Trends in Microbiology

CellPress OPEN ACCESS

Review

The impact of artificial sweeteners on bacterial physiology and the microbiome

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Artificial sweeteners are non-nutritive compounds that have a profound sweetening effect with a negligible to zero calorific contribution. Global initiatives to reduce sugar consumption to tackle health conditions such as obesity have led to a significant increase in their consumption in recent decades. Artificial sweeteners have undergone extensive testing to determine whether their consumption could impact human health; however, their impact on the microbiome and microbial physiology has been comparatively overlooked. Recent work has demonstrated that artificial sweeteners (e.g., Ace-K, saccharin, and aspartame) can influence the oral and gut microbiome and that they can significantly affect bacterial behavior and growth. In this review, we will contextualize these findings and explore their relevance to human artificial sweetener consumption.

Highlights

Artificial sweeteners have been shown to impact the human gut microbiome to varying levels, with saccharin having the most pronounced effect, influencing the human glycemic response via gut microbiome dysbiosis.

At environmentally relevant concentrations, a range of artificial sweeteners have been shown to impact bacterial conjugation and natural transformation.

Several artificial sweeteners have been shown to inhibit bacterial growth, including the growth of multidrug-resistant pathogens.

Certain artificial sweeteners, such as Ace-K and saccharin, have also shown antivirulence potential by inhibiting biofilm formation and increasing antibiotic susceptibility, highlighting their therapeutic potential.

Artificial sweeteners in the human diet

Artificial sweeteners are compounds that mimic sugar in taste but are typically nonnutritive or of low-calorie value, while having a higher sweetening intensity than sugar. Many of these sweeteners were originally fortuitous discoveries; for example, saccharin was discovered in 1879 by Constantin Fahlberg while working with the coal tar derivative benzoic sulfamide[i] [1]. Among the sweeteners currently in commercial use, there is a wide structural diversity, with some having a chemical structure similar to sucrose, such as the sugar alcohol stevia. By contrast, others have a comparatively distinct structure, such as aspartame, which has an aspartyl-phenylalanine methyl ester [2] (Table S1). Their mechanisms of action typically involve these sweeteners binding to sweet taste receptors (STRs) with higher affinity than sucrose, thereby producing an intensely sweet taste [3]. In some cases, the sweetness is attributed to these compounds being able to bind to unique binding sites on the STR instead of the main site used by sucrose [4]. While the effects of these compounds on eukaryotic cells have been extensively studied and used to set acceptable daily intake (ADI) amounts, their impact on prokaryotic cells has only begun to be investigated in recent decades [5,6].

Most artificial sweeteners pass through the body unmetabolized, allowing them to reach the gut and interact with the bacteria present before being excreted. Once through the digestive system, they then enter wastewater systems and have been increasingly detected in wastewater treatment plants worldwide [7]. Importantly, before this happens, while passing through the gut, these compounds interact with the gut microbiome. These interactions could facilitate dysbiosis, which is the imbalance or disruption of microbial communities, by altering the composition or function of the microbes that make up the system [8]. The gut microbiome has been shown to be highly responsive to the presence of artificial sweeteners in the diet, driving fluctuations in the species diversity and richness within the gut microbiota [9–12] (Figure 1). While there has been an increasing body of evidence revealing the impact of artificial sweeteners on the microbiome (Table 1), there has also been an emerging body of data indicating that these compounds can impact bacterial cellular behaviors, such as natural transformation, motility, biofilm

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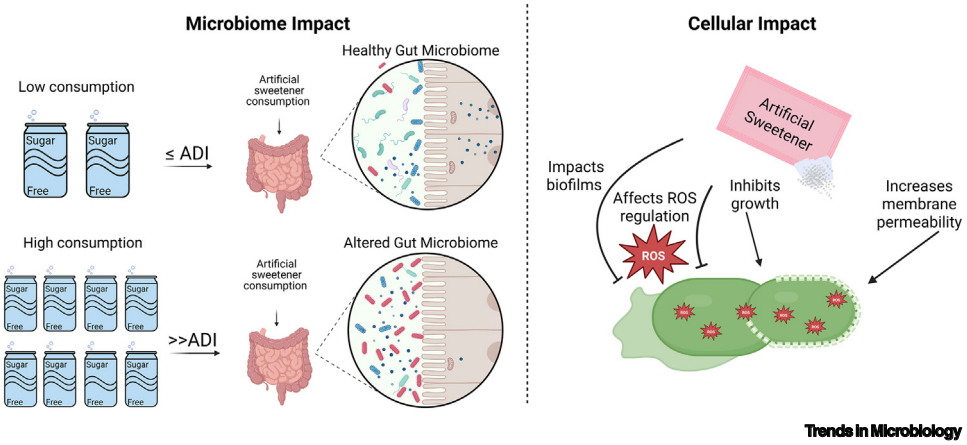

Figure 1. The impact of artificial sweeteners on the gut microbiome and bacterial physiology. On the left panel, the standard findings show that, when tested below the acceptable daily intake (ADI), microbiomes are stable. However, often, when the ADI is exceeded, the microbiome is altered. The right panel outlines the key cellular impacts that artificial sweeteners have on individual bacteria *in vitro*.

formation, and even bacterial viability (Table 2), perhaps explaining how some of these effects on the microbiome are being mediated. It is likely that sweeteners impact bacterial growth, which may alter the microbiome and, in turn, affect the host. For example, sweetener-induced alterations to the gut microbiome have been shown to be linked to host physiological responses, such as glucose tolerance, insulin sensitivity, and inflammation.

In this review, we explore three of the most widely used and well-studied artificial sweeteners—acesulfame potassium (Ace-K), aspartame, and saccharin—providing a comprehensive breakdown of their effects on bacterial physiology, microbiome, and host physiology.

Acesulfame potassium

Effects on the microbiome. One of the most commonly used artificial sweeteners, Ace-K (ADI of 15 mg/kg body weight), has been evaluated in several animal and human microbiome studies. Hanawa *et al.* [13] used C57BL/6J male mice (n = 4/group, 8-week-old mice), which consumed water spiked with Ace-K at a 150-mg/kg concentration (above ADI) for 8 weeks. They observed that α-diversity was significantly lower in the group consuming Ace-K as compared to the control group, using Shannon index values [13]. There were significant gut microbiota fluctuations seen at the phyla level, including an increase in Actinobacteria and Verrucomicrobia, as well as a decrease in Bacteroidetes, Deferribacteres, and Proteobacteria [13]. It was also observed that there was an increase in the proportion of Erysipelotrichaceae and a decrease in the proportion of Clostridiaceae, Lachnospiraceae, and Ruminococcaceae families. These changes in chronic Ace-K-exposed mice were coupled with an increased expression of several proinflammatory cytokines (interferon γ, interleukin 1β, and tumor necrosis factor α) within small intestine mucosa along with increased intestinal permeability, suggesting intestinal epithelial cell damage. Furthermore, Ace-K increased the expression of MadCAM-1, an adhesion protein that is involved in the adhesion of leukocytes to mucosal tissues. A key point in this study is that access to the Ace-K-spiked water was unregulated, so actual sweetener consumption levels cannot be accurately determined.

In another study, CD-1 mice were fed Ace-K by gavage (n = 10/group; 8-week-old; males and females) at a concentration of 37.5 mg/kg body weight per day (above ADI) for 4 weeks and an altered gut microbiome diversity was observed. Specifically, there was an increase in *Bacteroides*

Table 1. Effect of artificial sweeteners on the microbiome at different doses

Sweetener	Model	Daily intake	Alterations	Study methods	Publication reference
Ace-K	C57Bl/6J mice, n = 4/group, 8-week-old, males	150 mg/kg—above ADI	Increased Actinobacteria, Bacteroidetes, Deferribacteres, Proteobacteria, and Verrucomicrobia Increased proportion of Erysipelotrichacecae Decreased Clostridiaceae, Lachnospiraceae, and Ruminococcaceae	Unmonitored consumption for 8 weeks. Microbiota transplant	[13]
Ace-K	CD-1 mice, n = 5/group, 8-week-old, females (5) and males (5)	37.5 mg/kg—above ADI	Male: Increased *Bacteroides*, significant changes in genera *Anaerostipes* and *Sutterella* Female: increased the abundance of Mucispirillum; decreased the relative abundance of genera *Lactobacillus, Clostridium*, an unassigned Ruminococcaceae genus, and unassigned Oxalobacteraceae genus	Monitored consumption for 4 weeks. Metabolomics analysis	[14]
Ace-K	Human, n = 7/group, 18 years or older, females and males	1.7–33.2 mg—above ADI	No	Monitored consumption for 4 days.	[10]
Ace-K	C57Bl/6J mice, n = 9/group, 8-week-old, males	15 mg/kg—ADI limit	No	Monitored for 8 weeks. Metabolome analysis of cecum luminal contents	[9]
Ace-K	Wistar rats, n = 5/group, 70–day-old, females and males	40 mg/kg—above ADI	No	Monitored consumption for 27 days. Metabolomics of plasma and feces	[15]
Ace-K	Wistar rats, n = 5/group, 70–day-old, females and males	120 mg/kg—above ADI	No	Monitored consumption for 27 days. Metabolomics of plasma and feces	[15]
Saccharin	C57Bl/6 mice, n = 10/group, 10-week-old, males	6.3 g/kg—above ADI	Increased *Bacteroides* and Clostridial order Decreased *L. reuteri*	Unmonitored consumption for 11 weeks. Fecal transplantation; metagenomic analysis	[12]
Saccharin	C57BL/6J mice, n = 10/group, 8-week-old, males	15 mg/kg—ADI limit	After 3 months: Increased *Sporosarcina, Jeotgalicoccus*, *Akkermansia*, *Oscillospira* and *Corynebacterium* Decreased *Anaerostipes* and *Ruminococcus* After 6 months: Increased *Corynebacterium, Roseburia*, and *Turicibacter* Decreased *Ruminococcus, Adlercreutzia*, and *Dorea*	Monitored consumption for 3 or 6 months. Fecal gut microbiota analysis; fecal metabolite analysis	[16]
Saccharin	C57BL/6JRj mice, n = 6/group, 7-week-old, males	5 mg/kg—below ADI	Increased Bacteroides and Proteobacteria Decreased Firmicutes	Monitored consumption for 5 weeks. Fecal bacterial load analysis	[17]
Saccharin	Sprague–Dawley rats, n = 6/group, 6-week-old, females	22.4 mg/kg—above ADI	Increased *Rodentibacter* Decreased *Rothia*	Unmonitored consumption for 8 weeks. PICRUSt Metagenome prediction.	[18]
Saccharin	C57BL/6 mice, n = 10/group, 6-week-old, females	0.1 mg/ml—below ADI	Increased *Streptococcus, Prevotella, Fusibacter, Lachnospira, Anaerovorax, Psychrilyobacter, Psychromonas*,	Monitored consumption for 11 and 10 weeks. Metagenomics	[19]

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Table 1. (continued)

Sweetener	Model	Daily intake	Alterations	Study methods	Publication reference
			and *Trabulsiella* Decreased *Lactobacillus*		
Saccharin	Human, n = 20, 18–70 years, females and males	3 mg/kg below ADI	Increased *Blautia* Decreased *Ruminococcus* Decreased *Fusobacterium*	Monitored consumption for 28 days. Metagenomic sequencing; fecal microbiome transplantation into Swiss-Webster mice	[20]
Saccharin	C57Bl\6J mice, n = 23–28/group, 8-week-old, males	60 mg/kg—above ADI	No	Monitored consumption for 10 weeks. Fecal metabolomics	[21]
Saccharin	Human, n = 12/group, 18–45 years, females and males	15 mg/kg—ADI limit	No	Monitored consumption for 2 weeks. Fecal metabolomics	[21]
Aspartame	Sprague–Dawley rats, n = 10–12/group, males	5–7 mg/kg—below ADI	Increased Enterobacteriaceae and *C. leptum*	Unmonitored consumption for 8 weeks.	[22]
Aspartame	C57BL/6 mice, n = 6–12/group, 8-week-old, males	40 mg/kg—ADI limit	Increased *Prevotellaaceae* NK3B31 group and *Alloprevotella* Decreased *Candidatus Saccharimonas* and *Enterorhabdus*	Monitored consumption for 1 week	[23]
Aspartame	Human, n = 20, 18–70 years, females and males	4 mg/kg—below ADI	*Oral:* Decreased Porphyromonas and *Prevotella nanceiensis*	Monitored consumption for 28 days. Metagenomic sequencing; fecal microbiome transplantation into Swiss-Webster Mice	[20]
Aspartame	Human, n = 7/group, 18 years or older, females and males	62.7 mg/kg—above ADI	No	Monitored consumption for 4 days.	[10]
Aspartame	Human, n = 17, 18–45 years, females and males	7 mg/kg—below ADI	No	Monitored consumption for 12 weeks. Fecal SCFA analysis	[24]
Aspartame	Sprague–Dawley rats, n = 15/group, 8-week-old, females	5–7 mg/kg—below ADI	Maternal: Reduced abundance in Enterococcaceae, *Enterococcus*, *Parasutterella* Increased *Clostridium* cluster IV Offspring: Overabundant Porphyromonadaceae	Monitored consumption for 18 weeks. Fecal microbiota transplant.	[25]
Aspartame	BALB/c mice, maternal, n = 3/group; offspring, n = 14/group, 3-week-old	40 mg/kg—ADI limit	No	Unmonitored consumption for 2 weeks. Metabolomic analysis	[26]
Aspartame	BALB/c mice, maternal n = 3/group; offspring n = 14/group, 3-week-old	40 mg/kg—ADI limit	Slight impact on β-diversity	Unmonitored consumption for 2 weeks.	[27]
Sucralose	Human, n = 120, 18–70 years, females and males	1.7 mg/kg—below ADI	Increased *Bacteroides* Decreased *Blautia*	Monitored consumption for 28 days. Metagenomic sequencing; fecal microbiome transplantation into Swiss-Webster Mice	[20]
Sucralose	C57BL/6 mice, n = 5/group, 8-week-old,	5–15 mg/kg—ADI limit	Offspring—Increased *Akkermansia, Blautia, Corynebacterium,* and *Robinsoniella* Decreased *Alistipes, Barnesiella, Paraprevotella,*	Unmonitored consumption for 6 weeks.	[28]

Table 1. (continued)

Sweetener	Model	Daily intake	Alterations	Study methods	Publication reference
	females and males		*Saccharibacteria_genera_incertaesedis*, *Streptococcus*		
Sucralose	Sprague–Dawley rats, n = 6/group, 4-week-old, males	0.11 mg/kg—below ADI	Increased Acidaminococcaceae, Barnesiellaceae Decreased Lactobacillaceae, Akkermansiaceae, Streptococcaceae, and Muribaculaceae	Monitored consumption for 4 weeks. Predicted metabolic functions of cecal microbiota.	[29]
Sucralose	Sprague–Dawley rats, n = 6/group, 4-week-old, males	0.16 mg/kg—below ADI	Increased Barnesiellaceae Decreased Lactobacillaceae, Akkermansiaceae, and Streptococcaceae	Monitored consumption for 4 weeks. Predicted metabolic functions of cecal microbiota.	[29]
Sucralose	Wistar rats, n = 6/group, 5-week-old, males	1.5%	Increased *Bacteroides* Decreased *Lactococcus, Mucispirillum*, and *Bifidobacteria*	Unmonitored consumption for 4 months. Metagenomics analysis.	[30]
Sucralose	Human, n = 20/group, 18–35 years, females and males	0.25 mg/kg—below ADI	Increased *Blautia coccoides* Decreased Firmicutes and *Lactobacillus acidophilus*	Monitored consumption for 10 weeks.	[31]
Sucralose	C57BL/6 mice, n = 10/group, 6-week-old, females	5 mg/kg—below ADI	Increased *Streptococcus, Prevotella, Fusibacter, Lachnospira, Anaerovorax, Psychrilyobacter, Psychromonas*, and *Trabulsiella* Decreased *Lactobacillus*	Monitored consumption for 11 weeks. Metagenomic analysis.	[19]
Isomalt	Sprague–Dawley rats, n = 6/group, 7-week-old, males	10% of isomalt (w/w)—NA ADI	Increased *Akkermansia* and *Blautia* Decreased *Acinetobacter*	Unmonitored consumption for 6 weeks. Metabolomics analysis.	[32]
Isomalt	Human, n = 19, 21–53 years, females (12) and males (7)	30 g daily—NA ADI	Increased bifidobacteria	Monitored for 4 weeks.	[33]
Xylitol	Human, n = 13/group, 23–25 years, females and males	6.2 g daily—NA ADI	Decreased Prevotellaceae, *Streptococcus, Lautropia, Abiotrophia, Porphyromonas*, and *Actinomyces*	Monitored consumption for 2 weeks.	[34]
Xylitol	C57Bl/6J mice, n = 5/group, 3-week-old, males	40 NA ADI	Increased two *Clostridium* species and a *Faecalibaculum* genus Decreased *Clostridium* and *Barnesiella* genera	Monitored consumption for 16–18 weeks. Metabolome analysis of cecum luminal content	[35]
Xylitol	C57Bl/6J mice, n = 6/group, 3-week-old, males	194 NA ADI	Increased *Feacalibaculum* and *Prevotella* Decreased *Barnesiella* and *Bacteroides*	Monitored consumption for 16–18 weeks. Metabolome analysis of cecum luminal content	[35]
Xylitol	C57Bl/6J mice, n = 8/group, 8-week-old, males	2170 and 5420 mg/kg; NA ADI	Increased *Bifidobacterium* and Erysipelotrichaceae	Unmonitored consumption for 3 months. Metabolome analysis	[36]
Xylitol	Human, n = 29, 20–30 years, females (15) and males (14)	70% xylitol; NA ADI	No	Monitored consumption for 14 weeks.	[37]
Neotame	CD-1 mice, n = 5/group, 7-week-old, males	0.75 mg/kg—below ADI	Decreased Firmicutes, *Blautia, Dorea, Oscillospira*, and *Ruminococcus* Increased Bacteroidetes: *Bacteroides*	Monitored consumption for 4 weeks. Metabolomics analysis	[38]
Maltitol	Human, n = 40	22.8 g/50 g of chocolate daily; NA ADI	Increased *Bifidobacteria, Bacteroides, Lactobacilli, Atopobium* spp., *Eubacterium rectale, F. prausnitzii, Ruminococcus bromii, Ruminococcus*	Monitored consumption for 14 days.	[39]

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Table 1. (continued)

Sweetener	Model	Daily intake	Alterations	Study methods	Publication reference
			flavefaciens, and *Clostridium histolyticum/perfringens*		
Maltitol	Human, n = 48	NA ADI	Decreased *Actinomyces massiliensis* HOT 852 and *Lautropia mirabilis* HOT 022	Monitored consumption for 42 days	[40]
Maltitol	Human, n = 18/group, 18 years or older, females and males	1 g daily mg/kg; NA ADI	Decreased *Actinomyces naeslundii* HOT-176 and *Actinomyces* HOT-169	Monitored consumption for 2 weeks.	[41]
Lactitol	Human, n = 24/group, 45–65 years, females (7) and males (17)	15 g daily; NA ADI	Increased *Bifidobacterium longum, Bifidobacterium pseudocatenulatum, Bacteroides ovatus, Rothi mucilaginosa, Lactobacillus fermentium, Lactobacillus oris*, and *Lactobacillus salivarius* Decreased *K. pneumoniae,* Peptostreptococcaceae unclassified*, Roseburia inulinivorans*, and *E. rectale*	Monitored consumption for 4 weeks. Metagenomic and metabolomics analysis	[42]
Lactitol	Human, n = 29 18–75 years, females (22), and males (7)	20 g day 1, then 10 g daily; NA ADI	Increased Actinobacteria, Bifidobacteriales, Bifidobacteriaceae, and *Bifidobacterium*	Monitored consumption for 2 weeks.	[43]
Lactitol	C57BL/6J mice, n = 10/group, 6- to 8-week-old, males	6600 mg/kg; NA ADI	No	Monitored consumption for 1 weeks.	[44]
Lactitol	Human, n = 23, 65 years or older	10 g daily; NA ADI	No	Monitored consumption for 2 weeks.	[45]
Erythritol	*Cebus apella*, monkey, n = 1 females	60 mg/kg; NA ADI	No	Monitored consumption for 2 weeks.	[46]
Stevia	C57Bl/6J mice RebA n = 20/group RebD n = 17/group 8-week-old, males	50 mg/kg—above ADI	RebD: Increased *Feacalibaculum rodentium* RebA: No	Monitored consumption for 12 weeks. Metagenomic analysis.	[47]
Stevia	C57BL/6J Mice, n = 6/group, Maternal 21-week-old Offspring 8-week-old, females and males	0.5 mg/ml daily—below ADI	Increased maternal: *Lactobacillus apodemi* Offspring: *L. apodemi*	Unmonitored consumption for 8 weeks. Fecal microbiota transplantation. Metabolomics analysis	[48]
Stevia	Arbor acres broiler chicken, n = 128/group 1-day-old; males	100–3200—above ADI	Increased *Bifidobacteria* in the cecal digesta Decreased *E. coli*.	Unmonitored consumption for 42 days.	[49]
Stevia	Ross 308 broiler chicken n = 6/group, 1-day-old, males	250 mg/kg—above ADI	Increased Lactobacillales, Bacilli, *Lactobacillus*, and Lactobacillaceae Decreased Ruminococcaceae, Lachnospiraceae, Clostridia, and Clostridiales	Unmonitored consumption for 21 days.	[50]
Stevia	*Cebus apella*, monkey, n = 1 females	2 mg/kg—below ADI	No	Monitored consumption for 2 weeks.	[46]
Stevia	Sprague-Dawley rats, n = 8/group, 3-week-old, males	2–3 mg/kg—below ADI	No	Monitored consumption for 9 weeks.	[51]
Stevia	Mice n = 16/group,	194 mg/L daily—	No	Unmonitored consumption for	[52]

Table 1. (continued)

Sweetener	Model	Daily intake	Alterations	Study methods	Publication reference
	4- to 6-week-old	below ADI		15 weeks	
Stevia	C57BL/6J mice, n = 7–10/group, 4-week-old, males	50 mg/kg—above ADI	No	Monitored consumption for 4 weeks.	[53]
Stevia	Human, n = 27/group, 18–50 years, females (16) and males (11)	75.6 mg daily—above ADI	No	Monitored consumption for 4 weeks	[54]
Sorbitol	C57BL/6 mice, n = 7/group, 6-week-old, Male	45 mg/kg; NA ADI	Increased Clostridiales, Prevotellaceae, Rikenellaceae, Tannerellaceae, Helicobacteraceae, Clostridiales_vadinBB60_group, Defluviitaleaceae, Peptococcaceae	Monitored consumption for 4 weeks	[55]
			Relative abundances of 12 genera were comparatively decreased in the sorbitol intake group, while 23 genera were comparatively increased in the sorbitol intake group.		

in male mice only and a significant increase in *Anaerostipes* and *Sutterella* genera [14]. *Anaerostipes* species are involved in fermenting carbohydrates and are associated with obesity, whereas *Sutterella* species are associated with proinflammatory responses [14]. Conversely, the female mice were impacted differently, with an increased abundance of *Mucispirillum* and a decreased abundance of *Lactobacillus and Clostridium* genera and of Ruminococcaceae and Oxalobacteraceae families, which are involved in polysaccharide fermentation and food digestion [14]. There were other sex-specific changes observed in mice given Ace-K, such as male mice being twice the body mass, while female mice showed no significant changes in body mass compared with the water-fed control group. Furthermore, microbiome functional gene enrichment analysis revealed additional Ace-K-induced effects. For example, they observed increased relative abundances of genes associated with carbohydrate metabolism and fermentation pathways, which can be linked to the increase in *Bacteroides*. Female mice fed Ace-K showed decreased relative abundances of genes associated with energy metabolism pathways. It was also shown that the relative abundances of genes involved in carbohydrate absorption or transport were significantly decreased [14]. Female mice fed Ace-K also demonstrated increased relative abundances of genes linked to inflammation, specifically genes linked to lipopolysaccharide (LPS) synthesis [UDP-glucose:(heptosyl) LPS α-1,3-glucosyltransferase, ADP-L-glycero-D-manno-heptose 6-epimerase, amino-4-deoxy-L-arabinose transferase, UDP-D-GlcNAcA oxidase, and UDP-GlcNAc3NAcA epimerase]. In Ace-K-consuming male mice, only two genes participating in LPS biosynthesis displayed an increased relative abundance [14]. LPS is known to trigger inflammation, and increased levels are associated with an increased risk of chronic inflammation; however, the levels of gut epithelial inflammation were not investigated as part of this study. Several bacterial metabolism-related metabolites were shown to be affected in the fecal metabolome. Female mice displayed decreased lactic acid and succinic acid levels, whereas in male mice, there were increased pyruvic acid levels, which is a central metabolite of energy metabolism. This suggests that the weight gain observed in male mice may be due to increased energy metabolism and increased harvesting capacity caused by microbiome alterations.

A study using the FDA-recommended ADI for Ace-K of 15 mg/kg (administered via monitored fluid intake) in C57Bl/6J male mice (n = 9/group, 4-week-old mice) for 8 weeks showed no significant changes in gut microbiota from fecal or cecal content, luminal metabolites or body weight

Table 2. Effect of artificial sweeteners on the bacterial physiology and behavior

Sweetener	Bacteria species	Phenotype	Result	Reference
Ace-K	*S. mutans*	EPS and acid production	Inhibited	[11]
	S. mutans and *S. sanguinis*	Growth	Inhibited	[11]
	A. baumannii	Growth	Inhibited	[56]
	P. aeruginosa	Growth	Inhibited	[56]
	E. coli	Growth	Inhibited	[57]
	E. coli, *P. alloputida*	Plasmid conjugation	Promoted	[58]
	A. baylyi and *B. subtilis*	Transformation frequency	Elevated	[59]
	E. coli	Transformation frequency	Increased	[59]
	A. baumannii	Transformation frequency	Inhibited	[56]
	K. pnuemoniae	Live/dead	Significant dead/damaged increase	[60]
	E. coli	Live/dead	Significant dead/damaged increase	[60]
	B. subtilis	Live/dead	Significant dead/damaged increase	[60]
	E. coli and *P. alloputida*	Cell membrane permeability	Increased	[58]
	E. coli	Cell membrane permeability	Increased	[60]
	B. subtilis	Cell membrane permeability	Increased	[60]
	K. pnuemoniae	Cell membrane permeability	Increased	[60]
	E. coli	ROS production	Increased	[60]
	B. subtilis	ROS production	Increased	[60]
	K. pnuemoniae	ROS production	Increased	[60]
	A. baylyi	Cell membrane permeability	Increased	[59]
	E. coli and *P. alloputida*	ROS production	Increased	[58]
	P. aeruginosa	Biofilm formation	Inhibited	[56]
	A. baumannii	Biofilm formation	Inhibited	[56]
	A. baumannii	Twitching motility	Inhibited	[56]
	A. baumannii	Antibiotic efficacy	Increased	[56]
Aspartame	*E. coli* and *P. alloputida*	Plasmid conjugation	Promoted	[58]
	E. coli	Cell membrane permeability	Increased	[60]
	B. subtilis	Cell membrane permeability	Increased	[60]
	K. pnuemoniae	Cell membrane permeability	Increased	[60]
	E. coli and *P. alloputida*	Cell membrane	Increased	[58]

Table 2. (continued)

Sweetener	Bacteria species	Phenotype	Result	Reference
		permeability		
	A. baylyi	Cell membrane permeability	Increased	[59]
	E. coli	Live/dead	Significant dead/ damaged increase	[60]
	B. subtilis	Live/dead	Significant dead/ damaged increase	[60]
	K. pnuemoniae	Live/dead	Significant dead/ damaged increase	[60]
	E. coli and *P. alloputida*	ROS production	Increased	[58]
	E. coli	ROS production	Increased	[60]
	B. subtilis	ROS production	Increased	[60]
	K. pnuemoniae	ROS production	Increased	[60]
	E. coli	Epithelial cell adhesion	Increased	[61]
	E. coli	Epithelial cell invasion	Increased	[61]
	E. coli	Epithelial cell viability	Unaffected	[61]
	E. faecalis	Epithelial cell adhesion	Increased	[61]
	E. faecalis	Epithelial cell invasion	Increased	[61]
	E. faecalis	Epithelial cell viability	Increased	[61]
	E. coli	Biofilm formation	Increased	[61]
	E. faecalis	Biofilm formation	Increased	[61]
	E. coli and *E. faecalis*	Growth	No effect	[61]
	A. baylyi and *B. subtilis*	Transformation frequency	Elevated	[59]
Saccharin	*S. mutans*	Biofilm formation	Biomass reduction	[62]
	E. coli	Biofilm formation	Increased	[61]
	P. aeruginosa	Biofilm formation	Inhibited	[63]
	A. baumannii			
	S. aureus			
	E. coli and *P. alloputida*	Plasmid conjugation	Promoted	[58]
	E. coli	Live/dead	Significant dead/ damaged increase	[60]
	B. subtilis	Live/dead	Significant dead/ damaged increase	[60]
	K. pnuemoniae	Live/dead	Significant dead/ damaged increase	[60]
	E. coli and *P. alloputida*	Cell membrane permeability	Promoted in some cases	[58]
	A. baylyi	Cell membrane permeability	Increased	[59]

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Table 2. (continued)

Sweetener	Bacteria species	Phenotype	Result	Reference
	E. coli	Cell membrane permeability	Increased	[60]
	B. subtilis	Cell membrane permeability	Increased	[60]
	K. pnuemoniae	Cell membrane permeability	Increased	[60]
	E. coli	ROS production	Increased	[60]
	B. subtilis	ROS production	Increased	[60]
	E. coli and *P. alloputida*	ROS production	No effect	[58]
	K. pnuemoniae	ROS production	Increased	[60]
	A. baumannii	Growth	Inhibited	[56]
	S. aureus	Exponential growth phase	Delayed	[17]
	K. pnuemoniae	Exponential growth phase	Delayed	[17]
	P. aeruginosa	Exponential growth phase	Delayed	[17]
	B. cereus	Exponential growth phase	Delayed	[17]
	E. coli	Growth	Reduced	[61]
	E. faecalis	Growth	No effect	[64]
	P. aeruginosa	Growth	Inhibited	[63]
	E. coli			
	A. baumannii			
	K. pneumoniae			
	S. aureus			
	E. coli			[57]
	A. baylyi and *B. subtilis*	Transformation frequency	Increased	[59]
	A. baumannii and *P. aeruginosa*	Twitching motility	Inhibited	[63]
Sucralose	*F. prausnitzii*	Growth	No effect	[65]
	E. coli	Growth	No effect	[61]
	Streptomyces badius	Growth	Inhibited	[66]
	Ensifer arboris			
	Citrobacter murlinlae			
	Rhizobium borborid			
	Microbacterium sp. U 13			
	Stenotrophomonas sp. I_61			
	E. coli			[57]
	S. mutans	Biofilm production	Biomass reduction	[62]
	E. coli	Biofilm formation	Increased	[61]
	E. coli and *P. alloputida*	Plasmid conjugation	Promoted	[58]
	A. baylyi and *B. subtilis*	Transformation frequency	Elevated	[59]
	E. coli	Live/dead	Significant dead/	[60]

Table 2. (continued)

Sweetener	Bacteria species	Phenotype	Result	Reference
			damaged increase	
	B. subtilis	Live/dead	Significant dead/ damaged increase	[60]
	K. pnuemoniae	Live/dead	Significant dead/ damaged increase	[60]
	E. coli	ROS production	Increased	[58]
	E. coli	ROS production	Increased	[60]
	B. subtilis	ROS production	Increased	[60]
	K. pnuemoniae	ROS production	Increased	[60]
	E. coli and *P. alloputida*	Cell membrane permeability	Increased	[58]
	E. coli	Cell membrane permeability	Increased	[60]
	B. subtilis	Cell membrane permeability	Increased	[60]
	K. pnuemoniae	Cell membrane permeability	Increased	[60]
Xylitol	*S. mutans, Streptococcus sobrinus, Prevotella intermedia, Streptococcus salivarius, Streptococcus mitis, Bacteriods forsytus, Lactobacillus casei, Ligilactobacillus salivarius, Lactobacillus fermentum*	Bacterial concentration	Decreased except *B. forsytus*	[67]
	S. mutans	Zone of inhibition	No effect	[68]
	S. sanguinis			
	L. acidophilus			
	Staphylococcus pseudintermedius	Growth	Inhibited	[69]
	Staphylococcus schleiferi			
	S. aureus			
	S. pneumoniae and *S. mitis*	Growth	Inhibited	[70]
	P. aeruginosa, S. aureus, or coagulase-negative *Staphylococcus*	Growth	No effect	[71]
	S. mutans	Biofilm formation/growth	Inhibited	[72]
	E. coli and *Lactobacillus rhamnosus*	Biofilm formation	Inhibited	[73]
Neotame	*Escherichia coli* K802NR	Inhibitory activities	No effect	[74]
	E. coli and *E. faecalis*	Biofilm formation	Increased	[75]
	E. coli, Shigella, and *Enterococcus faecium*	Growth	No effect	[75]
Mannitol	*A. baumannii* and *P. aeruginosa*	Growth	No effect	[56]
	S. mutans and *S. sanguinis*	Zone of inhibition	No effect	[68]
	L. acidophilus			
	E. coli	Growth	Inhibited	[76]
Maltitol	*A. baumannii*	Growth	No effect	[56]
Maltitol	*Actinomyces oris*	Growth	No effect	[40]

(continued on next page)

Table 2. (continued)

Sweetener	Bacteria species	Phenotype	Result	Reference
	Actinomyces dentalis			
	Actinomyces johnsonii	Growth	Inhibited	[40]
	Actinomyces massiliensis			
	Actinomyces israelii			
	Actinomyces naeslundii			
	S. mutans	Growth	Inhibited	[77]
	S. pseudintermedius	Growth	No effect exept slight increase in *S. pseudintermedius*	[69]
	S. schleiferi			
	S. aureus			
Erythritol	*Porphyromonas gulae*	Growth	Inhibited	[78]
	Bacteroides thetaiotaomicron	Growth	No effect	[46]
	Bifidobacterium longum			
	C. leptum			
	E. coli			
	L. acidophilus			
	Blautia coccoides			
	S. pseudintermedius	Growth	Inhibited	[69]
	S. schleiferi			
	S. aureus			
	P. gingivalis	Growth	Inhibited	[79]
	Aggregatibacter actinomycetemcomitans			
	Actinomices viscosus			
	Streptococcus gordonii	Growth	Reduced except *P. micra* and *S. mutants*	[80]
	A. naeslundii			
	S. mutans			
	Fusobacterium nucleatum			
	Parvimonas micra			
	P. intermedia			
	P. gingivalis			
	B. abortus	Growth	Increased	[81]
	S. gordonii and *A. naeslundii*	Biofilm formation	Inhibited	[80]
	S. mutans	Biofilm eradication	No effect	[82]
Sorbitol	*S. pseudintermedius, S. schleiferi* and *S. aureus*	Growth	No effect except *S. pseudintermedius* slight increased	[69]
	S. mutans	Growth	Inhibited	[82]
	S. aureus	Growth	Inhibited	[83]
	E. coli			
	E. coli	Growth	Inhibited	[76]
	E. coli and *S. aureus*	Zone of inhibition	The zone increased up to 5% and then decreases after higher concentrations	[84]

Table 2. (continued)

Sweetener	Bacteria species	Phenotype	Result	Reference
	S. mutans, S. sanguinis, and *L. acidophilus*	Zone of inhibition	No effect	[68]
	Dietzia maris	Biomass	Biomass increased	[85]
Stevia	*Vibrio parahaemolyticus*	Growth	Inhibited	[86]
	S. aureus and *E. coli*	Growth	Inhibited	[87]
	Mycobacterium tuberculosis	Growth	Inhibited	[88]
	B. thetaiotaomicron	Growth	No effect	[46]
	B. longum			
	C. leptum			
	E. coli			
	L. acidophilus			
	B. coccoides			
	E. coli	Growth	Inhibited	[57]
	Lactobacilli and *Bifidobacteria*	Carbon source	Can be used as a carbon source to a limited extent	[89]
Lactitol	*Lactobacillus crispatus*	Growth	Increased	[90]
	Lactobacillus vaginalis, Lactobacillus gasseri, *Lactobacillus jensenii, Lactobacillus iners, Prevotella bivia, Gardnerella vaginalis, Mobiluncus curtisii,* and *Atopobium vaginae*	Growth	No effect	[90]
	Candida albicans	Growth	Inhibited	[90]
	Bifidobacterium pseudocatenulatum	Growth	Inhibited	[91]

[9]. Another study using higher concentrations of 40 mg/kg (above ADI) for 4 weeks, administered by gavage in Wistar rats (n = 5/group, 10-week-old rats, both sexes), also showed no significant changes in the gut microbiota. In female rats, α-diversity was similar to that of the control group, while male rats exhibited slightly higher α-diversity, when using the Shannon true diversity algorithm. β-Diversity showed that bacterial diversity between the Ace-K-treated and control groups was similar using Bray–Curtis analysis. There were no differences in water or food consumption and body weight between groups [15]. The same study also dosed animals with 120 mg/kg of Ace-K, and some changes in fecal metabolites were observed, including amino acids, complex lipids, fatty acids, and their derivatives. A difference between males and females was only seen in carbohydrate metabolites. In the plasma metabolome of males, there was a significant reduction in primary glycine-conjugated bile acids (glycochenodeoxycholic acid), secondary glycine bile acid conjugates (e.g., glycodeoxycholate and glycoursodeoxycholic acid), and the secondary bile acid ω-muricholic acid [15]. This highlights the need to study functional changes in the microbiome and changes in the microbiome composition.

There are comparatively few studies exploring the impact of Ace-K on the human gut microbiome. Frankenfeld *et al*. [10] completed a 4-day food record of 31 individuals, seven of whom consumed between 1.7 and 33.2 mg/d Ace-K (below to above ADI). Fecal samples were collected on day 5 for microbiome analysis, which revealed that there were no significant differences in β-diversity between those who consumed Ace-K and those who did not, using the UniFrac analysis [10]. There were no significant differences in the relative abundance of gene functions across treated and control samples. Furthermore, the study included 3 people who

had a combination of Ace-K and aspartame, which also showed no impact. The reason for the absence of impact in the study may be due to the low concentrations of Ace-K consumed or low cohort size. These studies suggest that at concentrations in or around the ADI, there is limited impact of Ace-K on the gut microbiome, but at higher concentrations, effects can be observed, and in some instances, the extent of these effects is specific to sex, at least in animal models. This raises an interesting question around consumption levels of artificial sweeteners among humans and the lack of accurate data available to be able to ascertain consumption levels across demographics. Given the unregulated nature of artificial sweetener intake and their proliferation on supermarket shelves, it is highly likely that some demographics are consuming in excess of the ADI. More research is necessary to quantify actual consumption concentrations and evaluate their effects in human microbiome studies.

Biological effects

Ace-K has been shown to impact bacterial physiology and behavior in a dose- dependent manner in isolated intestinal communities [92]. de Dios *et al*. [56] demonstrated that a concentration as low as 0.89% (wt/vol%) could inhibit the growth of nosocomial multidrug-resistant (MDR) clinical isolates of *Acinetobacter baumannii* and *Pseudomonas aeruginosa*, with 4.43 and 5.31%, respectively, completely inhibiting growth. While these concentrations are unrealistic with respect to human consumption, they could be achieved topically, highlighting the potential for Ace-K to be repurposed as an antimicrobial [56]. Bulge-mediated cell lysis was shown to be the underlying mechanism of action of Ace-K, with cells exhibiting an aberrant morphology and an increased cell envelope permeability. This aligns with the transcriptional response to Ace-K exposure with genes encoding membrane-associated proteins being dysregulated [56]. This increased cell envelope permeability induced by Ace-K was shown to potentiate antibiotic activity, resensitizing MDR *A. baumannii* to a range of antibiotics, including carbapenems (β-lactam family) [56]. Ace-K was also shown to have antivirulence properties, inhibiting biofilm formation, motility, and natural transformation at sublethal concentrations. The inhibition of these traits was associated with the downregulation of Type 4 pili, a component of these virulence mechanisms. *csu* genes, which are also involved in biofilm formation were also downregulated. The therapeutic potential was further validated by incorporating Ace-K into wound dressings and demonstrating robust antimicrobial activity in *ex vivo* wound infection models. The antimicrobial potential of Ace-K was also shown in a separate study, which demonstrated that the growth of *Escherichia coli, Klebsiella pneumoniae*, and *Bacillus subtilis* was inhibited by Ace-K [60]. *B. subtilis* can be found in the human gastrointestinal tract, where it is typically considered beneficial, supporting digestion and the immune system [93,94]. Therefore, these bacteria being impacted would be detrimental to the host. Most *E. coli* strains are commensal and harmless, whereas some strains are opportunistic or pathogenic. Commensal *E. coli* in the intestines aid digestion; therefore, impacting its growth would be detrimental to the host[ii]. On the contrary, if *E. coli* is causing disease, Ace-K-mediated growth inhibition would be beneficial to the host. *K. pneumoniae* is pathogenic, making its inhibition potentially beneficial. The study suggested that an overproduction of reactive oxygen species (ROS) was underpinning these antimicrobial effects, as it was shown that Ace-K could trigger an increase in ROS [60]. It was also observed that genes associated with ROS detoxification were upregulated in these strains upon Ace-K exposure. Ace-K was also shown to induce damage to the cell envelope; however, whether this damage was a result of the increased ROS produced is not clear, as other sweeteners, such as saccharin, were also able to cause similar cell envelope damage, without impacting ROS production [60].

Ace-K has also been shown to inhibit the development of cariogenic biofilms in the oral cavity [11]. Specifically, 5 μg/ml of Ace-K decreased bacterial adherence and biofilm mass in mixed cultures of *Streptococcus mutans* and *Streptococcus sanguinis.* Ace-K was shown to reduce acid and

extracellular polymeric substances (EPS) production in *S. mutans* [11]. EPS is made of exported polysaccharides, proteins, small molecules, and lipids, which form a physical barrier to protect groups of bacteria. It also helps with attachment to surfaces and cell–cell adhesion; therefore, a reduction in EPS is beneficial for the host [95]. It was also revealed that growth was suppressed in *S. mutans* and *S. sanguinis*, suggesting prophylactic potential beyond the external wound setting [11].

Yu *et al*. [58] demonstrated that Ace-K could promote plasmid-mediated conjugation in strains of *E. coli* and *Pseudomonas alloputida* derived from wastewater. Ace-K induced donor cell membrane permeability was shown to drive this increased conjugation efficiency [58]. The same team subsequently demonstrated that Ace-K could promote antibiotic resistance gene transfer via natural transformation in *Acinetobacter baylyi* ADP1, a classical model for studying competence and transformation [59]. Although this is completely opposite to what was observed in the study by de Dios *et al.* [56], this is likely due to the differences in concentrations that were used, as de Dios *et al*. used 0.66% compared with 0.03% in this study, suggesting a dose-dependent effect. The mechanism appears to be conserved, with increases in cell envelope permeability facilitating greater plasmid transfer; however, transcriptomic analysis also revealed an upregulation of genes encoding DNA uptake and translocation (Com) machinery [59]. Moreover, it was shown that Ace-K promoted plasmid persistence in the host, which could allow the spread of antimicrobial-resistant genes [59]. This suggests that Ace-K can influence bacterial physiology and behavior in a concentration-dependent manner. Low concentrations, such as those found in wastewater, were associated with driving increased antibiotic resistance gene uptake, which could have negative implications for the spread of antibiotic resistance genes. Contrastingly, high concentrations can completely inhibit growth, kill bacteria, or resensitize bacteria to antibiotics, potentially offering a novel therapeutic strategy to tackle recalcitrant infections.

Aspartame

Effects on the microbiome

Aspartame (ADI of 40 mg/kg body weight) is another common sweetener used in everyday food products and, as a result, is one of the most well-studied sweeteners with respect to its impact on the microbiome. Similar to what has been observed with Ace-K, the impacts on the microbiome vary considerably in the reported studies. Palmnäs *et al*. [22] conducted a study using male Sprague-Dawley rats fed standard or high-fat (HF) diets and then split into groups with or without water supplemented with 5–7 mg/kg (below ADI) aspartame (10–12 per group). This dose was well below the ADI, equating to 12.5–17.5%, but revealed that in both HF diet and standard chow groups, aspartame supplementation resulted in decreased food intake, reduced final body weight and increased fluid intake. The treatment duration was 8 weeks, and fluid intake and other anthropometrics were measured during week 7 to calculate the effective doses for each group of animals. Other anthropometrics, such as body fat, liver fat, fasting glucose, and plasma insulin levels, between standard chow with water or aspartame showed no significant differences. While not significant, aspartame consumption did result in a trend of increased fasting blood glucose levels. In the HF diet groups, it was observed that aspartame consumption significantly reduced plasma insulin levels to those of a standard chow diet. However, the combination of HF diet and aspartame also came with increased levels of circulating plasma free fatty acids [22]. Both aspartame diet groups had higher baseline blood glucose levels, which showed blunted reductions after insulin tolerance tests. Rats that had aspartame showed higher total levels of *Clostridium leptum* and Enterobacteriaceae than their water-consuming counterparts on both diets [22]. The combination of HF diet and aspartame resulted in an even further increase in Enterobacteriaceae and *Roseburia* spp. *Roseburia* spp. and *C. leptum* fall under *Clostridium*

cluster IV and are considered probiotic and essential for a healthy gut because of their contributions to short-chain fatty acid (SCFA) production [96–98]. It was also confirmed that the HF diets resulted in an increase in total Firmicutes and decrease in Bacteroidetes phyla, which has been linked with obesity, but it was noted that aspartame consumption was able to temper the increase in Firmicutes, with little effect on Bacteroidetes. Additionally, aspartame was able to mitigate the increase of *Clostridium* cluster XI caused by the HF diet [22]. Serum metabolomics analysis revealed significant increases in metabolites of bacterial origin, such as acetate and butyrate, only in standard chow diets with aspartame. Butyrate also saw a notable but not significant decrease in HF diet-fed rats given aspartame. Aspartame was also associated with increased propionate, a bacterial end product and highly gluconeogenic substrate. This may explain the link between aspartame and insulin tolerance.

Another study had 8-week-old Sprague-Dawley female rats (n = 9) consume a HF diet for 10 weeks initiated prior to conception. The rats were then given aspartame via drinking water, which was adjusted weekly based on their weight and fluid consumption rate to equate to 5–7 mg/kg (below ADI) throughout pregnancy and lactation (~6 weeks) [25]. The offspring of both aspartame- and water-consuming parent female rats (dams) showed no difference in their birth weights. These pups (8 female and 9 male) were subsequently monitored for 18 weeks. Once weaned, the offspring from dams that consumed aspartame gained weight faster and had a higher fat mass than offspring from dams fed water. In early life (6 weeks old), changes in microbiota compositions between offspring groups were most evident. Interestingly, as seen in some Ace-K studies, there were sex-specific effects of sweeteners on the microbiome and host. For aspartame, these effects were observed in *Clostridium* cluster I, *C. leptum, Lactobacilli*, *Faecalibacterium prausnitzii*, and *Akkermensia muciniphila* in male offspring and Enterobacteriacaea, *C. leptum, Collinsella aerifacuens*, and *Methanobrevibacter smithii* in female offsping. The microbiota shifts and increased weight gain normalized toward the end of the study (18 weeks). Weight gain normalization was sex specific and occurred at 12 weeks in male offspring and 18 weeks in female offspring. Cecal microbiome analysis done at the end of the study (18 weeks) showed that there was no difference in α-diversity between offspring using Shannon and Simpson indices and that there were only non-significant trends suggesting a potential difference in β-diversity. Linear discriminant analysis effect size showed that offspring of dams fed aspartame had only one bacterial family that was overabundant: Porphyromonadaceae, which is associated with disease progression, aberrant glucose intolerance, and weight gain in nonalcoholic steatohepatitis mouse models. However, in dams (at weaning), the levels of Enterococcaceae, *Enterococcus*, and *Parasutterella* decreased, while that of *Clostridium* cluster IV increased in abundance [25]. Interestingly, Enterococcaceae, *Enterococcus*, and *Parasutterella* are typically associated with negative health outcomes, whereas *Clostridium* cluster IV, which increased, is associated with healthy outcomes [98–100]. Both offspring groups from aspartame-fed dams had increased blood glucose, but male offspring also had lower insulin sensitivity [25]. In addition to these changes, offspring showed signs of mesolimbic alterations that were gender specific, but none of these alterations were seen in the dams. Gene expression analysis revealed that in male offspring, there was an increase in ventral tegmental area dopamine transporter mRNA from weaning until adulthood (18 weeks), and they had greater levels of nucleus accumbens (NAc) D2 receptor than water controls. In female offspring, maternal aspartame also caused an increase in NAc D2 receptor, and NAc μ-opioid receptor levels, but a decrease in ventral tegmental area tyrosine hydroxylase [25]. These changes suggest that maternal aspartame consumption could have long-term effects on offspring mesolimbic reward pathways, but it is important to note that this is in the context of obese maternal conditions. When fecal matter transplant (FMT) was carried out from the aspartame dam offspring to mature germ-free (GF) mice, they developed similar physiological conditions, such as increased blood glucose, weight gain, and

increases in body fat percentage and mass, as well as significantly greater glucose area under curve scores, than GF mice who received FMT from offspring of dams fed water. This was coupled with an increase in the relative abundance of Porphyromonadaceae. Offspring showed no differences between each other when it came to SCFA production. Aspartame-fed dams showed significantly increased cecal propionate, butyrate, isobutyrate, isovalerate, and valerate concentrations compared with water-fed ones. Interestingly, these metabolites largely align with those described as being altered upon aspartame consumption in the study by Palmnäs *et al*. [22], suggesting the production of a specific subgroup of SCFA may be particularly sensitive to aspartame in the diet. This study also highlights the lack of information on the influence of artificial sweetener consumption during pregnancy and the potential for there to be significant host and microbiota impacts on children.

In mice, there is evidence to suggest that, in certain disease conditions, aspartame can have negative health outcomes. For instance, a 2024 study found that aspartame by itself had no direct negative effects on mice, but when coupled with ulcerative colitis (UC), it worsened the condition . In that study, C57BL/6 male mice (n = 12/group, 8-weeks-old mice) were used to investigate the direct effects of aspartame consumption on healthy and induced UC mice [23]. The mice were given 40 mg/kg (ADI limit) of aspartame via gavage for 1 week, and at the end of the study, the mice showed no difference in body weight. The authors found increased α-diversity (Shannon index) in mice given aspartame compared to those given water. Both aspartame-fed healthy and induced UC mice clustered away from the control healthy mice when plotted using principal coordinate analysis of microbiota at the genus level [23]. The authors observed that aspartame on its own did not have any impact on healthy mice, but in the induced UC mice, aspartame significantly exacerbated outcomes. The negative outcomes within induced UC were evaluated using disease activity index (DAI), a scoring that includes visual signs of blood or bloody stool around the anus; fecal texture; shortened colon length; spleen index scores highlighting inflammation; and gut ulceration metrics such as severe damage to the muscular layer, intestinal mucosal tissue, and glands. Additionally, induced UC mice that consumed aspartame exhibited significantly worse colonic tissue damage than water control-induced UC mice. In the same study, another cohort of C57BL/6 male mice (n = 6/group, 8-weeks-old mice) was used to determine other signs of inflammation and gut damage due to UC. The levels of Claudin-3 and Occludin, two proteins that play an important role in gut epithelial barrier integrity and permeability via their function as tight junction proteins, were also evaluated [23]. Immunofluorescence of the two proteins revealed that mice with induced UC and fed aspartame had significantly less intense fluorescence than the induced UC and water-fed mice. This finding was then confirmed using Western blotting. Due to these findings, it would be expected that immune cells—namely neutrophils and macrophages associated with UC—should be higher, so this was investigated [23]. Quantitative analysis showed no difference in immune cell infiltration between healthy water-fed controls and aspartame-fed mice. In contrast, mice with induced UC exhibited significantly greater immune cell infiltration than both healthy groups. Notably, the combination of aspartame consumption and induced UC resulted in a further increase in immune cell infiltration. While this study provides key insights into the potential for specific clinical conditions to be exacerbated by aspartame consumption, a greater understanding of the role of the microbiome in mediating these exacerbations is needed.

A recent study gave pregnant BALB/c mice (n = 3, age not reported) unregulated access to aspartame at a concentration of 0.25 mg/ml on gestational day 7 [27]. They reported that the predicted dose was 40 mg/kg (ADI limit), as they expected the mice to consume 4 ml/d. Five water control dams were compared with the three dams given aspartame, and there were no significant differences in the number of pups delivered [27]. However, the dams that had aspartame

displayed significantly higher body weights than the water-fed dams. There were no differences in birth weight between pups, and this was true across sexes (seven each) between those born to aspartame- and water-fed dams. Offspring born to aspartame-fed dams were not significantly heavier than those of water-fed dams at the end of weaning (3 weeks). There was also no significant difference in bacterial composition at the phyla or genus level between the offspring. No difference in α-diversity (Chao1, Shannon, and Simpson indices) was reported between offspring of both dam groups. Maternal aspartame consumption was only shown to slightly affect β-diversity (UniFrac). The key finding in this study is that offspring of the dams fed aspartame showed significantly higher levels of serum IgE than those of the control group. They also had significantly different cytokine levels within the lungs. Offspring from the aspartame-fed dams had cytokine profiles skewed significantly more toward a Th2 and significantly less toward a Th1 response [27]. Nuclear factor (NF)-κB p65 levels were also significantly higher in the offspring of the aspartame-fed dams. These changes were similar regardless of the sex of the offspring. IgE was also shown to be elevated in the aspartame-fed dam's offspring. Alongside this, the increase in transcription factor NF-κB confirms that cells are being primed to produce cytokines to facilitate the Th2 response, which has been seen in the pathogenesis of asthma. These findings together suggest that maternal aspartame consumption prompts the lung immune cells of offspring to be more susceptible to the development of chronic inflammatory conditions like asthma. This effect appears to be mediated independently of the gut microbiome of the offspring, although it could be mediated at the functional level. Future work should not only explore this but also consider effects beyond the gut, such as the lung microbiome, which could be influencing these immune responses. Ho *et al.* [26] used the same experimental setup and dosing but reported that the dose is physiologically relevant, as allometric scaling reveals it is equivalent to roughly 3.5 mg/kg for a 60-kg person. They found that maternal aspartame consumption had a significant impact on arachidonic acid metabolism, pentose phosphate pathway, purine metabolism, and amino sugar and nucleotide sugar metabolism in offspring via Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analysis. Multiple bacterial groups exhibited strong statistical associations with specific metabolites. For example, with purine metabolism, *Enterocloster, Negativibacillus*, and *Parabacteroides_B_862066* were positively correlated with uric acid levels [26]. The authors also evaluated oxidative stress using three different biomarkers within the lungs [malondialdehyde (MDA), 8-hydroxy-2′-deoxyguanosine (8-OHdG), and glutathione (GSH)]. Each biomarker showed a significant increase in oxidative stress within offspring with maternal aspartame consumption. Notably, there were some sex-specific differences, primarily in one sex, the increased oxidative stress was more pronounced. MDA, which points to ROS damage of lipids in the cell membrane, had no sex-specific effect [26]. 8-OHdG, a marker of ROS damage to DNA, was more pronounced in females. Lastly, GSH, which is an intracellular antioxidant, was reduced further in males compared to females. Although the direct effect on the microbiome was minimal, the coinciding physiological consequences have raised further questions about potential direct immune-specific effects or the potential to impact more localized microbial communities, such as the lung microbiome.

In human studies, the effect of aspartame is contrary to that of the rodent models, again putting into perspective the value of these models with respect to understanding complex diet–microbiome interactions. Most studies show no to limited effect on the microbiome and the host. Frankenfeld *et al*. [10] studied the impact of aspartame on the gut microbiome of 31 humans of which 7 consumed 62.6 mg/kg of aspartame (above ADI) for 4 consecutive days, with fecal samples being collected on the fifth day. Food intake was monitored, and there was no significant difference between aspartame consumers and nonconsumers. Microbiome analysis showed no effect on the abundance of bacteria at the order or class level in the consumer group compared with those in the nonconsumer group [10]. There was also no difference in the gut microbial

functional capability between the consumer and nonconsumer groups. It could be that although the aspartame dose was above the ADI, the treatment duration was too short to yield any significant change in humans. This lack of effect on the gut microbiome was also seen in the randomized controlled trial by Suez *et al*. [20], with 20 participants per group. In this trial, each participant received aspartame sweetener in six sachets per day, with glucose included as a bulking agent over a 14-day period [20]. The daily aspartame consumption was approximately 4 mg/kg (below ADI). There were no impacts on the gut microbiome or glucose tolerance, including no significant effect on the glucose tolerance test (GTT) incremental area under the curve (iAUC). When the baseline abundances of metagenomic features were correlated with GTT-iAUC, *Bacteroides fragilis* and *Bacteroides acidifaciens* were positively associated with GTT-iAUC. Additionally, aspartame did alter gut microbiome function and caused a reduction in the abundance of *Porphyromonas* and *Prevotella nanceiensis* in the oral microbiome. It is possible that no significant effects in the gut were observed in this study due to the low dose compared with the ADI, which is reported as only 8%. Another reason that could explain why an effect is seen in the oral microbiome but not in the gut microbiome is because, unlike most artificial sweeteners, aspartame is digested in the small intestine into aspartic acid, phenylalanine, and methanol.

Ahmad *et al*. [24] conducted a study where 17 healthy adults (16 used for analysis) consumed 7 mg/kg (below ADI) of aspartame for 2 weeks, while collecting their fecal sample at multiple stages. Although the study was conducted over a 14-week period, the first 4 weeks were reserved for following an informed diet plan. Then, in weeks 5 and 6, aspartame was consumed alongside this diet. Weeks 7–10 was a washout period back to the standard diet plan, and weeks 11 and 12 was with consumption of the sweetener they had not previously consumed. Consumption of aspartame at approximately 14% (0.425 g) of the ADI did not have any observable effects on any of the 16 participants, including no changes in six fecal metabolites. There was no measurable effect on gut microbiota at phyla or genus levels, no effect on SCFA production, and no impact on glucose metabolism or insulin sensitivity.

Overall, there is limited evidence of a negative impact of aspartame on the gut microbiota in humans, and in healthy rodents, the data skew toward it having no significant effect when consumed. This suggests it is among the least disruptive of the commonly consumed artificial sweeteners. However, some rodent studies have highlighted two areas that need further investigation: the potential for sex-specific effects on microbiota, and the potential maternal influence of aspartame consumption on offspring.

Biological effects

There is an emerging body of compelling evidence that aspartame can impact bacterial behavior and physiology. Aspartame was amongthe sweeteners investigated for their impact on DNA uptake in the seminal study by Yu *et al*. [58], where it was shown that aspartame promoted plasmid-mediated conjugation. Aspartame increased the plasmid transfer rate by five-fold at a single-cell level [58]. Strikingly, despite the structural differences between aspartame and Ace-K, cell membrane permeability was increased by 3.7-fold in donor strains in the presence of aspartame, suggesting a conserved mechanism for the observed increase in conjugation. This was also confirmed by showing increased expression levels of conjugative transfer-related genes [58]. Furthermore, it was shown that there was an increase in ROS, which was concentration dependent.

Aspartame was also included in the two follow-up studies by Yu *et al.* [59,60], where it was shown that aspartame significantly promotes the transformation frequency of extracellular DNA in Gram-negative and Gram-positive bacteria at various concentrations, which are clinically and

environmentally relevant, ranging from 0.03 up to 300 mg/L [59]. The mechanism behind the increase in transformation frequency was also shown to be similar to Ace-K, with aspartame triggering an increase in cell envelope permeability and in the expression of genes associated with DNA uptake. The growth of *E. coli, K. pneumoniae*, and *B. subtilis* was also shown to be inhibited via increased cell envelope permeability, suggesting aspartame has antimicrobial potential [60]. This antimicrobial activity was further demonstrated when sweetener-decorated gold nanoparticles were applied to *K. pneumoniae, E. coli*, and *Enterobacter cloacae* cultures [64]. It was seen that saccharin, sucralose, and Ace-K did not show any notable antimicrobial activity in this formulation; however, aspartame-coated gold nanoparticles showed antimicrobial activity against all strains [64]. When exploring the mechanism of action underpinning this antimicrobial activity, increases in ROS and cell membrane permeability were observed, aligning with previous reports. Intriguingly, when tested individually, neither the gold nor the aspartame had antimicrobial activity [64].

When investigating the effect of aspartame on specific gut bacteria such as *E. coli* and *Enterococcus faecalis*, Shil and Chichger [61] observed no impact on *E. coli* or *E. faecalis* growth (up to 1000 μM). However, it was shown that *E. coli* biofilm formation was significantly increased when supplemented with aspartame [61]. Furthermore, it was shown that aspartame also impacted the ability of bacteria to adhere to mammalian gut epithelial cells [61]. Aspartame has also been shown to impact Gram-negative *N*-acyl homoserine lactone-mediated quorum sensing [74]. Specifically, it was shown that LasRI based quorum sensing was disrupted in *P. aeruginosa* PAO1, and motility was also attenuated in the presence of 1.36 mM of aspartame, suggesting aspartame may have some antivirulence potential [74].

Overall, aspartame has been shown to impact cell membrane permeability in various studies, and, as a result, it has been observed to increase transformation frequency. This raises concerns about whether aspartame could be promoting an increased spread of antimicrobial resistance or virulence-associated genes not only in the host but also in the environment. Intriguingly, aspartame also has antivirulence and antimicrobial properties by inhibiting quorum sensing and growth in certain bacteria. Inhibition of quorum sensing may also be occurring in the microbiome, impacting gut balance; therefore, further studies should be conducted to investigate the extent of this impact on other species, including oral and gut commensals.

Saccharin

Effects on the microbiome

Saccharin (ADI of 15 mg/kg body weight) is another common sweetener, which has been suggested to affect the microbiome. From the early 1980s, and prior to sequencing advances that facilitate in-depth microbiome analysis, there was evidence that high concentrations of saccharin (7.5%) in the diet could impact specific microbes in the rat cecal microbiome; however, the overall anaerobe numbers were unchanged [101]. Saccharin was included in one of the first major studies to evaluate the impact of artificial sweeteners on the microbiome, conducted in 2014 by Suez *et al.* [12]. In that study, saccharin was shown to significantly impact gut flora, leading to an elevated abundance of the *Bacteroides* genus and Clostridiales order and a reduction in *Lactobacillus reuteri* after 11 weeks of unregulated commercial saccharin consumption at 6.3 g/kg (above ADI) in CB7B1/6 wild-type male mice (n = 10/group, 10-week-old mice) [12]. These gut flora changes were associated with glucose intolerance following fecal transplantation. The study performed a metagenomics analysis of fecal samples and deduced that saccharin-consuming mice showed an increase in glycan degradation pathways, which are associated with energy harvest and obesity. Additionally, pathways for starch, sucrose, fructose, and mannose metabolism, as well as folate, glycerolipid, and fatty acid biosynthesis, were enriched,

which have previously been linked to type 2 diabetes in humans [12]. In line with this, the authors found that both commercial saccharin and pure saccharin (5 mg/kg, below ADI) caused impaired glucose tolerance in the mice. Commercial saccharin includes saccharin and bulking agents (glucose), whereas pure saccharin only contains saccharin. Suez *et al.* [12] also conducted a human trial where seven healthy volunteers consumed 5 mg/kg via three daily doses of commercial saccharin for 6 days, which resulted in four of the seven having poorer glycemic responses. Fecal transplantation from the top responders to saccharin into GF mice showed an increase in *Weisella cibaria* and *B. fragilis* and a decrease in *Candidatus arthromitus* in the microbiome. FMT from all seven saccharin consumers to germ-free mice caused an increase in glucose intolerance, with the top responders causing significant increases. This suggests that alteration of the microbiome by saccharin influences glucose intolerance.

Sünderhauf *et al*. [17] supplemented the drinking water of C57BL/6JRj male mice (n = 6/group, 7-week-old mice) with 5 mg/kg body weight (below ADI) saccharin for 5 weeks, and fluid consumption was tracked. Fecal microbiome analysis demonstrated a shift toward an increase in the relative abundance of Bacteroidetes and Proteobacteria and a decrease in Firmicutes, but no impact on α-diversity (Shannon and Chao1 indices). There was a significant difference between the β-diversity of saccharin versus water-treated mice (UniFrac). Obesity and metabolic syndrome in humans are associated with a decrease in Bacteroidetes and an increase in Firmicutes; however, the authors did not observe any differences in weight between the mouse cohort [17]. They also reported that saccharin decreased total bacterial 16S rRNA, suggesting the potential for it to mediate an anti-inflammatory effect, as bacterial overgrowth is widely linked to a corresponding increase in intestinal inflammation. This prompted the team to investigate whether saccharin was having any impact on mucosal damage or gut inflammation. Saccharin did not alter the intestinal barrier but did trigger an improvement in gut inflammatory parameters, with a reduction in the expression of *icam-1* and *kc* mRNA levels, which are markers of intestinal inflammation [17]. Subsequently, they tested whether this anti-inflammatory effect could help alleviate induced colitis, which is an inflammatory bowel disease. In the acute colitis mice, it was shown that 0.1 mg/ml saccharin, after colitis was induced, was able to lower AUC scores for DAI. They also measured other signs of inflammation such as *icam-1* mRNA levels in the ileum, *kc* mRNA in the colon, and colonic histology comparison, which showed significant inflammation reduction in the saccharin-treated mice compared with the control cohort. It was also shown that saccharin 0.1 mg/ml still possessed anti-inflammatory properties against induced chronic colitis if it was given to mice for 5 weeks prior to induction. Further investigation revealed that there were lower levels of dimeric IgA in the fecal samples of saccharin-supplemented mice that developed chronic colitis. It should be noted that the saccharin supplementation was halted before chronic colitis was induced, and the prophylactic effect lasted between 22 and 30 days and then wore off. This suggests that, at least in this model system, saccharin has an anti-inflammatory effect that may be dependent on its impact on bacteria [17].

Another study explored the longer-term impacts of saccharin consumption on the microbiome by providing C57BL/6J male mice (n = 10/group, 8-week-old mice) with water containing saccharin at 15 mg/kg (ADI limit, 0.3 mg/ml) for 6 months, while monitoring their water consumption [16]. At baseline, there was no significant difference in the relative abundance of bacteria between control and treatment, as was observed in the previous study. However, this study did report that several genera were impacted by saccharin exposure, with *Anaerostipes* and *Ruminococcus* significantly decreased in saccharin-treated mice after 3 months. At the same time, after 3 months, there was a significant increase in *Sporosarcina, Akkermansia, Oscillospira, Corynebacterium*, and *Jeotgalicoccus.* However, after 6 months, there was a significant decrease observed in *Dorea, Ruminococcus*, and *Adlercreutzia* [16]. Additionally at 6 months, a significant increase in

Turicibacter, Corynebacterium, and *Roseburia* was observed. Some of these bacteria, such as *Corynebacterium, Ruminococcus, Turicibacter, Anaerostipes*, and *Dorea*, are related to inflammation [16]. It was also suggested that changes in gut microbiota induce inflammatory responses, such as elevated inducible nitric oxide synthase and *tnf-α* gene expression in the mice liver [16]. The bacterial functional gene profile after 6 months of saccharin consumption revealed that bacterial inflammation-related pathways were highly elevated, including six multidrug-resistance orthologs, eleven fimbrial orthologs, six lipopolysaccharide biosynthesis orthologs, one flagellar assembly ortholog, and twenty three bacterial toxin orthologs. The gut metabolome was also altered, with inflammation-related metabolites, such as daidzein, being increased [16]. All these suggest that saccharin consumption alters the fecal microbiome and the metabolome, thereby increasing the risk of inflammation in the host, contrary to the study by Sünderhauf et al [17]. This may be explained by the dosing differences, variation in how a mouse responds to saccharin as compared with a rat or the differences in experiment duration. However, a key limitation of this study was that inflammation in the host was not explored phenotypically, so it is difficult to ascertain if these tissue-specific gene expression changes would have mediated a physiological host response, evidenced through histology, for example. Indeed, two other inflammatory markers, *il-1β* and *il-6*, were also quantified and shown not to be significantly elevated in response to saccharin exposure.

The oral microbiome in Sprague-Dawley rats has also been shown to respond to saccharin exposure. Female rats (n = 6/group, 6-week-old rats) were provided with unmonitored drinking water supplemented with 0.83 mg/ml saccharin for 12 h/d, and purified water for the 12 hours at night for 8 weeks [18]. The sucrose group was shown to have the most diverse community of bacteria, containing 372 genera. Meanwhile, the saccharin group was shown to have significantly less diversity, with only 186 genera comparatively. The study showed a shift in the composition ratio of the top four genera—*Rodentibacter, Rothia*, *Pasteurellaceae*, and *Streptococcus*, with the composition ratio of *Rodentibacter* and *Streptococcus* increasing and *Rothia* decreasing in rats consuming saccharin compared with the control [18]. *Rodentibacter* is commonly found in the human oral cavity; however, it is considered an opportunistic pathogen, so an increasing abundance could have negative impacts on oral health [18]. The saccharin group also exhibited a decrease in functional abundance of metabolic features, including carbohydrate transport and metabolism, amino acid transport and metabolism, inorganic transport and metabolism, cell wall/membrane/envelope biogenesis, replication, recombination and repair, transcription, translation, ribosomal structure and biogenesis, and energy production and conversion. However, when studying oral immunoglobulin content (SIgA and IgG), no impact was observed. Interestingly, control groups who had water supplemented with sucrose did have significantly higher levels of both immunoglobulins, and those supplemented with the artificial sweetener xylose also displayed increased SIgA and IgG levels. This suggests that, at least in this experimental setup, saccharin does not impact markers of oral inflammation but does impact the diversity of the oral microbiome as well as metabolic function.

Serrano *et al.* [21] demonstrated that using a normal diet in mice (n = 23–28/group, 8-week-old mice) supplemented with drinking water at 250 mg/ml (Above ADI) of saccharin, where consumption was regulated and monitored, reveals no significant changes in the relative abundance of bacterial taxa compared with the water control mice after 10 weeks of exposure using the Bray–Curtis analysis [21]. The study also observed no changes in the α-diversity indices or baseline differences among the treatment groups using the Shannon and Simpson indices. The mice did not show differences in food intake, weight gain, or glucose intolerance. In the same study, 12 people per group (male and female) were given maximum ADI levels of saccharin capsules for 15 days and were put on a strict diet to limit any confounding effects [21]. Their microbiota was

measured before and after saccharin supplementation, and all treatment groups had similar gut microbial α-diversity results using the Shannon and Simpson indices. The study further compared the same groups of *Bacteroides* genus and Clostridial order, which were seen to have been affected in the results of the study by Suez *et al.* [12], but no changes were observed in those groups in this study. No baseline differences were noted in the relative microbial abundance between groups at the genus or any other taxonomic rank. They also performed pairwise comparisons, which showed identical results. They also did not observe any impact on glucose tolerance, as glucose response, plasma excursions of insulin, C-peptide, glucagon, or glucagon-like peptide 1, were all comparable between groups. Both mouse and human studies confirmed that saccharin was detected in fecal matter, indicating it had reached the intestinal microbiota.

In 2022, Suez *et al*. [20] demonstrated a link between saccharin consumption, the gut microbiome, and an altered glycemic response. In this study, they conducted a randomized control trial with 20 people per group, and provided the sweetener via 6 sachets with glucose as the bulking agent [20]. The participants consumed 2 sachets dissolved in water 3 times daily for 14 days. The daily saccharin consumption was approximately 3 mg/kg (below ADI). This supplementation was subsequently shown to lead to an impaired glycaemic response, which the authors suggest was a result of an impact on the gut microbiome. Specifically, baseline levels of *Prevotella copri* and uridine monophosphate biosynthesis were positively associated with an impaired glycemic response and gradually increased during exposure, whereas baseline levels of *Bacteroides xylanisolvens* were negatively associated with an impaired glycemic response and increased during exposure. In the case of oral microbiome changes, they observed a reduction in the relative abundance of *Fusobacterium* [20]. While analyzing stool sample metagenomics, pathways related to glycolysis and glucose degradation were idenitifed in the saccharin-treated group. Furthermore, plasma levels of indoxyl sulfate increased in the saccharin-treated group and have been previously associated with vascular disease. To confirm that these effects were caused by the gut microbiome, a fecal transplantation was performed into mice using top responder stool samples and baseline samples from the same individual as a control. It was observed that the mice with the top responder sample, which consumed saccharin, showed a higher glycemic response. This elevated glycaemic response was also observed in the fecal transplantations of the bottom three responders. The fecal microbiome features showed a degradation of the cyclic amide caprolactam and biosynthesis of the branched-chain amino acid isoleucine, which has been previously associated with poorer metabolic health [20].

While there is a strong body of evidence in both human and animal studies that saccharin can impact the microbiome, there are also conflicting reports showing limited impacts. This highlights the need for larger studies, which explore key variables that may have impacted, the outcomes in conflicting studies. For example, when comparing the two key studies that explored impacts in humans—Serrano *et al* [21], which showed no impact on the gut microbiome to Suez *et al*. [20], which did—there are several differences in the experimental design as well as how the saccharin was administered (sachets vs capsules), which could all play a role in the confounding results. However, the findings, and particularly the links to an altered glycaemic response, highlight the urgent need for larger, carefully designed longitudinal human cohort studies to explore the impacts of saccharin on the gut microbiome and host metabolic outcomes.

Biological effects

Saccharin was tested in several of the studies highlighted for Ace-K and aspartame, with comparable impacts on bacterial physiology observed for saccharin, as was seen with these other sweeteners. For example, saccharin increased plasmid transfer rate by six fold at a single-cell level, and again, cell envelope permeability was demonstrated to be the likely mechanism.

Saccharin was also shown to promote the transformation frequency of extracellular DNA in Gram-negative and Gram-positive bacteria, and again, this phenotype was linked to an increase in cell envelope permeability [59]. Saccharin negatively impacted the cell envelope in *K. pneumoniae* and in a dose-dependent manner in *E. coli* and *B. subtilis* [60]. 5 mM saccharin supplementation was shown to impact the growth of *Staphylococcus aureus, K. pneumonia*, and *P. aeruginosa*, where a delay was observed in entering exponential growth from 2 to 6 hours [17]. A greater increase in sensitivity was seen for *Bacillus cereus* [17]. Shil and Chichger [61] also investigated the antimicrobial potential of saccharin and observed significant reductions in the growth of *E. coli* at high concentrations (1000 μM). Interestingly, biofilm formation was significantly increased in *E. coli* exposed to saccharin compared with that in vehicle control. Saccharin has also been shown to inhibit QS in *P. aeruginosa* via LasR interference by impacting protein solubility [74]. This study also validated the impact of this at a phenotypic level by demonstrating that *P. aeruginosa* had impaired motility, a key phenotype regulated by QS.

Recent work from De Dios *et al*. [63] has provided a greater mechanistic insight into the impact that saccharin can have on bacterial physiology. This study revealed that saccharin can alter DNA replication dynamics in *E. coli*. However, the impact on DNA replication dynamics appears to be nonlethal and is superseded by the effect that saccharin has on the bacterial cell envelope, with cells undergoing bulge-mediated cell lysis. Transcriptomics data showed a downregulation of the outer membrane porin OmpF, alongside an upregulation of peptidoglycan biosynthesis and O-antigen LPS biosynthesis, suggesting a response to cell envelope damage. KEGG pathway analysis revealed that β-lactam resistance mechanisms were significantly upregulated, further suggesting not only cell envelope damage but also potentially linking saccharin to β-lactam resistance mechanisms. MDR strains of *E. coli, S. aureus, K. pneumoniae, A. baumannii*, and *P. aeruginosa* were shown to be susceptible to killing by saccharin at concentrations between 1 and 2%; however, concentrations closer to 6% were required to completely inhibit the growth of *P. aeruginosa*. In this study, the therapeutic potential of saccharin was also highlighted, with it being able to disrupt polymicrobial biofilms, a major clinical challenge. When integrated into a wound dressing, it could outperform frontline silver based wound dressings, further showcasing its therapeutic potential. Strikingly, saccharin was able to resensitize carbapenem-resistant *A. baumannii* to a range of carbapenems. This was shown to be a result of increased cell envelope permeability, facilitating greater antibiotic transition into the cell and overwhelming native resistance mechanisms. This suggests that saccharin overcomes many of the known hurdles in antibiotic development, such as limited efficacy against biofilms or poor host tolerance. Further studies are necessary to fully explore the therapeutic potential of saccharin and determine, for example, the capacity for bacteria to evolve resistance to the antimicrobial effects of saccharin.

Concluding remarks

As evidenced throughout the review, there are contrasting examples of *in vivo* studies, demonstrating both robust and negligible impacts of different artificial sweeteners on the microbiome. Uebanso *et al.* [9] and Murali *et al.* [15] conducted experiments in rodents where there was limited to no effect on several parameters, such as body weight, microbiome changes, and metabolites associated with sweetener consumption. Contrastingly, Hanawa *et al.* [13] and Bian *et al.* [14] found that rodent consumption of sweeteners did cause substantial changes in the microbiome, glucose metabolism biomarkers, and metabolites. These variations in findings can possibly be linked to extensive differences in experimental design across the four studies. Examining the two studies that reported little to no effects of sweeteners on the microbiome, they both investigated Ace-K but had substantially different experimental designs, with the most pertinent difference being that the study by Murali *et al.*[15] used rats, while the study by Uebanso used mice [9]. While both studies used Ace-K, they tested different doses; for the rats, 40 and 120 mg/kg

Outstanding questions

What levels of artificial sweeteners are being consumed across different demographics?

How can animal microbiome studies more accurately represent artificial sweetener consumption in humans?

Are there sex-specific differences in how the microbiome responds to artificial sweetener consumption?

What are the consequences of long-term artificial sweetener consumption on the microbiome, metabolism, and overall health?

Are there lasting impacts of artificial sweetener consumption during pregnancy?

Can the microbiome changes caused by artificial sweeteners ultimately lead to the development of disease?

Can artificial sweeteners recapitulate their antimicrobial potential *in vivo*?

Can bacteria evolve resistance to the antimicrobial effects of artificial sweeteners?

of Ace-K were tested, but in the mice study, they used 1.5 and 15 mg/kg. Both monitored the dosing. In the mouse study by Uebanso *et al.*[9] (mouse), they achieved precise dosing by measuring the fluid intake of the animals and adjusting their water bottle sweetener concentration accordingly to achieve the target dose range. The rat study by Murali *et al.*[15] took a different approach; they administered the sweetener solution of appropriate concentration via gavage into the animals. Despite these extensive differences in study design, both reported limited to no effect of the sweeteners. There is a similar level of experimental design variability when examining the studies by Hanawa *et al.*[13] and Bian *et al.*[14] that did report a variety of host and microbiome impacts of Ace-K. The study by Hanawa *et al.*[13] used the same strain, C57BL/6J, as the study by Uebanso *et al.*[9], but the study by Bian *et al.*[14] used CD1 mice. That study delivered 37.5 mg/kg via gavage, while the study by Hanawa *et al.*[13] allowed the animals *ad libitum* access to the sweetener with a reported dose, which was 150 mg/kg, which is comparable with the 120-mg/kg used in the study by Murali *et al.*[13–15]. The study by Hanawa *et al.*[13] exclusively used only male mice, while the study by Bian *et al.*[14] used both males and females. Bian *et al.*[14] reported that there were sex-specific changes caused by sweetener consumption in the animals. As outlined, sex-specific differences have been seen in several studies of other sweeteners, suggesting that studies limited to one sex may risk missing key sex-specific impacts [25]. This is particularly pertinent when considering that studies report that sweetener consumption during gestation can impact offspring. This highlights the need for more studies to be done on female mice to fully understand how artificial sweeteners impact female hosts. It also highlights the need for better understanding in humans of how sweetener consumption during pregnancy impacts the mother and the child, an area that has thus far has not been explored in humans. It is interesting to note that there are limited to no restrictions on artificial sweetener consumption during pregnancy. Another difference that could be influencing the results of studies includes the age of the animals when they start the sweetener regimen and its duration. For instance, the study by Uebanso *et al.*[9] used a particularly young cohort of mice (4 weeks old) and did not observe any impacts on the gut microbiome, whereas studies with more mature cohorts, such as the study by Bian *et al.*[14], did report differences. Overall, these studies highlight the need for greater standardization in experimental design to fully elucidate the impacts of artificial sweeteners on the microbiome and the host.

As the field moves forward, there are some key questions that need to be addressed. The true effect of artificial sweeteners on the microbiome needs to be explored in larger human studies, as the current seminal works in this area have relatively modest cohort sizes. A greater understanding of human artificial sweetener consumption across age groups (particularly in the young) and geographic regions is needed to more accurately ascertain how these deviate from the ADI and whether certain population subgroups are consuming sweeteners at higher rates than recommended. This level of insight will also help guide future animal and human studies, so that they assess concentrations that are more representative of what is being consumed among a given group. This is particularly pertinent when you consider that several studies assessing concentrations in excess of the ADI did report impacts on the microbiome. There are several studies that point toward sex-specific differences; these also need to be explored in larger longitudinal animal and human studies to ascertain not only the real-world relevance of these initial findings but also any potential intergenerational consequences. Several microbial shifts have been linked to disease onset, such as obesity; however, the role of artificial sweeteners in specific disease onset needs greater clarification. For example, do higher glycemic responses and microbiome changes caused by saccharin ultimately lead to the development of type 2 diabetes, or do reported increases in specific opportunistic pathogens ultimately lead to infection and disease onset? Additionally the impact of artificial sweeteners can differ in specific disease states, as evidenced in the case of UC; greater

insight is needed into how specific disease states can influence the impact that artificial sweeteners have on the microbiome and the host.

There is a need for more standardized protocols to evaluate the impact of sweeteners on human and animal health. Whether it is the need for more accurate control of dosing, which is a particular issue in many of the published animal microbiome studies, or to standardize how the sweetener is delivered (liquid, food, and capsule), as all these variables have the potential to impact outcomes. Particularly, in human trials, variation in study design, dosage, exposure duration, diet, demographics, and interindividual variability are all factors that contribute to the heterogeneous effects of artificial sweeteners on microbial composition, function, and host physiological responses. Furthermore, there is a need for more validation within studies, such as performing FMT to evaluate whether the sweetener effects can be recapitulated in naïve cohorts. Mechanistic insight is also needed to understand how the effects on the microbiome are being mediated; indeed, greater integration is needed between teams working on the fundamental effects of sweeteners on microbial physiology and those working on microbiome-level impacts. High-resolution multiomics (host and bacterial transcriptomics, proteomics, and metabolomics) have also been used to great effect to pinpoint certain underlying mechanisms driving microbiome shifts; the wider exploitation of these technologies in artificial sweetener studies would facilitate more rapid mechanistic insights.

The therapeutic potential of artificial sweeteners is also an area that requires further study. For example, the potential anti-inflammatory properties of saccharin could have significant therapeutic potential if further validated in humans. One of the strongest cases for the therapeutic exploitation of artificial sweeteners lies in their potential to treat infections. Several studies have now confirmed that artificial sweeteners such as Ace-K and saccharin have the capacity to inhibit the growth of multidrug-resistant priority pathogens. Critically, these sweeteners also overcome some of the key hurdles that typically lead to the failure of novel therapeutic interventions in the antimicrobial pipeline, such as an inefficacy against biofilms, with both saccharin and Ace-K displaying potent antibiofilm activity. Strikingly, these sweeteners have also been shown to potentiate the efficacy of a wide range of antibiotics, overcoming native and acquired resistance mechanisms. This paves the way for their use as combination therapies, rejuvenating the efficacy of some frontline antibiotics. There have also been no reports of bacteria evolving resistance to the antimicrobial effects of sweeteners to date; however, this does need further investigation. Much of the therapeutic potential of these sweeteners has thus far been demonstrated *in vitro*, so there is now an impetus to confirm these findings *in vivo* and across a range of infection etiologies. More insight into the underlying mechanisms of action is also needed to fully understand how these sweeteners impact bacterial viability. It is widely recognized by the World Health Organization and the Centers for Disease Control that we are in urgent need of novel therapeutic interventions to tackle the growing prevalence and burden of antibiotic-resistant infections, with the cost (>$1 billion) and the timeframe (10–15 years) to bring a new antimicrobial to market being prohibitive to both established pharmaceuticals and start-ups. Given the wealth of human safety data available on artificial sweeteners, they perhaps represent a unique opportunity to accelerate the movement of a novel antimicrobial through the drug development pipeline.

Acknowledgments

RRMC is supported by a Biotechnology and Biological Sciences Research Council Awards (BB/V007823/1, UKRI1911) and the Academy of Medical Sciences/the Welcome Trust/ the Government Department of Business, Energy and Industrial Strategy/the British Heart Foundation/Diabetes UK Springboard Award (SBF006\1040) and Medical Research Council Grant MR/Y001354/1.

Declaration of interests

RRMC has several patents covering the therapeutic use of artificial sweeteners.

Supplemental information

Supplementary information associated with this article can be found online at https://doi.org/10.1016/j.tim.2026.01.010.

Resources

[i]https://www.fda.gov/food/food-additives-petitions/aspartame-and-other-sweeteners-food

[ii]https://www.cdc.gov/ecoli/about/index.html

References

1. Basson, A.R. *et al.* (2021) Artificial sweeteners: history and new concepts on inflammation. *Front. Nutr.* 8, 746247
2. Dragomir, N. *et al.* (2025) Beyond sugar: a holistic review of sweeteners and their role in modern nutrition. *Foods* 14, 3182
3. Shi, Z. *et al.* (2025) Structural and functional characterization of human sweet taste receptor. *Nature* 645, 801–808
4. Temussi, P.A. (2011) Determinants of sweetness in proteins: a topological approach. *J. Mol. Recognit.* 24, 1033–1042
5. Pavanello, S. *et al.* (2023) Non-sugar sweeteners and cancer: toxicological and epidemiological evidence. *Regul. Toxicol. Pharmacol.* 139, 105369
6. Wilk, K. *et al.* (2022) The effect of artificial sweeteners use on sweet taste perception and weight loss efficacy: a review. *Nutrients* 14, 1261
7. Li, J. *et al.* (2025) Artificial sweeteners in wastewater treatment plants: a systematic review of global occurrence, distribution, removal, and degradation pathways. *J. Hazard. Mater.* 494, 138644
8. Winter, S.E. and Bäumler, A.J. (2023) Gut dysbiosis: ecological causes and causative effects on human disease. *Proc. Natl. Acad. Sci. U. S. A.* 120, e2316579120
9. Uebanso, T. *et al.* (2017) Effects of low-dose non-caloric sweetener consumption on gut microbiota in mice. *Nutrients* 9, 560
10. Frankenfeld, C.L. *et al.* (2015) High-intensity sweetener consumption and gut microbiome content and predicted gene function in a cross-sectional study of adults in the United States. *Ann. Epidemiol.* 25, 736–742.e4
11. Zhu, J. *et al.* (2021) The effects of nonnutritive sweeteners on the cariogenic potential of oral microbiome. *Biomed. Res. Int.* 2021, 9967035
12. Suez, J. *et al.* (2014) Artificial sweeteners induce glucose intolerance by altering the gut microbiota. *Nature* 514, 181–186
13. Hanawa, Y. *et al.* (2021) Acesulfame potassium induces dysbiosis and intestinal injury with enhanced lymphocyte migration to intestinal mucosa. *J. Gastroenterol. Hepatol.* 36, 3140–3148
14. Bian, X. *et al.* (2017) The artificial sweetener acesulfame potassium affects the gut microbiome and body weight gain in CD-1 mice. *PLoS One* 12, e0178426
15. Murali, A. *et al.* (2022) Investigating the gut microbiome and metabolome following treatment with artificial sweeteners acesulfame potassium and saccharin in young adult Wistar rats. *Food Chem. Toxicol.* 165, 113123
16. Bian, X. *et al.* (2017) Saccharin induced liver inflammation in mice by altering the gut microbiota and its metabolic functions. *Food Chem. Toxicol.* 107, 530–539
17. Sünderhauf, A. *et al.* (2020) Saccharin supplementation inhibits bacterial growth and reduces experimental colitis in mice. *Nutrients* 12, 1122
18. Cheng, X. *et al.* (2021) Effect of different sweeteners on the oral microbiota and immune system of Sprague Dawley rats. *AMB Express* 11, 8
19. Shi, Z. *et al.* (2021) Impaired intestinal *Akkermansia muciniphila* and aryl hydrocarbon receptor ligands contribute to nonalcoholic fatty liver disease in mice. *mSystems* 6 e00985–20
20. Suez, J. *et al.* (2022) Personalized microbiome-driven effects of non-nutritive sweeteners on human glucose tolerance. *Cell* 185, 3307–3328.e19
21. Serrano, J. *et al.* (2021) High-dose saccharin supplementation does not induce gut microbiota changes or glucose intolerance in healthy humans and mice. *Microbiome* 9, 11
22. Palmnäs, M.S.A. *et al.* (2014) Low-dose aspartame consumption differentially affects gut microbiota-host metabolic interactions in the diet-induced obese rat. *PLoS One* 9, e109841
23. Zhong, M. *et al.* (2024) Acceptable daily intake of aspartame aggravates enteritis pathology and systemic inflammation in colitis mouse model. *J. Food Sci.* 89, 10202–10221
24. Ahmad, S.Y. *et al.* (2020) The effects of non-nutritive artificial sweeteners, aspartame and sucralose, on the gut microbiome in healthy adults: secondary outcomes of a randomized double-blinded crossover clinical trial. *Nutrients* 12, 3408
25. Nettleton, J.E. *et al.* (2020) Maternal low-dose aspartame and stevia consumption with an obesogenic diet alters metabolism, gut microbiota and mesolimbic reward system in rat dams and their offspring. *Gut* 69, 1807–1817
26. Ho, S.Y. *et al.* (2025) Maternal aspartame exposure induces neonatal pulmonary metabolic dysregulation and redox imbalance: a multiomics investigation of gut microbiota-host interactions. *J. Agric. Food Chem.* 73, 27012–27024
27. Chuang, H.C. *et al.* (2025) Maternal aspartame exposure alters lung Th1/Th2 cytokine balance in offspring through nuclear factor-κB activation. *Int. Immunopharmacol.* 145, 113800
28. Dai, X. *et al.* (2020) Maternal sucralose intake alters gut microbiota of offspring and exacerbates hepatic steatosis in adulthood. *Gut Microbes* 11, 1043–1063
29. Zhang, M. *et al.* (2021) Low doses of sucralose alter fecal microbiota in high-fat diet-induced obese rats. *Front. Nutr.* 8, 787055
30. Sánchez-Tapia, M. *et al.* (2020) The development of metabolic endotoxemia is dependent on the type of sweetener and the presence of saturated fat in the diet. *Gut Microbes* 12, 1801301
31. Méndez-García, L.A. *et al.* (2022) Ten-week sucralose consumption induces gut dysbiosis and altered glucose and insulin levels in healthy young adults. *Microorganisms* 10, 434
32. Yang, Z. *et al.* (2024) Isomalt attenuates hepatic steatosis in rats via modulating gut microbiota and its metabolic function. *J. Funct. Foods.* 112, 105963
33. Gostner, A. *et al.* (2006) Effect of isomalt consumption on faecal microflora and colonic metabolism in healthy volunteers. *Br. J. Nutr.* 95, 40–50
34. Wu, Y.F. *et al.* (2022) Xylitol-containing chewing gum reduces cariogenic and periodontopathic bacteria in dental plaque—microbiome investigation. *Front. Nutr.* 9, 882636
35. Uebanso, T. *et al.* (2017) Effects of consuming xylitol on gut microbiota and lipid metabolism in mice. *Nutrients* 9, 756
36. Xiang, S. *et al.* (2021) Xylitol enhances synthesis of propionate in the colon via cross-feeding of gut microbiota. *Microbiome* 9, 62
37. Rafeek, R. *et al.* (2019) Xylitol and sorbitol effects on the microbiome of saliva and plaque. *J. Oral Microbiol.* 11, 1536181
38. Chi, L. *et al.* (2018) Effects of the artificial sweetener neotame on the gut microbiome and fecal metabolites in mice. *Molecules* 23, 367
39. Beards, E. *et al.* (2010) A human volunteer study to assess the impact of confectionery sweeteners on the gut microbiota composition. *Br. J. Nutr.* 104, 701–708
40. Keijser, B.J.F. *et al.* (2018) The impact of maltitol-sweetened chewing gum on the dental plaque biofilm microbiota composition. *Front. Microbiol.* 9, 381
41. Prosdocimi, E.M. *et al.* (2017) Effect of maltitol-containing chewing gum use on the composition of dental plaque microbiota in subjects with active dental caries. *J. Oral Microbiol.* 9, 1374152
42. Lu, H. *et al.* (2021) Lactitol supplementation modulates intestinal microbiome in liver cirrhotic patients. *Front. Med. (Lausanne)* 8, 762930

43. Li, X.Q. *et al.* (2020) Beneficial effects of lactitol on the composition of gut microbiota in constipated patients. *J. Dig. Dis.* 21, 445–453
44. Wang, Y.N. *et al.* (2019) Effects of probiotics and prebiotics on intestinal microbiota in mice with acute colitis based on 16S rRNA gene sequencing. *Chin. Med. J.* 132, 1833–1842
45. Björklund, M. *et al.* (2012) Gut microbiota of healthy elderly NSAID users is selectively modified with the administration of *Lactobacillus acidophilus* NCFM and lactitol. *Age (Omaha)* 34, 987–999
46. Mahalak, K.K. *et al.* (2020) Impact of steviol glycosides and erythritol on the human and *Cebus apella* gut microbiome. *J. Agric. Food Chem.* 68, 13093–13101
47. Morissette, A. *et al.* (2024) Rebaudioside D decreases adiposity and hepatic lipid accumulation in a mouse model of obesity. *Sci. Rep.* 14, 3077
48. Ye, J. *et al.* (2025) Stevioside mitigates metabolic dysregulation in offspring induced by maternal high-fat diet: the role of gut microbiota-driven thermogenesis. *Gut Microbes* 17, 2452241
49. Wu, X. *et al.* (2019) Effect of dietary stevioside supplementation on growth performance, nutrient digestibility, serum parameters, and intestinal microflora in broilers. *Food Funct.* 10, 2340–2346
50. Jiang, J. *et al.* (2021) Dietary stevioside supplementation increases feed intake by altering the hypothalamic transcriptome profile and gut microbiota in broiler chickens. *J. Sci. Food Agric.* 101, 2156–2167
51. Nettleton, J.E. *et al.* (2019) Low-dose stevia (rebaudioside A) consumption perturbs gut microbiota and the mesolimbic dopamine reward system. *Nutrients* 11, 1248
52. Xi, D. *et al.* (2020) Rebaudioside affords hepatoprotection ameliorating sugar sweetened beverage-induced nonalcoholic steatohepatitis. *Sci. Rep.* 10, 6689
53. Kim, Y. *et al.* (2024) A combination of rebaudioside A and neohesperidin dihydrochalcone suppressed weight gain by regulating visceral fat and hepatic lipid metabolism in ob/ob mice. *Food Sci. Biotechnol.* 33, 913–923
54. Kwok, D. *et al.* (2024) Comparison of a daily steviol glycoside beverage compared with a sucrose beverage for four weeks on gut microbiome in healthy adults. *J. Nutr.* 154, 1298–1308
55. Li, C.H. *et al.* (2022) Long-term consumption of the sugar substitute sorbitol alters gut microbiome and induces glucose intolerance in mice. *Life Sci.* 305, 120770
56. de Dios, R. *et al.* (2023) Artificial sweeteners inhibit multidrug-resistant pathogen growth and potentiate antibiotic activity. *EMBO Mol. Med.* 15, e16397
57. Wang, Q.P. *et al.* (2018) Non-nutritive sweeteners possess a bacteriostatic effect and alter gut microbiota in mice. *PLoS One* 13, e0199080
58. Yu, Z. *et al.* (2021) Nonnutritive sweeteners can promote the dissemination of antibiotic resistance through conjugative gene transfer. *ISME J.* 15, 2117–2130
59. Yu, Z. *et al.* (2022) Artificial sweeteners stimulate horizontal transfer of extracellular antibiotic resistance genes through natural transformation. *ISME J.* 16, 543–554
60. Yu, Z. and Guo, J. (2022) Non-caloric artificial sweeteners exhibit antimicrobial activity against bacteria and promote bacterial evolution of antibiotic tolerance. *J. Hazard. Mater.* 433, 128840
61. Shil, A. and Chichger, H. (2021) Artificial sweeteners negatively regulate pathogenic characteristics of two model gut bacteria, *E. coli* and *E. faecalis*. *Int. J. Mol. Sci.* 22, 5228
62. Giacaman, R.A. *et al.* (2013) Cariogenic potential of commercial sweeteners in an experimental biofilm caries model on enamel. *Arch. Oral Biol.* 58, 1116–1122
63. de Dios, R. *et al.* (2025) Saccharin disrupts bacterial cell envelope stability and interferes with DNA replication dynamics. *EMBO Mol. Med.* 17, 993–1017
64. Liu, H. *et al.* (2023) A potential strategy against clinical carbapenem-resistant Enterobacteriaceae: antimicrobial activity study of sweetener-decorated gold nanoparticles in vitro and in vivo. *J. Nanobiotechnol.* 21, 409
65. Loayza, J.J.J. *et al.* (2023) Effect of food additives on key bacterial taxa and the mucosa-associated microbiota in Crohn's disease. The ENIGMA study. *Gut Microbes* 15, 2172670
66. Omran, A. *et al.* (2013) Metabolic effects of sucralose on environmental bacteria. *J. Toxicol.* 2013, 372986
67. Cocco, F. *et al.* (2020) Concentration in saliva and antibacterial effect of xylitol chewing gum: in vivo and in vitro study. *Appl. Sci.* 10, 2900
68. Evans, A. *et al.* (2015) Inhibitory effects of children's toothpastes on *Streptococcus mutans*, *Streptococcus sanguinis* and *Lactobacillus acidophilus*. *Eur. Arch. Paediatr. Dent.* 16, 219–226
69. Tochio, T. *et al.* (2023) Topical erythritol combined with L-ascorbyl-2-phosphate inhibits staphylococcal growth and alleviates staphylococcal overgrowth in skin lesions of canine superficial pyoderma. *Pol. J. Vet. Sci.* 26, 647–655
70. Kontiokari, T. and Uhari, M. (1995) Effect of xylitol on growth of nasopharyngeal bacteria in vitro. *Antimicrob. Agents Chemother.* 39, 1820–1823
71. Zabner, J. *et al.* (2000) The osmolyte xylitol reduces the salt concentration of airway surface liquid and may enhance bacterial killing. *Proc. Natl. Acad. Sci. U. S. A.* 97, 11614–11619
72. Loimaranta, V. *et al.* (2020) Xylitol and erythritol inhibit real-time biofilm formation of *Streptococcus mutans*. *BMC Microbiol.* 20, 184
73. Kwiecińska-Piróg, J. *et al.* (2024) How xylitol, gluten, and lactose change human gut microbiota *Escherichia coli* and *Lactobacillus rhamnosus* GG biofilm. *Nutrition* 124, 112446
74. Markus, V. *et al.* (2021) Inhibitory effects of artificial sweeteners on bacterial quorum sensing. *Int. J. Mol. Sci.* 22, 9863
75. Shil, A. *et al.* (2024) The artificial sweetener neotame negatively regulates the intestinal epithelium directly through T1R3-signaling and indirectly through pathogenic changes to model gut bacteria. *Front. Nutr.* 11, 1366409
76. Gasser, T.C. and Madsen, P.O. (1993) Influence of urological irrigation fluids on urothelial bacterial adherence. *Urol. Res.* 21, 401–405
77. Haewpetch, P. *et al.* (2022) Enzymatic synthesis of maltitol and its inhibitory effect on the growth of *Streptococcus mutans* DMST 18777. *Biomolecules* 12, 167
78. Tochio, T. *et al.* (2022) The bacteriostatic effect of erythritol on canine periodontal disease–related bacteria. *Pol. J. Vet. Sci.* 25, 75–82
79. Zhang, J.L. *et al.* (2019) Antibacterial activity of erythritol on periodontal pathogen. *Shanghai Kou Qiang Yi Xue* 28, 362–367
80. Coluccia, A. *et al.* (2022) In vitro study on green propolis as a potential ingredient of oral health care products. *Antibiotics* 11, 1764
81. Her, M. *et al.* (2010) The development of a selective medium for the *Brucella abortus* strains and its comparison with the currently recommended and used medium. *Diagn. Microbiol. Infect. Dis.* 67, 15–21
82. Lim, J.H. *et al.* (2017) Spontaneous detachment of *Streptococcus mutans* biofilm by synergistic effect between zwitterion and sugar alcohol. *Sci. Rep.* 7, 8107
83. Sun, A. *et al.* (2018) Screening small metabolites from cells as multifunctional coatings simultaneously improves nanomaterial biocompatibility and functionality. *Adv. Sci.* 5, 1800341
84. Lin, R. *et al.* (2023) Dual cross-linked starch hydrogel for eugenol encapsulation and the formation of hydrogen bonds on textural hydrogel. *Carbohydr. Polym.* 316, 121044
85. Bakhtiyari, A.S. *et al.* (2021) Enhancement of biomass and total carotenoid content of a UV-resistant strain of *Dietzia maris* in response to different carbon and nitrogen sources. *BioTechnologia* 102, 197–207
86. Liu, J. *et al.* (2022) Natural inhibitors targeting the localization of lipoprotein system in *Vibrio parahaemolyticus*. *Int. J. Mol. Sci.* 23, 14352
87. Dong, Z. *et al.* (2024) Injectable, thermo-sensitive and self-adhesive supramolecular hydrogels built from binary herbal small molecules towards reusable antibacterial coatings. *RSC Adv.* 14, 2027–2035
88. Kataev, V.E. *et al.* (2011) Synthesis and antituberculosis activity of derivatives of *Stevia rebaudiana* glycoside steviolbioside and diterpenoid isosteviol containing hydrazone, hydrazide, and pyridinoyl moieties. *Russ. J. Bioorg. Chem.* 37, 542–551
89. Kunová, G. *et al.* (2014) Utilisation of steviol glycosides from *Stevia rebaudiana* (Bertoni) by lactobacilli and bifidobacteria in in vitro conditions. *Folia Microbiol. (Praha)* 59, 251–255

90. Collins, S.L. *et al.* (2018) Promising prebiotic candidate established by evaluation of lactitol, lactulose, raffinose, and oligofructose for maintenance of a *Lactobacillus*-dominated vaginal microbiota. *Appl. Environ. Microbiol.* 84 e02200–17
91. Biagi, G. *et al.* (2013) Effect of feeding a selected combination of galacto-oligosaccharides and a strain of *Bifidobacterium pseudocatenulatum* on the intestinal microbiota of cats. *Am. J. Vet. Res.* 74, 90–95
92. Pfeffer, M. *et al.* (1985) Acesulfame K, cyclamate and saccharin inhibit the anaerobic fermentation of glucose by intestinal bacteria. *Z. Ernahrungswiss* 24, 231–235
93. Garvey, S.M. *et al.* (2022) The probiotic *Bacillus subtilis* BS50 decreases gastrointestinal symptoms in healthy adults: a randomized, double-blind, placebo-controlled trial. *Gut Microbes* 14, 2122668
94. Lin, D. *et al.* (2021) *Bacillus subtilis* attenuates hepatic and intestinal injuries and modulates gut microbiota and gene expression profiles in mice infected with *Schistosoma japonicum*. *Front. Cell Dev. Biol.* 9, 766205
95. Poulin, M.B. and Kuperman, L.L. (2021) Regulation of biofilm exopolysaccharide production by cyclic di-guanosine monophosphate. *Front. Microbiol.* 12, 730980
96. Tamanai-Shacoori, Z. *et al.* (2017) *Roseburia* spp.: a marker of health? *Future Microbiol.* 12, 157–170
97. Barber, T.M. *et al.* (2023) The effects of the Mediterranean diet on health and gut microbiota. *Nutrients* 15, 2150
98. Grenda, T. *et al.* (2022) Probiotic potential of *Clostridium* spp.—advantages and doubts. *Curr. Issues Mol. Biol.* 44, 3118–3130
99. Henneke, L. *et al.* (2022) A dietary carbohydrate–gut *Parasutterella*–human fatty acid biosynthesis metabolic axis in obesity and type 2 diabetes. *Gut Microbes* 14, 2057778
100. Dubin, K. and Pamer, E.G. (2017) Enterococci and their interactions with the intestinal microbiome. *Microbiol. Spectr.* 5, 10–128
101. Anderson, R.L. and Kirkland, J.J. (1980) The effect of sodium saccharin in the diet on caecal microflora. *Food Cosmet. Toxicol.* 18, 353–355