Formulation and design of synbiotics: rationale, strategies, and challenges

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GLOBAL PREBIOTIC ASSOCIATION

Food Science and Technology Department Impacting the World Three Times a Day | FOOD for HEALTH | University of Nebraska Lincoln

Disclosures: Funding from companies that sell/use probiotics and prebiotics
Board of Directors of ISAPP
Founding partner/owner of Synbiotic Health

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Outline

- Past and present
- Why probiotics and prebiotics may not be the answer
- The potential of synbiotics
- The science of developing synbiotics
- Examples in the marketplace
- Examples from clinical trials
- Future

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THE JOURNAL OF NUTRITION 1995

Dietary Modulation of the Human Colonic Microbiota: Introducing the Concept of Prebiotics

GLENN R. GIBSON and MARCEL B. ROBERFROID*

"One approach that may be encouraged for future research is the combination of both probiotics and prebiotics as synbiotics, which may be defined as ***a mixture of probiotics and prebiotics*** *that beneficially affects the host by improving the survival and implantation of live microbial dietary supplements in the gastrointestinal tract, by selectively stimulating the growth and/or by activating the metabolism of one or a limited number of health-promoting bacteria, and thus improving host welfare."*

"Beyond nutritional benefits, probiotics, prebiotics and (**perhaps most importantly**) **synbiotics** have potential pharmaceutical applications."

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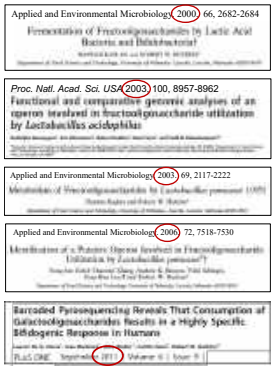
Metabolism of prebiotics by gut microbes

- Hutkins lab has been studying prebiotics for > 20 years
- Especially interested in how probiotics and other gut microbes degrade and transport prebiotics
- Prebiotics studied include GOS, FOS, inulin, PDX, xylan, and XOS
- Research includes both pre-clinical and human clinical trials



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- Introduced the concept of **Complementary** and **Synergistic** Synbiotics
- Suggested *in vitro* approaches to identify prebiotics that best supported specific probiotics strains
- Recommended RCTs to establish efficacy including probiotic and prebiotic arms as controls
- Recommended effective doses be determined

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Being clear about the differences between complementary and synergistic

Complementary

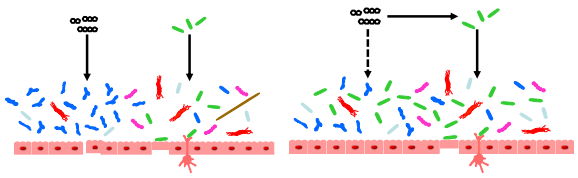
Prebiotic chosen to boost resident microbes

Probiotic chosen for specific beneficial effects

Synergistic

Prebiotic stimulates growth and activity of the cognate probiotic

Probiotic chosen for specific beneficial effects



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The 2011 paper was certainly an improvement

So why did ISAPP revisit the definition?

- Original definition too wordy and lacked precision
- Original definition was too restrictive
- Synbiotics were being used in clinical studies and in commercial products, without conforming to **any** particular definition or rationale
- **Consensus Panel Goal:** to propose a scientifically valid, clear and concise definition of 'synbiotics' for relevant stakeholders

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ISAPP Synbiotic Consensus Panel
Antwerp, Belgium, May 2019

Kelly Swanson, University of Illinois
Glenn Gibson, University of Reading
Robert Hutkins, University of Nebraska
Raylene Reimer, University of Calgary
Gregor Reid, University of Western Ontario
Karen Scott, University of Aberdeen
Hannah Holscher, University of Illinois
Meghan Azad, University of Manitoba
Nathalie Delzenne, UC Louvain
Kristin Verbeke, KU Leuven
Mary Ellen Sanders, ISAPP

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So why not Synbiotic = Probiotic + Prebiotic?

- May be true, but not always
- By definition, both probiotics and prebiotics must each provide a health benefit
- But for a synbiotic, the microbe doesn't have to be probiotic nor does the substrate have to be prebiotic
- The only requirement is that the **combination must provide a health benefit**
- For example, it is possible that a synbiotic could be functional at doses below that necessary for the individual components

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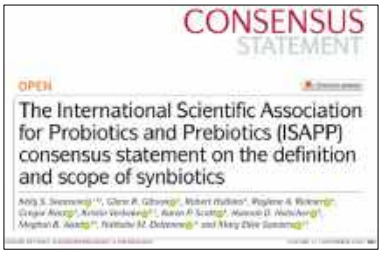

"a mixture comprising live microorganisms and substrate(s) selectively utilized by host microorganisms that confers a health benefit on the host"

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What else was in the Consensus Paper?

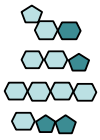
1. Synbiotics should be well described

Microbe (or probiotic)

- Current taxonomic nomenclature
- Number (of each strain)
- Genome sequence and annotation
- Safety status

Substrate (or prebiotic)

- Structure
- Purity and amount
- Supplier



Stability (or shelf-life)

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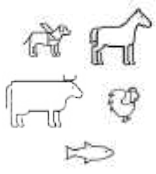
2. Target sites and hosts can vary

Gut is not the only target

- Oral synbiotics
- Vaginal synbiotics
- Topical synbiotics

Humans are not the only hosts

- Companion animals
- Livestock and poultry
- Aquaculture



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3. Just as for prebiotics and probiotics, appropriate RCT study design is critical

- Participant population: host species, age, sex, health status
- Intervention description: strains and substrate
- Complementary or synergistic
- Primary and secondary outcomes
- Crossover or parallel-arm
- Placebo/control options
- Statistics and statistical power
- Microbiota analysis
- Document safety – CONSORT guidelines

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Goal of gut health products:
Improve host health by modifying the microbiota

Why Synbiotics?

Because ***modifying the gut microbiota*** is not easy

- **Colonization resistance**: the sum of those factors that contribute to the inability of foreign organisms to implant in the host GI tract
- Thus, under ordinary circumstances, it is difficult for transient organisms, **including beneficial microbes**, to displace the resident microbiota

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How to overcome colonization resistance and enrich for beneficial microbes in the gut

- Be consumed regularly at high doses
- Give those strains a competitive advantage
- **Do both**

That's where synbiotics come in

"the ability of a probiotic strain to persist when ***specific niche-defining resources*** *are available* ***reinforces the potential of the synbiotic concept***"

Maldonado-Gomez, Walter et al., 2016, Cell Host & Microbe, 20, 415-417

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Personalizing your microbiota with synbiotics

- Microbiomes are unique to the individual
- That's why, in large part, individuals respond differently to gut health interventions.
- Every prebiotic study has non-responders, likely because keystone members are missing

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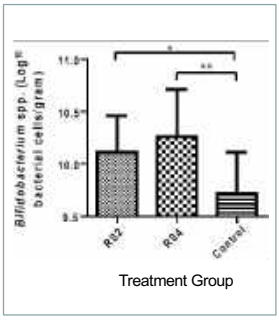
Resistant starch is bifidogenic, but not for all subjects: There were responders and non-responders



Subject	RS2 vs control	RS4 vs control
KD	231%	656%
IM	98%	355%
JH	192%	559%
JW	694%	-5%
NP	654%	1033%
DB	5%	295%
RL	2%	22%
MH	140%	568%
KLo	-32%	55%
PVK	133%	23%
Average	**+212%**	**+356%**

Adapted from Martinez et al., 2010

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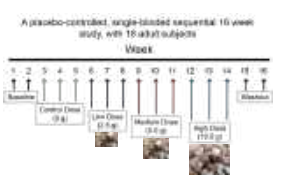
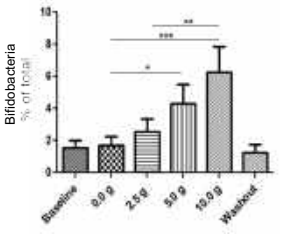
Bifidogenicity of GOS among healthy adults

Study design: GOS feeding study, 18 subjects, increasing doses for 12 weeks





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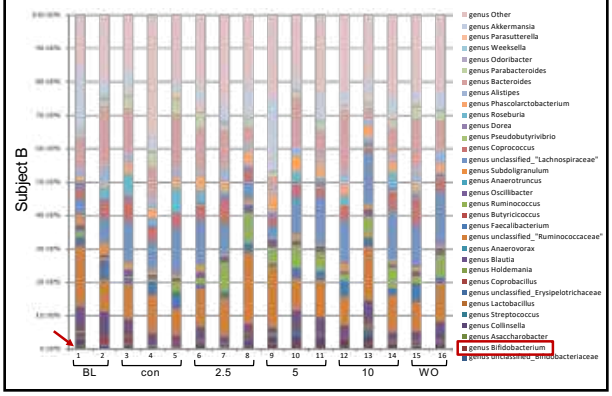
Some individuals are GOS non-responders



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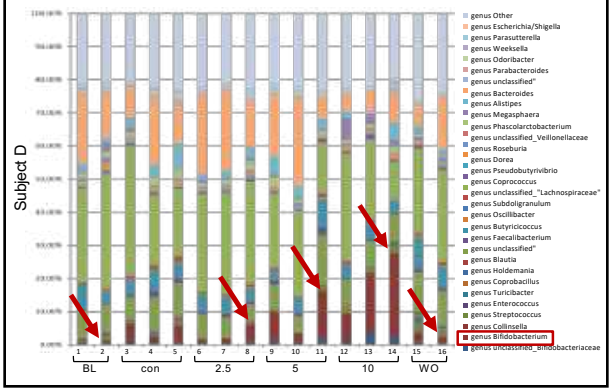
Others are GOS responders



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Personalizing your microbiota with synbiotics

- Microbiomes are unique to the individual
- That's why, in large part, individuals respond differently to gut health interventions.
- Every prebiotic study has non-responders, likely because keystone members are missing
- **Synbiotics that deliver the prebiotic AND the microbe that uses that prebiotic would be expected to improve responder rates**

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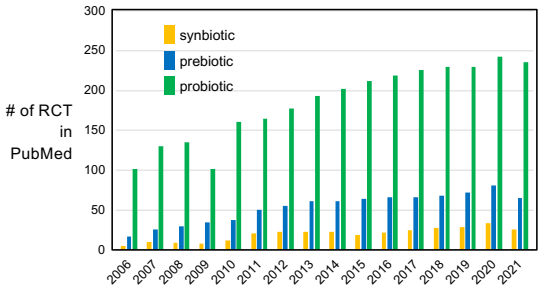
Despite their potential, far fewer synbiotic RCTs



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Synbiotics realities

- Most commercial synbiotics have not been formulated according to the suggested guidelines (esp. doses)
- Instead, prebiotic-probiotic combinations are often formulated based on convenience, cost, etc.
- Experimental evidence for synergistic or additive effects is lacking (beyond *in vitro* experiments)

➡ In general, prebiotic-probiotic combinations have not been selected on a rational basis

Adapted from Kolida and Gibson, 2011 and Krumbeck et al., 2018

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Formulating synbiotics: More than just one from column A and one from column B?

Probiotics

Prebiotics

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This approach is common. But 'prebiotic' doses for most synbiotics are < 1 g, far less than needed to be effective

15 strains + **315 mg** inulin | 9 strains + **200 mg** konjac root | 3 strains + **60 mg** FOS | 7 strains + **130 mg** FOS + inulin | 11 strains + **150 mg** prebiotics

1 strain + **1 g** psyllium | 7 strains + **300 mg** mannan | 5 strains + **25 mg** FOS | 14 strains + **25 mg** HMO + others | 7 strains + **200 mg** guar gum

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Challenges for synbiotic supplements

- How to squeeze enough prebiotic into a capsule
- Even large capsules (size 000) accommodate < 1g
- Minimum prebiotic doses are usually 2 - 5 g
- Alternative deliveries: straws, sachets, gummies
- Or consider delivery via foods and beverages

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Although some products contain up to 6 g, others don't even state an amount

1 strain + **2.5 g** inulin | 2 strains + **2 g** inulin | 1 strain + **3 g** FOS | 1 strain + **6 g** guar gum

6 strains + supernatant + **unstated mg** inulin | 3 strains + **unstated mg** prebiotic | 3 strains + **unstated mg** chicory root

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In theory, synbiotic (or pro + pre) foods may provide opportunities for more effective prebiotic doses

3 g chicory root fiber (inulin)

3 g inulin

0.6 g inulin

unstated mg yacon root

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Examples of synbiotics in clinical studies

1. Perhaps the best success story for synbiotics


24 AUGUST 2017 | VOL 548 | NATURE | 407

A randomized synbiotic trial to prevent sepsis among infants in rural India

***L. plantarum* ATCC 202195 + FOS significantly reduced sepsis and respiratory tract infections in infants from rural areas**

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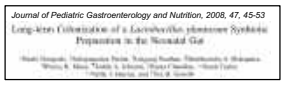
24 AUGUST 2017 | VOL 548 | NATURE | 407

A randomized synbiotic trial to prevent sepsis among infants in rural India


Journal of Pediatric Gastroenterology and Nutrition, 2008, 47, 45-53

Study highlights

- Two-arm, placebo v. synbiotic
- Synbiotic = *L. plantarum* 202195 + 150 mg FOS
- **Rationale**: Strain selected based on its ability to colonize the infant gut and block adherence and translocation of Gram-negative bacteria

- But **no rationale** for using FOS or if the strain was able to grow on FOS
- Also, no evidence that the strain was enriched *in vivo*

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2. Another synbiotic with a health effect but still no rationale


J Cosmet Dermatol. 2021;20:2841–2850.

Synbiotics supplement is effective for Melasma improvement

Piyanat Piyavatin MD[1] | Sirinthip Chaichalotornkul MD, PhD[1] | Thamthiwat Narasatwanchai MD, PhD[1] | Akkarach Bunrunggert PhD[2] | Tawee Saiwichai PhD[2]

- Primary outcomes: treatment of melasma (facial blemishes)
- Two-arm, placebo v. synbiotic
- Synbiotic = 3 LAB, + 3 bifidobacterial + FOS
- **No rationale, no strain information, no FOS dose**
- Result: synbiotics improved the severity of melasma score.

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3. Yet another synbiotic with an effect but no rationale


Australian Dental Journal, 2020; 65: 210–219

Effect of synbiotics in the treatment of smokers and non-smokers with gingivitis: randomized controlled trial

- Primary outcomes: gingival crevicular fluid levels of IL-6, IL-8 and IL-10
- Four-arm: smoker v. non-smoker, placebo v. synbiotic
- Synbiotic = 6 microbes + 239 mg FOS
- **No rationale, no strain information**
- Result: Synbiotics reduced subclinical therapeutic outcome

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4. Would improved synbiotic stability confer synergism?


Factors affecting the production of synbiotic fermented milk tablets containing jerusalem artichoke powder and *Lacticaseibacillus casei* TISTR 1463

"*The combination of inulin-rich Jerusalem artichoke powder plus L. casei demonstrated a synergistic effect on probiotic viability during fermentation and drying.*"

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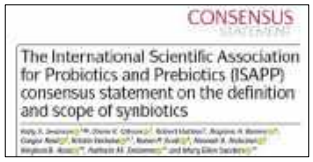
One of the main goals of the ISAPP statement:


CONSENSUS

The International Scientific Association for Probiotics and Prebiotics (ISAPP) consensus statement on the definition and scope of synbiotics

*"This proposed definition of a synbiotic should encourage **innovation** in formulations by not requiring that component parts meet the strict definitions of either a probiotic or a prebiotic."*

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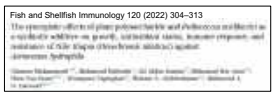
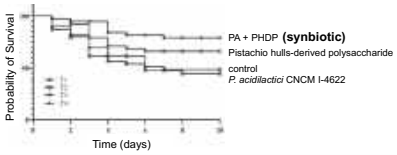
A few examples of synbiotic innovations

1. Disease-resistance in fish


Fish and Shellfish Immunology 120 (2022) 304–313

Survival rates (%) of tilapia after infection with *Aeromonas hydrophila* were **enhanced** by a **putative synbiotic**

***Pediococcus acidilactici* + pistachio polysaccharide**



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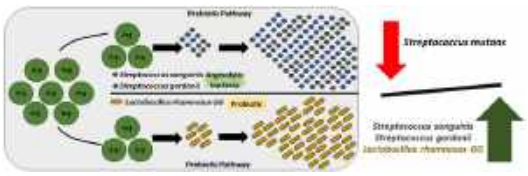
2. A putative synbiotic inhibits cariogenic *S. mutans*

LGG + arginine



Scientific Reports (2020), 7951

Effect of a novel synbiotic on *Streptococcus mutans*

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According to Swanson et al., what makes a synbiotic synergistic?

1. The substrate is selectively utilized by the microbe
2. The measured health benefit is greater than the estimated effects of each component separately

Additional distinctions?

3. *The responder rate is increased*
4. *Persistence or activity is enhanced*
5. *Rational basis for expecting synergism*

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Rationale for synbiotic pairing

- Based on previous probiotics clinical trials
- Based on pre-clinical, animal, or *in vitro* data
- Based on biochemical compatibility
- For many published studies there simply isn't one

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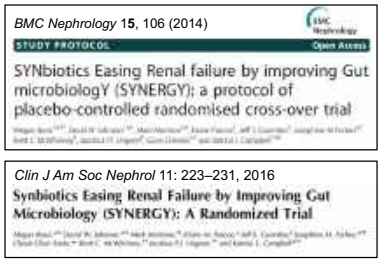
A few examples of a clearly stated rationale


BMC Nephrology **15**, 106 (2014)

STUDY PROTOCOL Open Access

SYNbiotics Easing Renal failure by improving Gut microbiologY (SYNERGY): a protocol of placebo-controlled randomised cross-over trial

Clin J Am Soc Nephrol 11: 223–231, 2016

Synbiotics Easing Renal Failure by Improving Gut Microbiology (SYNERGY): A Randomized Trial

*"The **underlying rationale** for selecting the bacterial strains in the synbiotic formulation is the **mechanistic inhibition of bacterial production of** uremic toxins"*

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- Subjects = Caesarean-delivered, mix-fed formula + bf
- Primary outcomes: metagenome, metabolome, other-omics
- Three-arm: control v. prebiotic v. synbiotic (+ reference group)
- Synbiotic = GOS:FOS + *Bifidobacterium breve* M-16 V
- **Rationale based on previous pre-clinical and clinical trials**

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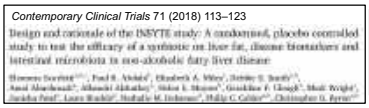

- Primary outcomes: liver fat content, markers of liver fibrosis
- Two-arm: control v. synbiotic
- Synbiotic = BB-12 + 8 g FOS
- **Rationale:** 1. synbiotic *chosen to maximize beneficial effects*
  2. *BB-12 was chosen based on specific beneficial effects on the host*
  3. *FOS was chosen to specifically stimulate growth and activity of BB-12 and to* ***improve its survival in the host***
- Main results: synbiotic altered the microbiome but did not reduce liver fat content or markers of liver fibrosis.

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- Two-arm: control v. synbiotic
- Synbiotic = *B. animalis* LMG P-28149 + 5 g FOS
- **Rationale**: Previous literature with *Bifidobacterium*/synbiotics
- Outcomes: synbiotic reduced duration of abdominal discomfort
  synbiotic reduced proinflammatory cytokines

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Requirements for Synergistic Synbiotics (Note: it's a high bar)

- The microbe of interest must selectively utilize and outcompete other microbes for the substrate
- Clinical benefit must be greater than the placebo **AND** the individual components
- Pre-clinical studies can be useful for formulating and testing synbiotic pairs for potential synergism

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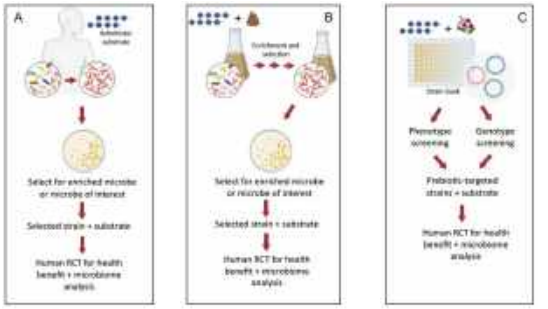
Pre-Clinical Platforms for Rational Design of Synergistic Synbiotics

***In vivo* selection- IVS** | ***In vitro* enrichment- IVE** | **Gene-trait matchmaking**



Krumbeck al., 2015, AEM, 81:2455 | Kok et al., 2019, AEM, 85:e01073 | Fuhren et al., 2020, AEM, 86:e01081-

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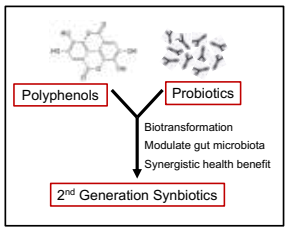
Thinking more broadly about synbiotics

Case-in-point: enhancing bioactivity of polyphenolics



Screening of prebiotic polyphenols

↓ *Inhibition of pathogenic bacteria*
Not inhibitory to probiotic bacteria

In vitro screening of potential synbiotic combinations

↓ *Synergistic effects*

In vivo evaluation

↓ *Synergistic health benefits*
Microbiome modulation

Development of second generation synbiotics

Adapted from Sharma and Padwad, 2020, Nutrition 77

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Conclusions

- Synbiotic design should conform to the definition
- Rationally designed synergistic synbiotics may be an effective strategy to personalize one's microbiota
- Pre-clinical studies can be useful for formulating and testing synbiotic pairs for potential synergism

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Thanks to recent students, collaborators, and sponsors

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Questions?

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