Growth of Beneficial *Bifidobacterium* on Unique Oligosaccharides

S.M. Holt*, C. Skory, and G.L. Cote. *Western Illinois University, Biological Sciences, Macomb, IL &
USDA, National Center for Agricultural Utilization Research, Peoria, IL

ABSTRACT

Prebiotics are dietary supplements used to selectively stimulate the growth of beneficial intestinal bacteria such as Bifidobacterium in humans and animals. Maintenance of beneficial intestinal bacteria may provide ecological-based, health-promoting factors such as disease suppression and improved metabolism. A promising source of potential prebiotic supplements is through enzymatic synthesis of oligosaccharides using microbial glucansucrase enzymes. Glucansucrases can be used to prepare a seemingly endless variety of potential prebiotic oligosaccharides containing various linkage structures and monosaccharide components. In this study, six oligosaccharide products synthesized by bacterial glucansucrase were assessed for ability to support bacterial growth (growth rate, μ h-1) and biomass (cell dry weight, mg ml-1) formation using two Bifidobacterium species commonly associated with the gastrointestinal tract of animals.

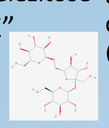
METHODS

Oligosaccharide preparations were obtained from the USDA, ARS, NCAUR, Peoria, IL. Each growth assessment was performed anaerobically using sealed Balch Glass Test Tubes (17 ml) in triplicate and reported as the mean. Bifidobacterium adolescentis ATCC 15703 and Bifidobacterium pseudocatenulatum ATCC 27919 were obtained from ATCC. *Bifidobacterium* species were grown anaerobically on modified DSM 58 medium (Marx et al., 2000) containing 2% w/v of each oligosaccharide preparation as the sole carbohydrate source. DSM medium was pre-treated with Oxyrase 60 min before inoculation and incubation to maintain anaerobiosis. Each culture was incubated at 37C for 24-48 hours. Growth was measured at A660nm and was converted to biomass (cell dry weight, mg ml-1) using the equation 364.77 • A660 + 6.7 • A660 (Gerhardt, 1999). A plot of growth (cdw log10) versus incubation time (hours) was used to determine growth rate (μ h^{-1} and maximum biomass, mg ml^{-1}.

Oligosaccharide Types Used In This Study

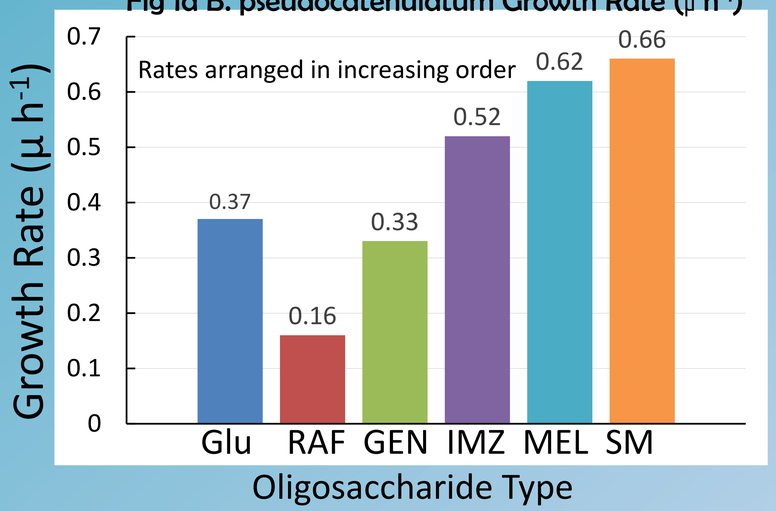
Acceptor Molecule	Acceptor Product Composition & Structure
Gentiobiose "GEN"	84% DP3, α-D-Glc-(1→6)β-D-Glc-(1→6)-D-Glc 9% DP4, α-D-Glc-(1→6)α-D-Glc-(1→6)β-D-Glc-(1→6)-D-Glc 7% DP4, α-D-Glc-(1→3)α-D-Glc-(1→6)β-D-Glc-(1→6)-D-Glc
Maltitol "MAL"	50% DP3, α-D-Glc-(1→6)α-D-Glc-(1→4)α-D-Glucitol (panitol) 25% 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 "Sucromalt" "SM"	67% DP3, 6^2-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% DP 4, 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-Fru (raffinose) <5% DP5 and higher, structures not determined

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



RESULTS

Fig. 1a &1b. Influence of Oligosaccharide Type on B. pseudocatenulatum Growth Rate (μ h^{-1}) and Biomass (mg ml^{-1}) Formation



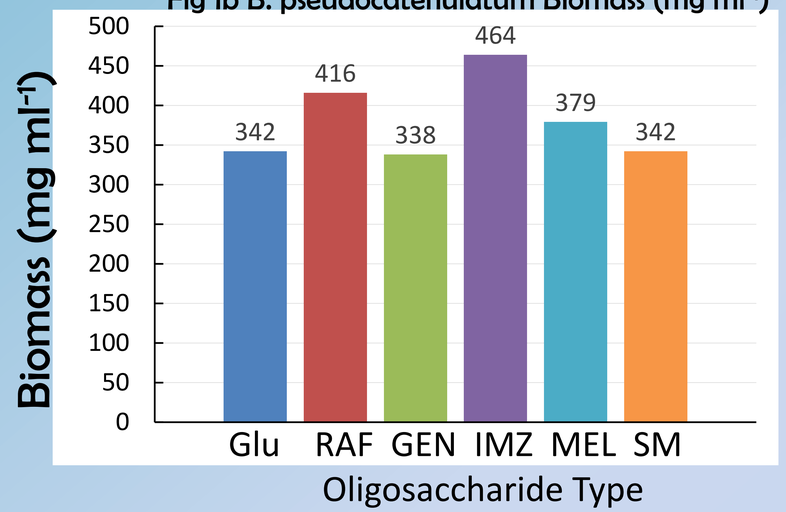
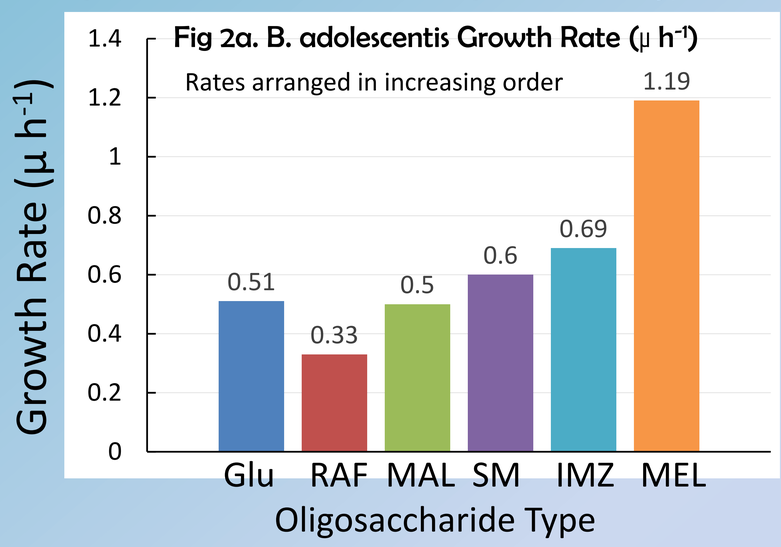
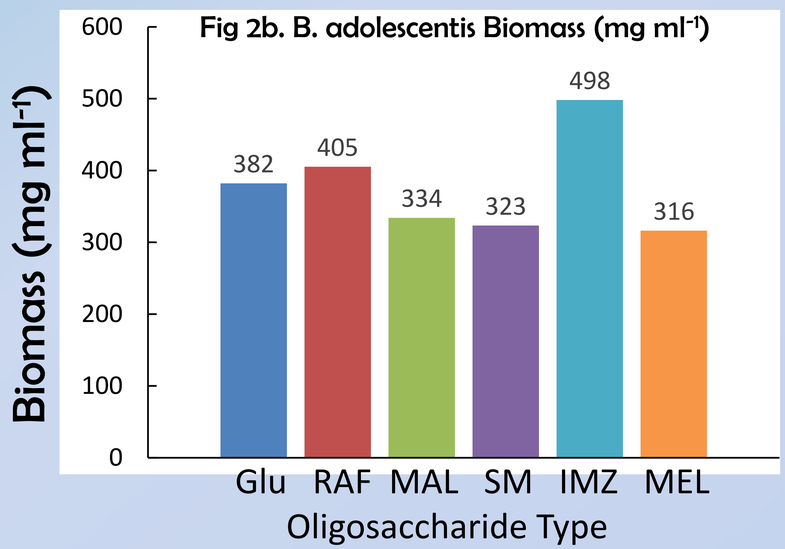


Fig. 2a & 2b. The Influence of Oligosaccharide Type on B. adolescentis Growth Rate (μ h^{-1}) and Biomass (mg ml^{-1}) Formation





RESULTS • CONCLUSIONS • SUMMARY

For B. pseudocatenulatum, the highest growth rates were achieved with IMZ (0.52 μ h^{-1}), MEL Oligos (0.62μ h^{-1}), and Sucromalt (0.66μ h^{-1}). The highest Biomass occurred with MEL (379 mg ml^{-1}), RAF (416 mg ml^{-1}), and IMZ (464 mg^{-1})

For B. adolescentis, the highest growth rates were achieved with SM (0.60 μ h^{-1}), IMZ (0.69 μ h^{-1}), and MEL Oligos (1.19 μ h^{-1}). The highest Biomass formation occurred with Glu (382 mg ml-1), RAF Oligos (403 mg ml-1), and IMZ (498 mg ml-1).

RAF & GENT supported the lowest growth rates for the listed species. In addition to growth rate, IMZ also supported the highest biomass for both *Bifidobacterium* species (498, 464 mg ml-1). RAF supported the second highest biomass for both species (404, 416 mg ml-1) even though the growth rates were lowest indicating a slow but steady metabolism of this supplement.

In summary, the Sucromalt, isomelezitose, melibiose, and raffinose products supported significant growth of intestinal *Bifidobacterium* and are good candidates for further studies of their prebiotic potential.