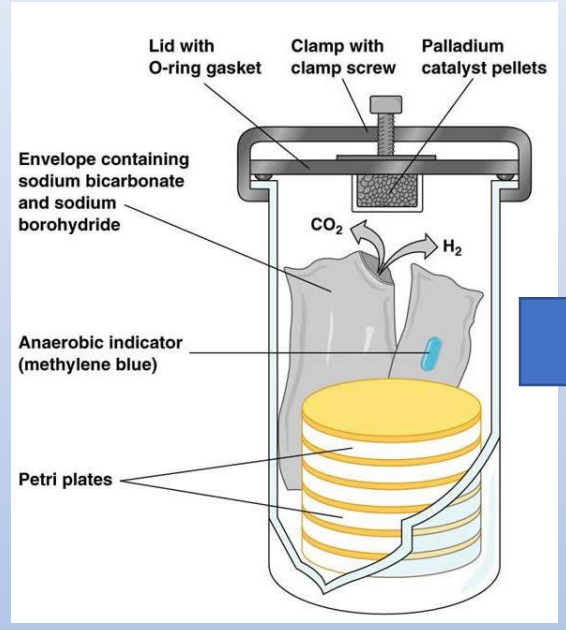


Actinobacteria; Coryneform & Propionic Acid Grp

Genus: Bifidobacterium

- obligate anaerobic
- GI warm-blooded animals
- generally beneficial
- use nondigestible oligosaccharides
  onions, bean, cruciferous
- probiotics in foods

Determine Growth of Bifidobacterium on Oligosaccharides: Methods



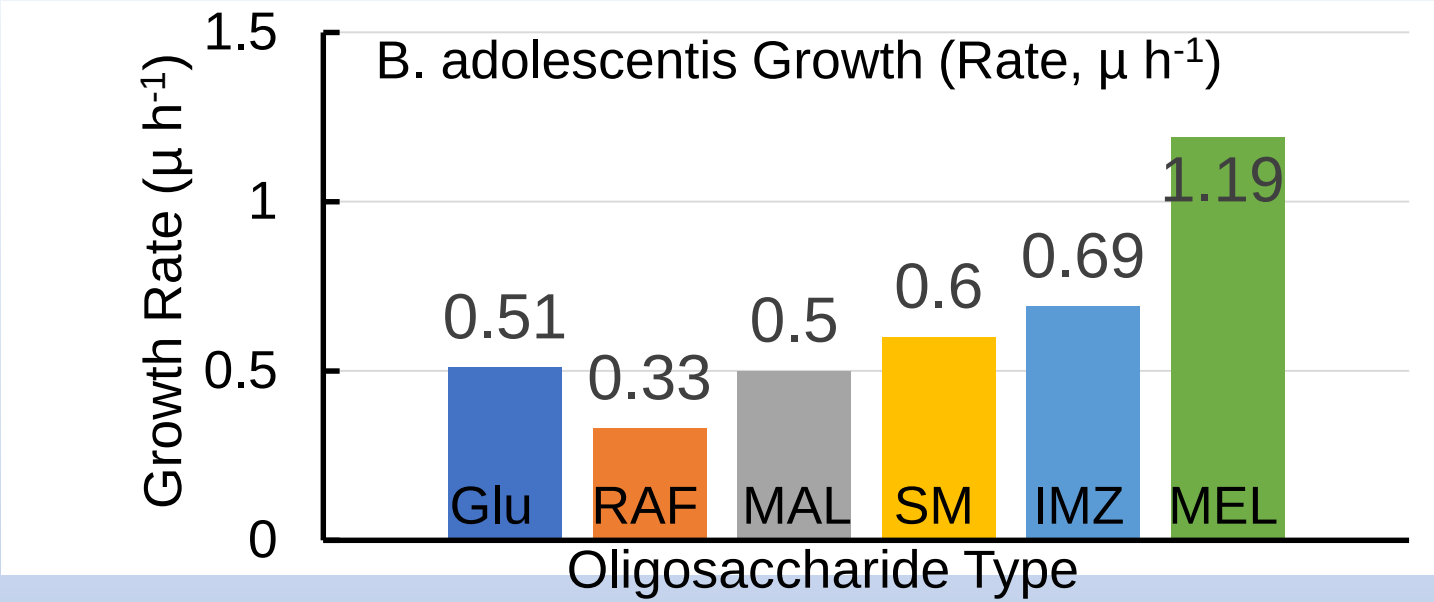
Pre Culture

Test Culture

DSM 58
Oxyrase

Growth Rate
Biomass

Influence of Oligosaccharide Type on Bifidobacterium Growth

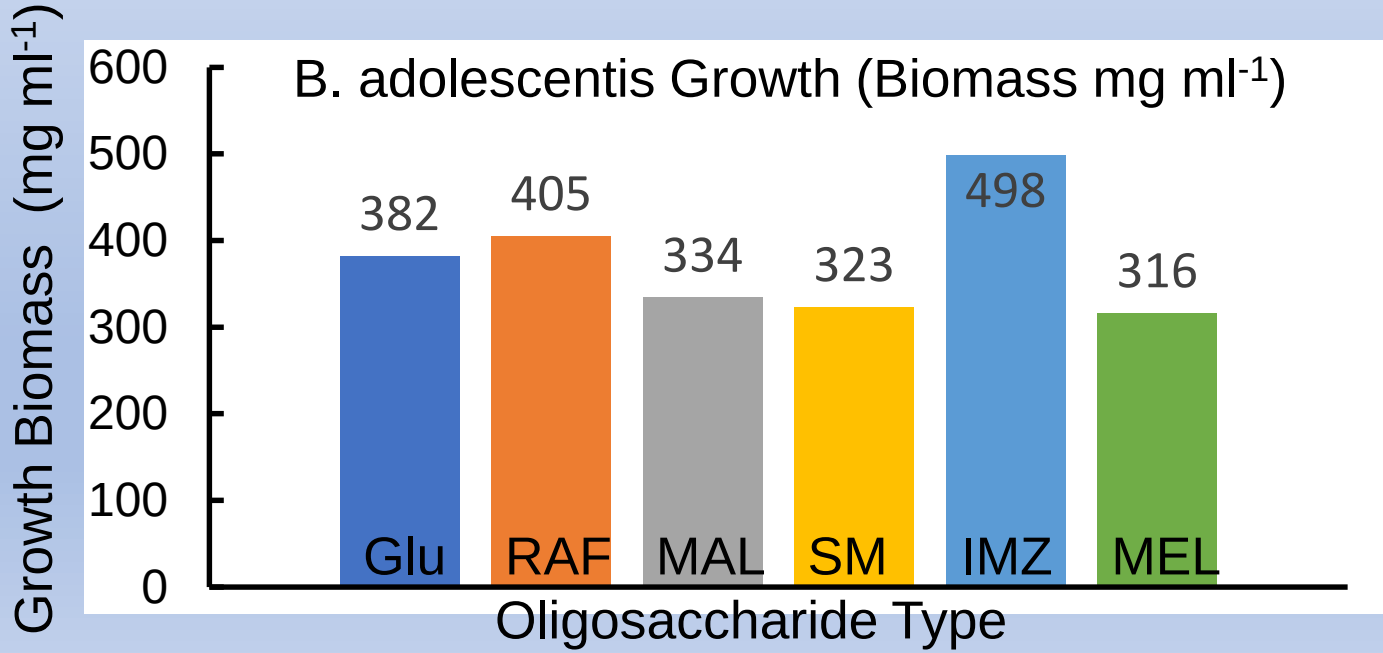


highest growth rates;

SM (0.60 μ h^{-1}),

IMZ (0.69 μ h^{-1})

MEL Oligos (1.19 μ h^{-1}).



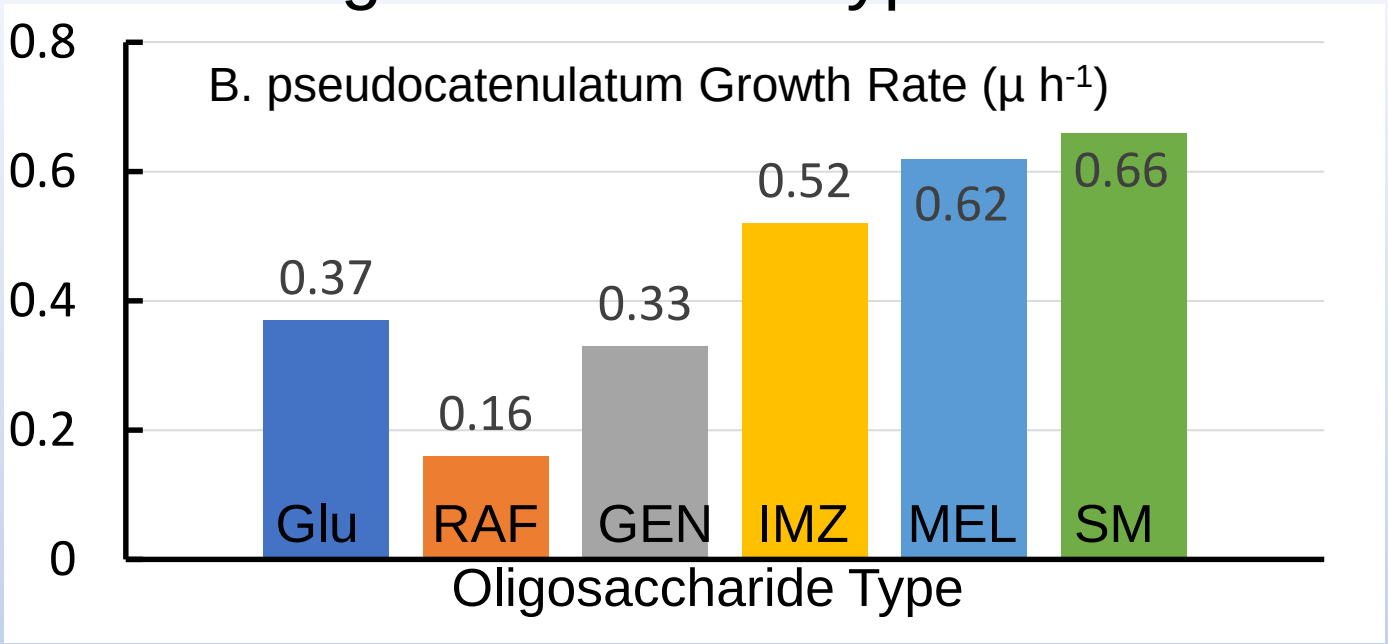
highest Biomass formation

Glu (382 mg ml^{-1})

RAF Oligos (403 mg ml^{-1})

IMZ (498 mg ml^{-1}).

Influence of Oligosaccharide Type on Bifidobacterium Growth

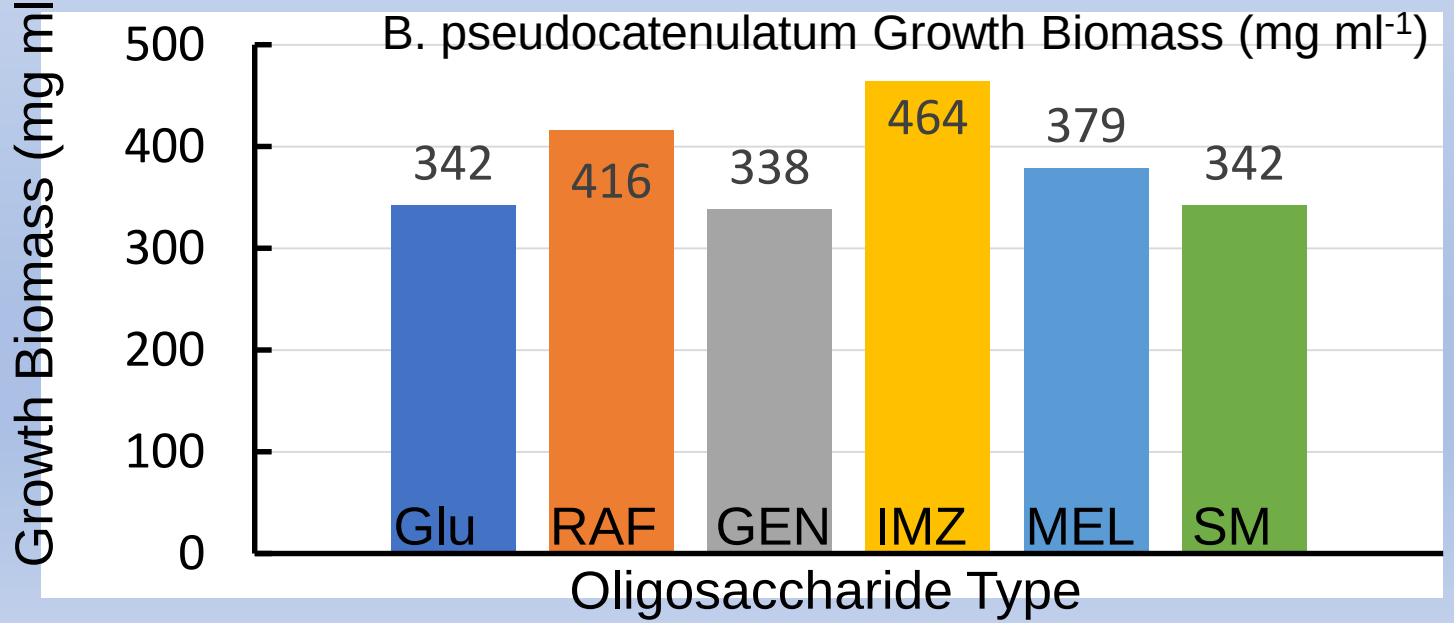


highest growth rates

IMZ (0.52 μ h^{-1}),

MEL Oligos (0.62μ h^{-1})

Sucromalt (0.66μ h^{-1})



highest Biomass Formation

MEL (379 mg ml^{-1})

RAF (416 mg ml^{-1})

IMZ (464 mg ml^{-1})

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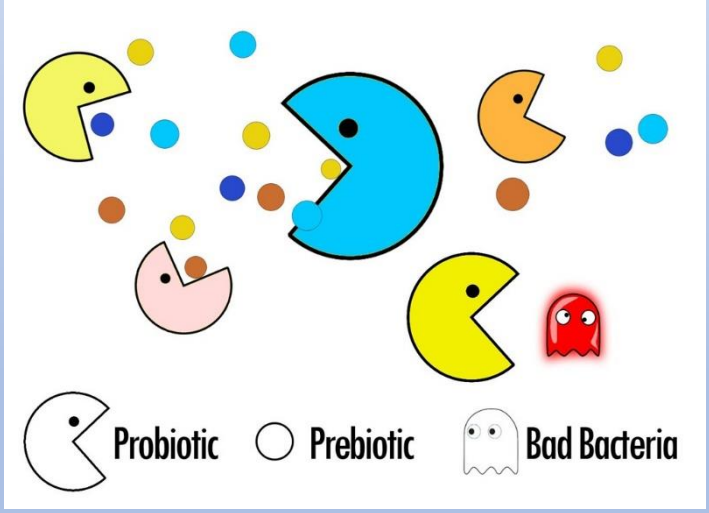
Summary Oligosaccharide Research

Some oligosaccharides supported significant growth of intestinal *Bifidobacterium*
Oligosaccharide use depends on species
Some Oligos may be good candidates for further studies of their prebiotic potential



http://www.mega-sun.vn/upload/images/pacman-ghosts(1).jpg

Prebiotic

- a non-digestible food or feed ingredient
- selectively stimulates the growth or activity of beneficial bacteria in the colon
  Bifidobacterium or *Lactobacillus*
- improves host health
  reduce GI disease, aid digestion, boost immunity?