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REVIEW

The Use of Defined Microbial Communities To Model Host-Microbe Interactions in the Human Gut

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SUMMARY The human intestinal ecosystem is characterized by a complex interplay between different microorganisms and the host. The high variation within the human population further complicates the quest toward an adequate understanding of this complex system that is so relevant to human health and well-being. To study host-microbe interactions, defined synthetic bacterial communities have been introduced in gnotobiotic animals or in sophisticated *in vitro* cell models. This review reinforces that our limited understanding has often hampered the appropriate design of defined communities that represent the human gut microbiota. On top of this, some communities have been applied to *in vivo* models that differ appreciably from the human host. In this review, the advantages and disadvantages of using defined microbial communities are outlined, and suggestions for future improvement of host-microbe interaction models are provided. With respect to the host, technological advances, such as the development of a gut-on-a-chip system and intestinal organoids, may contribute to more-accurate *in vitro* models of the human host. With respect to the microbiota, due to the increasing availability of representative cultured isolates and their genomic sequences, our understanding and controllability of

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the human gut "core microbiota" are likely to increase. Taken together, these advancements could further unravel the molecular mechanisms underlying the human gut microbiota superorganism. Such a gain of insight would provide a solid basis for the improvement of pre-, pro-, and synbiotics as well as the development of new therapeutic microbes.

KEYWORDS animal model, gut-on-a-chip, *in vitro* model, intestinal microbiota, minimal microbiota

INTRODUCTION

Given its involvement in metabolic, nutritional, physiological, and immunological processes, the human intestinal microbiome can be regarded as an essential organ of the human body (1). Further strengthening its clinical relevance, the intestinal microbiome has been linked to numerous disease conditions, including metabolic and immune disorders, cancer, and neurodegenerative diseases (2). However, apart from a remarkable increase in the amount of genome sequence data of the human gut microbiota, progress in functional insight has been hampered by its complexity: the existence of more than 1,000 prevalent species (3), combined with the high interpersonal variation within the human population in terms of genetics, environment, and habits, results in a complex entity termed the human microbiome superorganism (4). The number of known host-microbe interactions has grown rapidly over the past decades, yet many aspects still remain obscure.

To solve this complexity, there is a need for a reductionist approach in which both host and microbiome are simplified to the extent that experimental variables can be tightly controlled and deliberately manipulated. Regarding the microbiota, synthetic or defined communities have been proposed as useful models to study microbial ecology (5). In recent years, the number of cultivable gastrointestinal microbial species has rapidly expanded (3) by the use of sophisticated or brute-force culturomics approaches (6, 7). These strategies have allowed for the design of defined communities that are representative of the normal human intestinal microbiota. With respect to the human host, laboratory animals, notably mice, have proven valuable models for developing human medicine. The colonization of germfree (GF) animals with defined bacterial communities, resulting in gnotobiotic animals, has already been applied for decades. During the 1960s and 1970s, it was recognized that the intestines of GF animals display aberrant histological, anatomical, and physiological characteristics compared to conventional laboratory animals (8). The development of the Schaedler cocktail for colonization of the murine gut (9) marked one of the first attempts to normalize GF mice. An altered version has been widely adopted as a standardized gut microbiota by animal breeders and biomedical researchers ever since. Over time, various other defined communities have been designed to generate gnotobiotic animals for purposes beyond standardization; they have proven to be a valuable *in vivo* tool to study microbial ecology (e.g., microbial invasion, microbe-microbe interactions, and metabolism) and host-microbe interactions. However, mice and other animal models have various limitations that hamper their use as models for the human microbiome, as was recently reviewed (10, 11). Interesting alternatives concern the development of sophisticated *in vitro* models, such as organ-on-a-chip systems and organoids.

This review summarizes existing models of host-microbe interactions in which defined communities, as models of the (human) gut microbiota, were applied. We aim to present all *in vivo* studies that used defined microbial communities representing the intestinal microbiota of healthy individuals and in which host parameters were considered. The designs of these model communities, as well as the selection of their host, are compared and critically evaluated. The potential uses of defined communities in *in vitro* (cellular) models, as a surrogate host, are outlined as well. We conclude by discussing the increased value, opportunities, and possible obstacles when applying defined communities in to-be-developed *in vitro* host-microbe interaction models.

DEFINED COMMUNITIES MIMICKING THE NORMAL INTESTINAL MICROBIOTA *IN VIVO*

A number of recent studies addressed host-microbe interactions *in vivo* by using defined communities representative of the healthy human gut microbiota (Tables 1 to 3). These include various mouse studies with more- or less-defined intestinal microbiota, which are summarized below. Studies in which animals were antibiotic treated before bacterial colonization are excluded from our analysis, as their reproducibility and gnotobiology cannot be ensured (12). The following section first discusses the specifically named defined communities applied in rodents (Table 1) ($n = 31$), followed by non-specifically-named communities in rodents (Table 2) ($n = 16$). Finally, the defined communities administered to nonrodent models are discussed (Table 3) ($n = 6$).

(Altered) Schaedler Flora

In 1965, Russel W. Schaedler colonized GF mice with a defined microbial community composed of strains isolated from normal mice, to study the fate of the bacteria in the gastrointestinal tract (GIT) and their effect on cecum size. With respect to these parameters, it turned out that the Schaedler flora (SF) was able to, at least partially, normalize the cecum size of GF mice in comparison with animals raised under conventional conditions (9). The defined microbial population was supplied to animal vendors to serve as a community that could limit infection of ex-GF rodents with opportunistic pathogens. Schaedler developed several different bacterial cocktails over time. In 1978, Roger P. Orcutt set out to standardize and improve the SF flora, but in view of the monitoring costs, the total number of bacterial species was limited to eight. Orcutt made a selection of bacterial species (altered Schaedler flora [ASF]) based on their representation and stable colonization in the murine gut, their ease of identification (morphologically), and their presence in or interference with isolator contaminants. For instance, the cocci and spore-forming, blunt-ended rods were eliminated, which represented the majority of isolator contaminants. Also, the amount of facultative anaerobes was limited, as they outgrew aerobic isolator contaminants and thus impeded the ability to detect the latter (13). The ASF consists of six *Firmicutes* (*Clostridium* species [ASF356], *Lactobacillus intestinalis* or *Lactobacillus acidophilus* [ASF360], *Lactobacillus murinus* or *Lactobacillus salivarius* [ASF361], *Eubacterium plexicaudatum* [ASF492], *Pseudoflavonifractor* sp. [ASF500], and *Clostridium* sp. [ASF502]), one *Bacteroidetes* species (*Parabacteroides distasonis* [ASF519]), and one *Deferribacteres* species (*Mucispirillum schaedleri* [ASF457]).

The ASF has been used multiple times as a reference or minimal defined microbiota, and its applications were extensively reviewed elsewhere (14). Several studies involving ASF in mice (or other animals) reported its effect on host parameters (Tables 1 to 3). The list is probably not exhaustive, given the wide application of ASF mice as a control or minor population in studies, which makes these studies harder to identify.

The applications of ASF in rodents varied from wild-type strains (mostly C57BL/6 but also C3H/HeN and Swiss Webster mice) to models prone to diseases, including irritable bowel disease (IBD) (15–17), type I diabetes (18), or colorectal cancer (19). The ASF lacks *Proteobacteria*, a phylum shared by mice and humans, whereas some researchers introduced *Proteobacteria* to ASF mice, such as *Oxalobacter formigenes* (20) and *Escherichia coli* (21). Other studies included only selected members of the ASF, because not all members were found to successfully colonize the murine cecum (18) or to test the levels of colonization resistance of different combinations of ASF members (22). Overall, the application of ASF for the study host-microbe interactions has been quite diverse, regarding host strain, gut region of interest, and host parameters studied.

Although the ASF has been used multiple times as a reference microbiota and has aided in the establishment of other defined microbiota, such as Oligo-MM and the Bristol microbiota, its representability of the normal gut microbiota has been criticized (23), as discussed below.

TABLE 1 Studies using specifically named defined communities to study host-microbe interactions *in vivo* ($n = 31$)[a]

Consortium (no. of species[b])	Division of phyla[e]	Strain source(s)	Host species (strain)	Part of the gut studied[f]	No. of animals/ group	Chow	Sex	Age (collection time[s][c])	Study outcome(s)[d]	Reference
Schaedler flora (5 species) 2 *Lactobacillus* spp., anaerobic *Streptococcus* sp. (group N), *Bacteroides* strain, *Enterococcus* sp., coliform strain		Mouse	Mouse (NR)		20	NR	NR	4 wk (3 wk–4 mo)	Colonization pattern; cecal size	9
ASF (8 species) ASF356: *Clostridium* species ASF360: *Lactobacillus intestinalis* or *Lactobacillus acidophilus* ASF361: *Lactobacillus murinus* or *Lactobacillus salivarius* ASF457: *Mucispirillum schaedleri* ASF492: *Eubacterium plexicaudatum* ASF500: *Pseudoflavonifractor* sp. ASF502: *Clostridium* sp. ASF519: *Parabacteroides distasonis*		Mouse	Mouse (HA/ICR)		30	NR	Both	Adult (14–56 days)	Death after *C. botulinum* infection; fecal *C. botulinum* toxin excretion; colonization pattern of *C. botulinum*	36
		Mouse	Rat (F344)		1–5	Sterile food (Charles River) *ad libitum*	M	NR (2 wk)	Hepatic genotoxicity of mononitrotoluene isomers; metabolic activation of 2NT by intestinal bacteria; cecal bacterial content	169
		Mouse	Mouse (scid C.B-17)		4–6	Autoclaved pelleted diet *ad libitum*	NR	NR (8–12 wk postreconstitution $CD4^+$ T cells)	After *Helicobacter hepaticus* infection, rectal prolapse; clinically severe disease; grossly thickened colon, cecum, and rectum on necropsy; colonic inflammation score; colonic epithelial cell proliferation; histopathology	16
		Mouse	Rat (HLA-B27 on 33-3/F344)		7–11	NR	At least M	2 mo (1 mo)	Gross gut score, levels of MPO and IL-1B in cecal tissue; histological inflammatory score of cecum and antrum	15
		Mouse	Mouse (C3H/HeN)		4–8	Irradiated diet (Harlan Teklad)	NR	6–8 wk (9–14 wk)	After colonization with *Helicobacter bilis* or *Brachyspira hyodysenteriae*, cecal pathological gross and histological scores; serum IgG1 + IgG2a Ab response	170
		Mouse	Mouse (C3H/HeN)		7–10	Irradiated diet (Harlan Teklad)	NR	6–8 wk (10 wk)	Fecal bacterial contents (after *H. bilis* infection); cecal pathological scores; cecal histological changes; serum immunoglobulin	171
		Mouse	Mouse (SW)		2–5	NR	NR	6–9 wk (NR)	Presence of Th17 cells and $Foxp3^+$ regulatory cells in LP of small intestine	172
		Mouse	Mouse (C57BL/6)		NR	NR	NR	NR	Total intestinal IgA and intestinal IgA, anti-CBir1; proliferation of splenic CBir1 TgT cells after CBir1 gavage	173

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TABLE 1 (Continued)

Consortium (no. of species[b])	Division of phyla[e]	Strain source(s)	Host species (strain)	Part of the gut studied[f]	No. of animals/ group	Chow	Sex	Age (collection time[s][c])	Study outcome(s)[d]	Reference
		Mouse	Mouse(B6.Rag$^{-/-}$)		NR	NR	F	8–10 wk (10 days)	Homeostatic and spontaneous proliferation of TCR TgT cells in LP	174
		Mouse	Mouse (C57BL/6)		5–8	Autoclaved chow	NR	8 wk (at least 3 dpi)	After infection, *S.* Typhimurium levels in mesenteric lymph nodes, spleen, cecum, and feces; cecal pathology score; cecal microbiota density; bacterial content and microbiota complexity in feces	97
ASF (8 and 9 species) 8 species: ASF 9 species: ASF + *Escherichia coli* HA108 or HA107	9 species: 	Mouse	Mouse(C57BL/6)		3	NR	NR	NR (119 days)	No. of IgA plasma cells per intestinal villus in duodenum, jejunum, ileum, and colon; IgA-bacterium binding in intestine; anti-*E. coli* IgA titer	21
ASF (8 species)		Mouse	Mouse (NMRI, C57BL/6, BALB/c, NIH Swiss, SW, NMRI, MyD88$^{-/-}$ Ticam1$^{-/-}$, SMARTA, C57BL/6.CD45.1^{+})		3–10	NR	NR	NR (up to 28 days)	Cecal bacterial contents; colonic Treg cell response and relative IL-10 expression in spleen, MLN, Peyer's patches, colonic and small intestinal LP, thoracic duct lymph; IL-17 production; relative abundance of strains; microscopic localization in colon and small intestine	175
		Mouse	Mouse (Nod1$^{-/-}$ and Nod2$^{-/-}$ on C57BL/6 background)		NR	NR	NR	6–9 wk (NR)	Cecal bacterial contents; intestinal tissue conductance and Cr-EDTA flux; E-cadherin protein expression and RegIII-gamma mRNA expression in colon; survival, colitis disease severity, histology score, and myeloperoxidase activity after DSS induction; colonic IL-6, IL-10, MCP-1, IFN-c, TNF-α, IL-12p70 levels	17
		Mouse	Mouse (C57BL/6)		NR	Autoclaved food	Both	8–12 wk (8–12 wk)	RegIII-gamma RNA and protein expression in ileum and colon	176

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TABLE 1 (Continued)

Consortium (no. of species[b])	Division of phyla[e]	Strain source(s)	Host species (strain)	Part of the gut studied[f]	No. of animals/ group	Chow	Sex	Age (collection time[s][c])	Study outcome(s)[d]	Reference
		Mouse	Mouse (C57BL/6 and C57BL/6 TSLPR$^{-/-}$)		3–5	NR	NR	NR (28 days)	Expression of thymic stromal lymphopoietin mRNA in intestinal epithelial cell or colonic LP (LP); % of CD4^{+} T cells secreting IL-17A and IFN gamma in the colonic LP and MLN; expansion of colonic Treg cells in colonic LP and MLN; expression of receptor for TSLP by CD4^{+} and regulatory T cells	177
		Mouse	Mouse (NIH Swiss)		4	NR	NR	3 days (3 days)	Structure of myenteric plexus, nerve density, average no. of HuC/D-positive myenteric neurons per ganglion, cell body size, and average no. of nNOS-positive neurons per myenteric ganglion in duodenum, jejunum, and ileum; small intestinal motility (frequency and amplitude of muscle contractions) in duodenum, jejunum, and ileum before and after general neural or specific nitrergic blockade	178
		Mouse	Mouse (C57BL/6)		5–14	Autoclaved mouse breeder's diet (Harlan), unlimited access	Both	6–12 wk (3 wk)	Colonic histology, inflammatory (MPO) activity, enteropathy (presence of fecal albumin), and cytokine expression; fecal microbiota profiles; colonic gene expression; proportion of T-cell subtypes in colonic LP and other mucosal and systemic immune compartments	81
ASF (8 species) (Oligo-MM[12] was also used, but no host parameters were assessed)		Mouse	Mouse (C57BL/6)		3 (ASF), 5–23 (Oligo-MM)	NR	Both	NR	Thicknesses of total colon and colon inner mucus (ASF); mucus turnover time (ASF); alpha diversity in colon and cecum (Oligo-MM)	26

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TABLE 1 (Continued)

Consortium (no. of species[b])	Division of phyla[e]	Strain source(s)	Host species (strain)	Part of the gut studied[f]	No. of animals/ group	Chow	Sex	Age (collection time[s][c])	Study outcome(s)[d]	Reference
ASF (8 and 9 species) 8 species: ASF 9 species: ASF + *Oxalobacter formigenes*	9 species: 	Mouse	Mouse (SW)		4–7	LM-485 autoclavable rodent diet, free access	M (no gender effect observed)	3–9 mo (3–9 mo + 6 wk)	Bacterial levels in stomach, cecum, proximal colon, and cecal mucosa; body wt; dietary oxalate intake; cecal and fecal oxalate levels; urine vol; urinary metabolite levels; cecal wet wt; cecal water metabolites	20
Partial ASF (6 species) ASF356, -361, -492, -502, -519, and -500 (ASF360 and -457 did not colonize)		Mouse	Mouse (NOD.MyD88KO)	None	9–23	NR	Both	NR (up to 30 wk)	Incidence of diabetes; histological scores of pancreatic islet destruction	18
Partial ASF (4 and 5 species) 4 species: ASF360, ASF361, ASF457, ASF519 5 species: 4 species + *Butyrivibrio fibrisolvens* (type I, ATCC 19171; type II, ATCC 51255)	4 species:  5 species: 	Mouse and bovine	Mouse (BALB/c)		4–5	Autoclaved low-fiber diet (5SRZ, catalog no. 1813680), high-fiber diet (5SVL, catalog no. 1813901), or tributyrin diet (5AVC, catalog no. 1814961)	NR	NR (2.5–5 mo after colorectal cancer induction)	Colorectal tumor multiplicity, tumor size, and tumor grade; levels of LDHA, lactate, butyrate, H3ac, and total H3 in colonic tissue and tumors; luminal SCFA levels; H3ac and expression levels of Fas, p21, and p27 genes in colonic tissue and tumors; apoptosis and cell proliferation levels in colonic tissue and tumors	19
Partial ASF (4, 5, 7, and 7 species) 4 species: ASF356, ASF360, ASF361, and ASF519 5 species: ASF360, ASF361, ASF457, SB2 (ASF502), and ASF519 7 species: ASF356, ASF360, ASF361, ASF457, ASF500, SB2 (ASF502), and ASF519 7 species: 4 species + *E. coli* Mt1B1, *Streptococcus danieliae* ERD01G, *Staphylococcus xylosus* 33-ERD13C (more with Oligo-MM [see below])	4 species:  5 species:  7 species:  7 species: 	Mouse	Mouse (C57BL/6)		4–6	NR	Both	0 or 8–12 wk (8–12 wk or 40 days)	Fecal bacterial content; bacterial load of *S.* Typhimurium in feces, cecum, and MLN; relative cecal wt; functional genomic analysis of bacteria	22

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TABLE 1 (Continued)

Consortium (no. of species[b])	Division of phyla[e]	Strain source(s)	Host species (strain)	Part of the gut studied[f]	No. of animals/ group	Chow	Sex	Age (collection time[s][c])	Study outcome(s)[d]	Reference
Oligo-MM (12, 15, and 17 species) 12 species, Oligo-MM: *Acutalibacter muris* KB18, *Flavonifractor plautii* YL31, *Clostridium clostridioforme* YL32, *Blautia coccoides* YL58, *Clostridium innocuum* I46, *Lactobacillus reuteri* I49, *Enterococcus faecalis* KB1, *Bacteroides caecimuris* I48, *Muribaculum intestinale* YL27, *Bifidobacterium longum* subsp. *animalis* YL2, *Turicimonas muris* YL45, *Akkermansia muciniphila* YL44 15 species: 12 species + 3 facultative anaerobes (*E. coli* Mt1B1, *Streptococcus danieliae* ERD01G, *Staphylococcus xylosus* 33-ERD13C) 17 species: 12 species + 5 ASF members (ASF360, ASF361, ASF457, SB2 [ASF502], ASF519)	12 species:  15 species:  17 species: 	Mouse	Mouse (C57BL/6)		4–6	NR	Both	0 (8–12 wk)	Fecal bacterial content; bacterial load of *S.* Typhimurium in feces, cecum, and MLN; relative cecal wt; functional genomic analysis of bacteria	22
Oligo-MM (12 and 13 species) 12 species: Oligo-MM 13 species: 12 species + *Clostridium scindens* ATCC 35704	12 species:  13 species: 	Mouse	Mouse (C57BL/6)		5–8	NR	NR	0 (6–12 wk)	Fecal and cecal bacterial contents; cecal levels of lipocalin-2; calprotectin expression in cecal tissue; histopathology of cecum; cecal bile acid metabolome	
SIHUMI(x) (7 and 8 species): SIHUMI: *Anaerostipes caccae* DSM 14662 or DSM 14667, *Bacteroides thetaiotaomicron* DSM 2079, *B. longum* NCC 2705, *Blautia producta* DSM 2950, *Clostridium ramosum* DSM 1402, *E. coli* K-12 MG1655, *Lactobacillus plantarum* DSM 20174 SIHUMI(x) *Clostridium butyricum* DSM 10702	7 species:  8 species: 	Human	Rat (Sprague-Dawley)		3–21	Sterilized standard chow (225 g/kg of body wt protein, 50 g/kg crude fat, 65 g/kg ash, 135 g/kg moisture, 480 g/kg N-free extract), fermentable-fiber-free diet, inulin diet, pectin diet, and high-fat and low-fat diets	Both	0–3 mo (2–38 wk)	Stability of microbiota in offspring; SCFA concn and pH in cecum, colon, and feces; bacterial counts in cecum, colon, and feces; Midtvedt criteria	27

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TABLE 1 (Continued)

Consortium (no. of species[b])	Division of phyla[e]	Strain source(s)	Host species (strain)	Part of the gut studied[f]	No. of animals/ group	Chow	Sex	Age (collection time[s][c])	Study outcome(s)[d]	Reference
SIHUMI(x) (8 and 9 species) 8 species: SIHUMI(x) 9 species: 8 species + *A. muciniphila* ATCC BAA-835	9 species: 	Human	Mouse (C3H)		5–10	NR	NR	12 wk (5–15 days)	Bacterial cell no. and proportions in cecum and colon; cecal and colonic histopathology scores; expression of proinflammatory cytokines in cecal and colonic mucosa; serum protein levels of proinflammatory cytokines; no. of *S.* Typhimurium cells in MLN and spleen; size; macrophage infiltration in cecal tissue; localization of *A. muciniphila* and *S.* Typhimurium; mucin formation, mucus thickness, mucus composition, and no. of mucin-filled cells	28
SIHUMI(x) (8 and 9 species) 8 species: SIHUMI(x) 9 species: 8 species + *Fusobacterium varium* ATCC 8501	9 species: 	Human	Mouse (C3H/HeOuJ)		12	Irradiated standard chow R03-40	F	0 (8 wk)	Body wt; dry mass of cecum and colon; bacterial content in cecum and colon; polyamine concn in cecum and colon; SCFA concn in cecum and colon; histology of cecum and distal colon (thicknesses of crypt, epithelial layer, mucosa, submucosa, muscularis externa); mitosis and apoptosis of cecal and distal colonic tissue	30
		Human	Mouse (Prm/Alf, C3H/He)		12–13	Sterilized pelleted standard chow R03-40	F	0 (56 ± 1 days)	Lengths of small, large, and whole intestines; thicknesses of muscle, crypt, and villi in proximal and small intestine and colon; fecal and cecal microbial contents; cecal concn of SCFAs and polyamines	31

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TABLE 1 (Continued)

Consortium (no. of species[b])	Division of phyla[e]	Strain source(s)	Host species (strain)	Part of the gut studied[f]	No. of animals/ group	Chow	Sex	Age (collection time[s][c])	Study outcome(s)[d]	Reference
SIHUMI(x) (7 and 8 species) 7 species: SIHUMI(x) without *C. ramosum* 8 species: SIHUMI(x)	7 species:  8 species: 	Human	Mouse (C3H/HeOuJ)		3–9	Irradiated low-fat or high-fat diet *ad libitum*	M	0 (16 wk)	Body wt; % body fat; adipose tissue wt (epididymal, mesenteric, and subcutaneous); energy intake; food efficiency; digestibility of high-fat diet; digestible energy; cecal and colonic bacterial contents per species; blood glucose; leptin gene expression in epididymal tissue; liver wt; liver triglyceride levels; liver glycogen contents; expression of genes involved in lipid transport, lipid synthesis, cholesterol synthesis, and lipid catabolism; gene expression of proteins involved in small intestinal glucose uptake; SCFA formation in cecum, colon, and portal vein plasma; gene expression of SCFA-related proteins in colonic mucosa; gene expression of lipid transport and storage proteins in ileum; parameters of intestinal permeability and low-grade inflammation	32
SIHUMI(x) (8 and 9 species) 8 species: SIHUMI(x) 9 species: 8 species + *A. muciniphila* ATCC BAA-835	9 species: 	Human	Mouse (C57BL/ 6.129P2-Il10tm1Cgn)		5–6	Irradiated standard chow (fortified type 1310; Altromin, Lage, Germany) *ad libitum*	M	0 or 8 wk (3 wk)	Body wt; histopathology scores in submucosa, LP, surface epithelium, and lumen; colon length; relative mRNA levels of *Tnfa*, *Ifng*, and *Reg3g*; fecal lipocalin-2 concn; fecal and cecal bacterial levels; cecal histology; no. of goblet cells per 100 epithelial cells in cecum and colon; mucus layer thickness in colon; relative Muc2 mRNA levels in distal small intestine, cecum, and colon	29

[a]Abbreviations: M, male; F, female; SW, Swiss Webster; LP, lamina propria; MLN, mesenteric lymph nodes; MPO, myeloperoxidase; NR, not reported; SCFA, short-chain fatty acid; Treg cell, regulatory T cell; IL-1B, interleukin-1B; Ab, antibody; TCR, T-cell receptor; dpi, days postinfection; DSS, dextran sodium sulfate; IFN, interferon; TNF-α, tumor necrosis factor alpha; 2NT, 2-nitrotoluene; H3ac, pan-histone 3 acetylation; LDHA, lactate dehydrogenase A; nNOS, neuronal nitric oxide synthase; TgT cells, transgenic T cells; MCP-1, monocyte chemoattractant protein 1.

[b]Two different strains tested are counted as one species. Strains were not always reported. Pathogenic species, in the case of an infection model, are not included.

[c]The colonization time includes the time from colonization (time zero in the case of transfer of microbiota to offspring) until and including the time of sacrifice or the end of experimental (e.g., dietary) manipulations, in cases where this is clearly stated in the paper. If age is given and animals are colonized at birth, the age is included in the colonization time.

[d]Study outcomes are reported only for animals colonized with the defined community of interest.

[e], *Firmicutes*; , *Bacteroidetes*; , *Actinobacteria*; , *Proteobacteria*; , *Verrucomicrobia*; , other.

[f]The color codes from left to right in the illustration are as follows: , stomach; , duodenum; , jejunum; , ileum; , cecum; , colon; , rectum; , feces.

TABLE 2 Studies using non-specifically-named defined communities in rodents to study host-microbe interactions *in vivo* ($n = 16$)[a]

Consortium (no. of species[b])	Division of phyla[f]	Strain source(s)	Host species (strain)	Part of the gut studied[g]	No. of animals/ group	Chow	Sex	Age (collection time[s][c])	Study outcome(s)[d]	Reference
NA, F-strains, and N-strains (2, 9, 11, 41, and 130 species) 2 species: *E. coli* C25 + *Lactobacillus* 9 species: 2 species + *Enterococcus* + *Lactobacillus* + *Candida* + 4 morphologically different strains of Gram-negative anaerobes 11 species: 9 species + 2 strains of Gram-negative anaerobes with fusiform morphology 41 species: 11 species + 30 additional strains of Gram-negative anaerobes 130 species: 50 strains of Gram-negative strict anaerobes (N) + 80 facultative anaerobes (F)	2 species:  Others not specified	Mouse	Mouse (CD-1)		4–57	Autoclaved Lobund diet L-356 or pelleted sterile diet from Charles River Mouse Farms	NR	NR (1–60 days)	Cecal number of *E. coli* C25 bacteria; cecal size; histopathology of stomach, small intestine, cecum, and colon	33
N- and F-strains (60, 96, and 97 species) 60 species: N-strains + 14 facultative anaerobes + *E. coli* C25 96 strains: F-strains + *E. coli* C25, *E. coli* 40T, or *Shigella* 97 strains: F-strains + *E. coli* C25 + *Shigella* or *E. coli* 40T	Not specified	Mouse	Mouse (CD-1)		5–75	Sterilized Lobund diet L-356, Charles River formula 7RF, Lobund diet L-485, or Purina breeder chow	NR	NR (4 wk)	Cecal size; cecal levels of fatty acids; cecal levels of *E. coli*; pH of cecal contents	34
NA (4 species) *Lactobacillus* species 1 and 2, *Bacteroides* sp., *Streptococcus* group N		Rat?	Rat (Sprague-Dawley)	None	2	Autoclaved standard diet[e] supplemented with caffeic acid	NR	NR	Urinary metabolites of caffeic acids	179

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TABLE 2 (Continued)

Consortium (no. of species[b])	Division of phyla[f]	Strain source(s)	Host species (strain)	Part of the gut studied[g]	No. of animals/ group	Chow	Sex	Age (collection time[s][c])	Study outcome(s)[d]	Reference
NA (2, 2, 2, 2, 3, 3, 4, 5, 6, 6, 6, 8, 8, 9, 13, 15, and 17 species) 2 species: *Actinobacillus* s3 + *Streptococcus* s1 2 species: *Bacteroides* s8 + *Actinobacillus* s3 2 species: *Eubacterium* s10 + *Micrococcus* s6 2 species: *Clostridium* C1 + C2 3 species: *Bacteroides* s8 + *Actinobacillus* s3 + *E. coli* s7 3 species: *Shigella flexneri* + C5 + C6 4 species: C1–C4 4 species: *S. flexneri* + C3–C5 6 species: C1–C6 6 species: *Actinobacillus* s3 + *Streptococcus* s1 + *Lactobacillus* s4 + *Corynebacterium* s5 + *Micrococcus* s6 + *Streptococcus* s2 6 species: *S. flexneri* + C5–C9 8 species: 6 species (*Actinobacillus*, etc.) + *Bacteroides* s8 + *E. coli* s7 8 species: *S. flexneri* + C3–C9 9 species: C1–C9 13 species: C1–C13 15 species: C1–15 17 species: 8 species (*Actinobacillus*, etc.) + C1–C9	2 species (2×):  2 species:   3 species:   6 species:    8 species:   4, 9, 13, and 15 species:  17 species: 	Human and mouse	Mouse (CD-1)		≥2	Sterilized commercial diet (Usine d'Alimentation Rationnelle) *ad libitum*	Both	2–5 mo (4 wk after last inoculation)	No. of IgA plasmocytes in duodenum	38

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TABLE 2 (Continued)

Consortium (no. of species[b])	Division of phyla[f]	Strain source(s)	Host species (strain)	Part of the gut studied[g]	No. of animals/ group	Chow	Sex	Age (collection time[s][c])	Study outcome(s)[d]	Reference
NA (2, 2, 2, 2, 2, 2, 2, 2, and 3 species) 2 and 2 species: *Clostridium* E or P with *E. coli* K-12 2 species (×6): *Clostridium* E + *E. coli* S, *Proteus mirabilis*, *Klebsiella pneumoniae*, *Bacteroides* (*Alistipes*) *putredinis*, *Veillonella alcalescens*, or *Clostridium perfringens* 3 species: *Clostridium* E and P + *E. coli* K-12	2 species (5×):  2 species:  2 species (2×):  3 species: 	Mouse, rat, human	Mouse (C3H)		2–6	Autoclaved commercial diet	NR	Adult (up to 51 days)	Fecal bacterial counts; (mucosal) histology of stomach, jejunum, ileum, cecum, and colon	180
UW-GL (9 species) Genera *Lactobacillus*, *Bacillus*, *Clostridium* and *Corynebacterium* Species not defined	 ? = phyla known, but exact composition not defined	NR	Mouse (BALB/c)		Total of 3	Sterilized Ralston Purina diet 5010C	Both	0 (60–90 days)	Cecal levels of bacteria and *Candida albicans*; histology of tongue and stomach	37
NA (6 species) *Streptococcus* (*Enterococcus*) *faecalis*, *Lactobacillus brevis*, *Aerobacter aerogenes*, *Staphylococcus epidermidis*, *Bacteroides spurius* (?), a yeast fungus	 ? = phylum could not be retrieved	NR	Mouse (BALB/c/ABOMf)	None	3–6	Sterilized food (2 different procedures)	NR	0 (14 wk)	Serum levels of IgG1, IgG2, IgM, and IgA	108
Partial or complete UW-GL (2, 3, and 9 species) 2 species: *Lactobacillus* + *Clostridium* 3 species: 2 species + *Bacillus* 9 species: UW-GL	2, 3 species: 	NR and mouse	Mouse (HA/ICR)		10–48	NR	Both	Adult (14–56 days)	Death after *C. botulinum* infection; fecal *C. botulinum* toxin excretion; colonization pattern of *C. botulinum*	36

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TABLE 2 (Continued)

Consortium (no. of species[b])	Division of phyla[f]	Strain source(s)	Host species (strain)	Part of the gut studied[g]	No. of animals/ group	Chow	Sex	Age (collection time[s][c])	Study outcome(s)[d]	Reference
NA (2 species) *B. thetaiotaomicron* VPI-5482 + *Desulfovibrio piger* ATCC 29098		Human	Mouse (NMRI/KI)		4–5	Autoclaved polysaccharide-rich diet (B&K) *ad libitum*	M (subset)	Adult or 12 wk (14–28 days)	Bacterial contents in cecum and distal colon; bacterial gene expression; glycan levels in cecum; SCFA production in cecum; serum acetate; liver triglycerides; epididymal fat pad	109
NA (2, 6, and 10 species) 2 species: *Staphylococcus epidermidis* + *Veillonella parvula* 6 and 10 species: anaerobic strains isolated from a conventional male mouse (not specified)	2 species: 	Mouse	Mouse (B10.BR)		45–73	Sterilized ST1 (Institute of Physiology AS CR)	M	21 days (12 mo)	Occurrence of ankylosing enthesopathy of the ankle; colon histology; bacterial contents in ileum and colon	181
NA (2 species) *B. thetaiotaomicron* + *Eubacterium rectale*		Human	Mouse (NMRI/KI)		4–5	Irradiated standard low-fat, plant polysaccharide-rich diet (diet 2018 from Harland Teklad); high-fat, "high-sugar" Western-type diet (catalog no. 96132; Harlan Teklad); or low-fat, high-sugar diet (catalog no. 03317; Harlan Teklad)	M	11 wk (14 days)	Bacterial gene expression; cecal colonization levels; fermentation efficiency in cecum; colonic gene expression; protein expression in cecum	85
NA (3, 8, 9, and 10 species) 3 species: *E. coli* HS, *Bacteroides vulgatus* DSM 1447, *B. thetaiotaomicron* DSM 2079 8 species: 3 species + *B. longum* NCC2705, *Blautia hansenii* DSM 20583, *C. scindens* DSM 5676, *Eubacterium ventriosum* DSM 3988, *Lactobacillus rhamnosus* NCC4007 9 species: 8 species + *Collinsella aerofaciens* DSM 3979 (colonized most mice) 10 species: 9 species + *Faecalibacterium prausnitzii* DSM 17677 (did not colonize mice)	3 species:  8 species:  9 species:  10 species: 	Human	Mouse (C3H/HeN)		15 in total	Sterile standard chow diet or switch to high-fat diet *ad libitum*	Both	7 wk (70 days after 1st inoculation)	Fecal and cecal bacterial cell counts; body wt; metabolites in urine and plasma	39

(Continued on next page)

TABLE 2 (Continued)

Consortium (no. of species[b])	Division of phyla[f]	Strain source(s)	Host species (strain)	Part of the gut studied[g]	No. of animals/ group	Chow	Sex	Age (collection time[s][c])	Study outcome(s)[d]	Reference
NA (15 and 19 species) 15 species: *Bacteroides caccae*, *Bacteroides ovatus*, *B. thetaiotaomicron*, *Bacteroides uniformis*, *B. vulgatus*, *Bacteroides* strain WH2, *C. scindens*, *Clostridium spiroforme*, *C. aerofaciens*, *Dorea longicatena*, *E. rectale*, *F. prausnitzii*, *Parabacteroides distasonis*, *Ruminococcus obeum*, *Ruminococcus torques* (strain information not accessible) 19 species: 15 species + *Bifidobacterium animalis* subsp. *lactis* CNCM I-2494, *Lactobacillus delbrueckii* subsp. *bulgaricus* CNCM I-1632 + CNCM I-1519, *Lactococcus lactis* subsp. *cremoris* CNCM I-1631, and *Streptococcus thermophilus* CNCM I-1630	15 species:  19 species: 	Human	Mouse (C57BL/6J)		5	Autoclaved low-fat, plant polysaccharide-rich diet (B&K rat and mouse autoclavable chow, catalog no. 7378000)	M	6–8 wk (42 days)	Fecal and cecal bacterial content; bacterial gene expression; urinary metabolites	40
NA (2 species) *B. thetaiotaomicron* VPI-5482 (ATCC 29148) + *F. prausnitzii* A2-165 (DSM 17677)		Human	Rat (F344)		6–16	Irradiated polysaccharide-rich diet (R03, SAFE)	M	<3 mo (30 days after inoculation of *F. prausnitzii*)	Host gene expression in colonic epithelium; cecal SCFA concn; oxidoreduction potential in cecal contents; colonic crypt depth; total cells/crypt in colon; expression of differentiation proteins of secretory lineage (KLF-4, ChgA); Muc2 production in colonic epithelium; colonic mucin glycosylation	182

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TABLE 2 (Continued)

Consortium (no. of species[b])	Division of phyla[f]	Strain source(s)	Host species (strain)	Part of the gut studied[g]	No. of animals/ group	Chow	Sex	Age (collection time[s][c])	Study outcome(s)[d]	Reference
NA (2 species) *B. thetaiotaomicron* VPI-5482 + *B. longum* NCC2705		Human	Mouse (SW)		3	Standard diet (Purina LabDiet 5K67)	NR	NR (10 days)	Fecal bacterial content; metabolites in feces and urine	86
NA (2, 8, and 9 species) 2 species: *B. thetaiotaomicron* + *D. piger* 8 species: *B. thetaiotaomicron*, *B. caccae*, *B. ovatus*, *E. rectale*, *Marvinbryantia formatexigens*, *C. aerofaciens*, *E. coli*, *Clostridium symbiosum* 9 species: 8 species + *D. piger*	2 species:  8 species:  9 species: 	Human	Mouse (NMRI)		4–20	Irradiated low-fat/high-plant-polysaccharide or high-fat/high-simple-sugar diet *ad libitum*, HF/HS diet with modified sulfate concn (600-fold range), or HF/HS diet supplemented with chondroitin sulfate	M	7–8 wk (2 wk)	Fecal bacterial relative abundance; fecal metatranscriptome; gene expression of *D. piger*; gene expression of mouse proximal colon; cecal metabolites	42
NA (14 species) + viruslike particles *C. aerofaciens* ATCC 25986, *B. caccae* ATCC 43185, *B. ovatus* ATCC 8483, *B. thetaiotaomicron* VPI-5482 + 7330, *Bacteroides uniformis* ATCC 8492, *Bacteroides vulgatus* ATCC 8482, *Bacteroides cellulosilyticus* WH2, *Parabacteroides distasonis* ATCC 8503, *C. scindens* ATCC 35704, *C. symbiosum* ATCC 14940, *C. spiroforme* DSM 1552, *D. longicatena* DSM 13814, *E. rectale* ATCC 33656, *R. obeum* ATCC 29174		Human	Mouse (C57BL/6J)		5	Autoclaved low-fat/high-plant-polysaccharide diet (B&K) *ad libitum*	NR	8 wk (46 days)	Gut barrier and immune function; overall health status; body wt and adiposity; no. of $CD4^+$ and $CD8^+$ T cells in spleens and MLN; fecal bacterial content and viral abundance; genetic changes upon viral attack (phage resistance); bacterial contents of proximal and distal small intestines, cecum, and colon; prophage activation	45

(Continued on next page)

TABLE 2 (Continued)

Consortium (no. of species[b])	Division of phyla[f]	Strain source(s)	Host species (strain)	Part of the gut studied[g]	No. of animals/ group	Chow	Sex	Age (collection time[s][c])	Study outcome(s)[d]	Reference
NA (14 species) *B. ovatus* DSM 1896, *Bacteroides uniformis* DSM 8492, *B. thetaiotaomicron* DSM 2079, *B. caccae* DSM 19024, *Barnesiella intestinihominis* YIT11860, *Roseburia intestinalis* 14610 (L1-82), *E. rectale* DSM 17629 (A1-86), *F. prausnitzii* DSM 17677 (A2-165), *Marvinbryantia formatexigens* DSM 14469 (I-52), *C. symbiosum* DSM 934, *C. aerofaciens* DSM 3979, *E. coli* HS, *A. muciniphila* DSM 22959 Muc, *D. piger* ATCC 29098		Human	Mouse (SW)		Total of 51	Autoclaved standard fiber-rich (15% dietary fiber), fiber-free, or prebiotic (addition of purified soluble glycans) diet *ad libitum*	Both	8–9 wk (54 days)	Microbial composition in feces, cecum, colonic lumen, and mucus layer; bacterial CAZyme expression in cecum; mucin-specific transcripts in *B. caccae*, *A. muciniphila*, and *B. thetaiotaomicron*; cecal microbial enzyme activities; levels of SCFAs and organic acids; colonic mucus layer thickness; colonic expression of mucus-production-related genes; no. of goblet cells in colon; histopathology; body wt; fecal lipocalin; colon length; cecal transcriptome; after infection with *C. rodentium*, histological scores of cecum and colon, area of inflamed tissue in cecum, survival, ascending and descending colon and rectum, adherent *C. rodentium* bacteria in colon	43

[a]Abbreviations: NA, consortium name not available/applicable; AS CR, Academy of Sciences of the Czech Republic; ChgA, chromogranin A; KLF-4, Kruppel-like factor 4; LP, lamina propria; MLN, mesenteric lymph nodes; NR, not reported; SCFAs, short-chain fatty acids; HF/HS, high fat/high sugar; CAZyme, carbohydrate-active enzyme; s3, species 3.

[b]Two different strains tested are counted as one species. Strains were not always reported. Pathogenic species, in the case of an infection model, are not included.

[c]The colonization time includes the time from colonization (time zero in the case of transfer of microbiota to offspring) until and including the time of sacrifice or the end of experimental (e.g., dietary) manipulations, in cases where this is clearly stated in the paper. If age is given and animals are colonized at birth, the age is included in the colonization time.

[d]Study outcomes are reported for the animals colonized with the defined community of interest.

[e]See reference 7.

[f], *Firmicutes*; , *Bacteroidetes*; , *Actinobacteria*; , *Proteobacteria*; , *Verrucomicrobia*; , other.

[g]The color codes from left to right in the illustration are as follows: , stomach; , duodenum; , jejunum; , ileum; , cecum; , colon; , rectum; , feces.

TABLE 3 Studies using defined communities in nonrodents to study host-microbe interactions *in vivo* ($n = 6$)[a]

Consortium (no. of species[b])	Division of phyla[e]	Strain source	Host species (strain)	Part of the gut studied[f]	No. of animals/ group	Chow	Sex	Age (collection time[s][c])	Study outcomes[d]	Reference
Bristol (3 and 4 species), modified ASF (6 and 7 species) 3 species: *Lactobacillus amylovorus* DSM 16698^T, *Clostridium glycolicum*, and *Parabacteroides* sp. (ASF519) 4 species: 3 species + *R. intestinalis* 6 species: *Clostridium* sp. (ASF356), *Lactobacillus* sp. (ASF360), *Lactobacillus animalis* (ASF361), *E. plexicaudatum* (ASF492), *Parabacteroides* sp. (ASF519), and *Propionibacterium* sp. 7 species: 6 species + *Staphylococcus* sp. or *Bacillus* sp.	3 species:  4 species:  6 species:  7 species: 	Pig	Pig (commercial hybrid and Babraham)		2–6	Evaporated milk	NR	0–17 days (14–21 days after 1st inoculation)	Presence of bacteria and mean total bacterial content in proximal and distal jejunum, terminal ileum, cecum, and colon; serum immunoglobulin concn	52
Bristol (3 species)		Pig	Pig ([Great York × Pie] × 'Dalland' cross)		6	Pasteurized sow colostrum (first hours), *ad libitum* milk replacer diet (days 0–4), moist diet (remaining)	NR	Neonates (26–37 days)	Relative OR51E1 expression in jejunum	59
Bristol (3 species)		Pig	Pig ([Great York × Pietrain] × 'Dalland' cross)		6	Sow serum or pasteurized sow colostrum, followed by *ad libitum* milk replacer diet (days 0–4), followed by a control diet or medium-chain-fatty-acid diet	NR	1 day (2–3 wk)	Oxyntic mucosa transcriptome	60

(Continued on next page)

TABLE 3 (Continued)

Consortium (no. of species[b])	Division of phyla[e]	Strain source	Host species (strain)	Part of the gut studied[f]	No. of animals/ group	Chow	Sex	Age (collection time[s][c])	Study outcomes[d]	Reference
DMF (7 and 8 species) 7 species: *Bifidobacterium adolescentis*, *B. longum*, *B. thetaiotaomicron*, *E. faecalis*, *L. brevis*, *Streptococcus bovis*, and *C. clostridioforme* 8 species: DMF + *E. coli* Nissle	7 species:  8 species: 	Pig	Pig (Landrace × Yorkshire × Duroc cross-bred)		3–6	NR	NR	7 days (35 days)	Fecal virus shedding; mean duration of diarrhea; diarrhea severity and % of diarrhea; gene expression levels of ChgA, Muc2, PCNA, SOX9, and villin in jejunal intestinal epithelial cells	62
		Pig	Pig (NR)		3–5	NR	NR	5 days (14–35 days)	Bacterial content in rectum, duodenum, jejunum, ileum, colon, and feces/ rectal swabs; diarrhea and virus shedding after virulent human rotavirus challenge	63

[a]NR, not reported; ChgA, chromogranin A.

[b]Two different strains tested are counted as one species. Strains were not always reported. Pathogenic species, in the case of an infection model, are not included.

[c]The colonization time includes the time from colonization (time zero in the case of transfer of microbiota to offspring) until and including the time of sacrifice or the end of experimental (e.g., dietary) manipulations, in cases where this is clearly stated in the paper. If age is given and animals are colonized at birth, the age is included in the colonization time.

[d]Study outcomes are reported only for the animals colonized with the defined community of interest.

[e]■, *Firmicutes*; ■, *Bacteroidetes*; ■, *Actinobacteria*; ■, *Proteobacteria*; ■, *Verrucomicrobia*; ■, other.

[f]The color codes from left to right in the illustration are as follows: ■, stomach; ■, duodenum; ■, jejunum; ■, ileum; ■, cecum; ■, colon; ■, rectum; ■, feces.

Oligo-MM

Another defined community of murine microbiota, Oligo-MM^{12}, was constructed in an attempt to provide full colonization resistance against *Salmonella enterica* serovar Typhimurium (22). Twelve strains were selected to represent the five most prevalent and abundant phyla of the laboratory mouse intestine, i.e., the *Firmicutes* members *Acutalibacter muris*, *Flavonifractor plautii*, *Clostridium clostridioforme*, *Blautia coccoides*, *Clostridium innocuum*, *Lactobacillus reuteri*, and *Enterococcus faecalis*; the *Bacteroidetes* members *Bacteroides caecimuris*, and *Muribaculum intestinale*; the *Actinobacteria* member *Bifidobacterium longum* subsp. *animalis*; the *Proteobacteria* member *Turicimonas muris*; and the *Verrucomicrobia* member *Akkermansia muciniphila*. Colonization resistance of ASF mice or mice colonized with Oligo-MM^{12} and/or (a subset of) ASF strains was compared to that of conventional mice. ASF was used as a reference because of its wide usage in gnotobiotic mouse research. Oligo-MM^{12} mice conferred increased, but not full, resistance compared to mice colonized with a subset of ASF strains with and without Oligo-MM. Functional genomic analysis of Oligo-MM and whole ASF revealed that both consortia together cover 66.6% of the KEGG modules of a conventional mouse microbiota. The addition of three facultative anaerobes (*E. coli*, *Streptococcus danieliae*, and *Staphylococcus xylosus*), underrepresented in Oligo-MM^{12}, increased coverage and furthermore conferred full colonization resistance (22). C57BL/6 mice stably colonized with Oligo-MM^{12} have been designated stable defined moderately diverse microbiota mice (sDMDMm2). The designers of Oligo-MM^{12} stressed the importance of expanding the amount of available mouse-derived strains, as initiated recently (24), in favor of the design of functionally defined and simplified microbial consortia for application in gnotobiotic animals (22). Because Oligo-MM^{12} was found to lack the enzymatic pathway to carry out 7α-dehydroxylation, an important bile acid transformation step, the addition of *Clostridium scindens* (a 7α-dehydroxylating bacterium) was tested in another study. This modification normalized large intestinal bile acid composition in mice, which was accompanied by colonization resistance against *Clostridium difficile* and decreased intestinal pathology (25). Finally, Oligo-MM^{12} served as a defined reference microbiota to verify the significant difference between the bacterial compositions in the large intestinal outer mucus layer and the lumen (26), but host parameters were not assessed. Note that the latter two studies that applied Oligo-MM^{12} left out the three additional facultative anaerobes that were found to be crucial for full colonization resistance.

SIHUMI(x)

Because the ASF was found to poorly represent the dominant intestinal bacteria and ASF mice hardly differed from GF mice in a key set of microbial biochemical activities (23) (Midtvedt criteria [see below]), a simplified human intestinal microbiota (SIHUMI) was established in rats to provide a highly standardized animal model to study host-microbe interactions. Species were selected according to their prevalence in humans, their fermentative capacity, the availability of their genomic sequence, and their ability to stably colonize the rodent gut. SIHUMI(x) includes four *Firmicutes* (*Anaerostipes caccae*, *Lactobacillus plantarum*, *Blautia producta*, and *Clostridium ramosum*), one *Bacteroidetes* species (*Bacteroides thetaiotaomicron*), one actinobacterium (*B. longum*), and one proteobacterium (*E. coli*). All seven members successfully colonized the rat intestinal tract, and total bacterial numbers in fecal samples did not differ from those in human feces. The amount of short-chain fatty acids (SCFAs) produced, however, was dramatically smaller than that in humans, probably owing to the smaller number of species. An eighth species was added to the consortium (SIHUMIx), *Clostridium butyricum*, which led to increased butyrate production. All members of the SIHUMIx consortium were successfully transferred to offspring. Dietary interventions varying in fiber and fat contents resulted in responses (partially) reflecting those observed in mice and humans (27).

In other studies, SIHUMIx served as a resident community to study the effect of the addition or removal of species. For instance, the inclusion of *A. muciniphila*, a mucin-

degrading commensal, was found to worsen intestinal inflammation induced by *S.* Typhimurium in mice (28). The same researchers recently showed, however, that in a colitis-prone mouse model colonized with SIHUMI, *A. muciniphila* did not induce or exacerbate intestinal inflammation (29). In two other studies, the polyamine-producing organism *Fusobacterium varium* was added to the low-polyamine-producing SIHUMIx in mice, which disclosed that gut morphology was not affected by either increased putrescine concentrations (30) or higher levels of other polyamines and SCFAs (31). Additionally, the mechanism underlying the obesogenic potential of *C. ramosum* in a SIHUMIx-associated animal model was further investigated by including or excluding this bacterium in SIHUMIx-associated mice fed a high- or a low-fat diet. The increased body fat deposition in the presence of *C. ramosum* was suggested to be due to the upregulation of small intestinal glucose and fat transporters (32). It should be noted that although SIHUMI was originally established in rats, all other studies applied the community in mice.

Toward a Normal Model Gut Microbiota

Since the generation of the Schaedler flora in the 1960s, other defined gut microbiotas have been developed in an attempt to normalize GF animals or generate animal models harboring a bacterial community representative of the human gut microbiome. During the 1970s, Syed et al. aimed to normalize GF mice with respect to cecum size, cecal numbers of *E. coli* cells, histology of the intestinal tract, and the development of a mucosa-associated microbiota in the stomach and large intestine (33). A mixture of 50 strictly anaerobic organisms (later designated "N-strains" [34]) and 70 facultative anaerobes ("F-strains") was found to generate a normal mouse phenotype, whereas less-complex bacterial communities led to intermediate phenotypes with respect to the parameters studied, including cecum size, cecal *E. coli* levels, GIT histology, and the development of a mucosa-associated microbiota in the stomach and large intestine (33). The exact taxonomic classification of the species within the F- and N-strains was limited by a lack of characterization at that time (33). It was considered likely that a number of the isolates used were identical. Based on morphology and fatty acid production, the total numbers of different strains were estimated to be rather on the order of 35 (N-strains) and 60 (F-strains) (34). The N-strains alone could not control the *E. coli* population and cecum size when associated with mice fed a crude instead of refined diet, but this could be restored by additional association with the F-strains (34). The F-strains were exploited as an indigenous gut microbiota to investigate *E. coli* plasmid transfer *in vivo* (35), but other studies using the N- or F-strains could not be identified.

At the end of the 1970s, the use of the UW-GL (University of Wisconsin Gnotobiote Laboratory flora) was reported, which was used as the intestinal microbiota of heterozygous athymic mice (36). This defined bacteriome consisted of nine Gram-positive species from the genera *Lactobacillus*, *Bacillus*, *Clostridium*, and *Corynebacterium* (37) and additionally two Gram-negative species that were not further specified (36). It was used to study colonization resistance against *Candida albicans* (37) and *Clostridium botulinum* (36). The latter study compared UW-GL with other defined microbiotas, including ASF and a partial UW-GL. Whereas death rates significantly dropped compared to those of GF mice, only the complete UW-GL fully prevented *C. botulinum* infection (36). The use of the UW-GL microbiota has not been reported since.

Logically, the conception of a healthy or "normal" microbiota is dependent on the available knowledge on conventional animals and/or healthy human subjects, and thus, the composition varies per study. While testing the effect of bacterial species on intestinal IgA immune system development, Moreau et al. paid specific attention to communities of *Clostridium* species, which were considered a dominant microbiota of the digestive tract of adult conventional mice (38). In studies using defined communities with human-derived gut bacteria, species were selected based on their prevalence in (healthy) human feces (39, 40) and/or their representation of the major three or four dominant phyla of the human gut microbiota (40–42). Next to the designers of

Oligo-MM12, only a few studies acknowledged the presence of five phyla (including *Verrucomicrobia*) of the human gut microbiota. A recently designed 14-membered synthetic microbiota that collectively possessed important core metabolic capabilities was applied to study *in vivo* foraging of host-derived mucus glycoproteins during fiber deprivation (43). Similarly, other studies took into account the functional capabilities of species. For instance, one study included species that are able to break down complex dietary polysaccharides not accessible to the host (*B. thetaiotaomicron*, *Bacteroides ovatus*, and *Bacteroides caccae*), to consume oligosaccharides and simple sugars (*Eubacterium rectale*, *Marvinbryantia formatexigens*, *Collinsella aerofaciens*, and *E. coli*), to ferment amino acids (*Clostridium symbiosum* and *E. coli*), or to remove the end products of fermentation by reducing sulfate (*Desulfovibrio piger*) or generating acetate (*Blautia hydrogenotrophica*) (41). This community has been frequently exploited to study host-microbe interactions or microbe-microbe interactions by the same research group or adopted by others albeit in different combinations ranging from 8 to 15 species (40, 42, 44–50). Recently, a more diverse, complex defined community comprising no fewer than 92 species was developed (51). The consortium consisted of phylogenetically diverse, human-derived bacterial strains, which had previously been cultured and sequenced. It also included strains representing species that were demonstrated to be age and/or growth discriminatory in models of microbiota development during the first years of life. Of all strains, 44 comprised a core group that could be detected in fecal samples of all colonized mice, independent of dietary intervention (51). No host parameters, however, were assessed in this study.

Remaining inclusion criteria for defined communities are the availability of the genomic sequence and the cultivability of the species. Obviously, both criteria make each individual species more easily traceable. If the entire genetic repertoire of the defined community is known, gene expression of the whole community as well as its individual members can easily be assessed (28, 40), and their function can be more precisely predicted. Interestingly, although the ASF has been used for over 50 years, publications on the replication of the four extremely-oxygen-sensitive ASF members on a defined medium are still lacking (14).

Defined Communities in Nonrodents

The above-discussed defined microbiotas were either isolated from rodents or applied to them. Laycock et al. stressed the need for a well-established intestinal colonization microbiota for pigs, given the high representability of these animal models in early immune development studies (52): in pigs, there is no transfer of maternal immunoglobulin G *in utero* (53, 54) and a poorly developed mucosal system in neonates (55). Furthermore, pigs are genetically more similar to humans than mice (56), and their digestive physiology is comparable to ours (57). Colonization of germfree piglets with ASF members turned out to be largely unsuccessful, and only the most consistently colonizing ASF member (*Parabacteroides* sp.) was incorporated into the novel "Bristol" microbiota. Additional strains were selected based on their representation of the major phylogenetic groups in gut sections of 12- to 18-week-old pigs and either their ability to grow on a wide range of metabolic carbohydrate structures (*Roseburia intestinalis*) or their presence in unweaned pigs (*Clostridium glycolicum* and *Lactobacillus amylovorus*). Except for *R. intestinalis*, the novel microbiota successfully colonized the GIT after administration to germfree piglets, with high clinical safety and an expected increase in serum immunoglobulin levels (52). The Bristol microbiota was exploited by other researchers as a simplified starter microbiota to study additional effects of a complex microbiota on early-life microbiota development (58), the intestinal expression of a butyrate-sensing olfactory receptor (59), and the gastric transcriptome (60). Note that in the latter three studies, the piglets were not maintained in a sterile environment, hampering comparison of the effects of the Bristol microbiota on host parameters between studies. A different 10-membered porcine gut microbiota, originally designed as a competitive-exclusion culture for pigs, was used to investigate antibody repertoire development in ex-germfree newborn piglets (61). Another "de-

fined commensal microflora" (DMF) included seven porcine bacterial species and had a composition similar to that of the ASF. Species were originally isolated from the cecal contents of 6-week-old healthy pigs and administered to germfree pigs to evaluate the interactions between intestinal commensals, antibiotics, probiotics, and human rotavirus. This model was primarily applied as a model commensal gut microbiota of neonates (62, 63).

OTHER DEFINED COMMUNITIES *IN VIVO*

Apart from the defined communities as models for the normal (human) gut microbiome to study host-microbe interactions, other kinds of communities have been composed for application in gnotobiotic animals. These communities, however, are not listed in Tables 1 to 3, and their application goes beyond the scope of this review, as they did not aim to represent the normal microbiota. For instance, these communities include disease-specific consortia, e.g., IBD related (15, 64–67). Others are age-specific, such as the human baby microbiota (68–70), the DMF (62, 63), and a recently developed *Bifidum*-dominated model consortium (71). Finally, some communities were developed for therapeutic or probiotic purposes. A well-studied and globally marketed multispecies probiotic is the bacterial cocktail VSL#3, which was recently characterized at the genomic level and has been used to treat various gastrointestinal disorders (72–74). Other communities were designed to treat infections (among others, *C. difficile* infection [CDI] [75–77] and colitis [78]) or to facilitate recovery from cholera (79). Two remarkable applications of defined communities, which were not *per se* meant to model the normal human gut microbiota, are discussed in more detail below.

Therapeutic Communities

Although the concept is not new and was pioneered 30 years ago (75), the interest in fecal transplantations has recently increased, and the avenue of synthetic microbiotas as stool substitutes has been suggested (80). A particular example of such a stool substitute is microbial ecosystem therapeutic 1 (MET-1), designed as a synthetic stool mixture to treat recurrent CDI. Sixty-two species were recovered from the stool of a healthy 41-year-old female donor, of which 33 species were selected that were sensitive to a range of antimicrobials and were easy to culture. Two CDI patients that were "rePOOPulated" with MET-1 returned to their normal bowel pattern within a few days and remained symptom-free for at least 6 months. The use of a synthetic stool mixture has several advantages over conventional stool transplants: (i) the bacterial composition is known, controllable, and reproducible; (ii) a pure consortium is more stable than stool; (iii) the formulation is safe, owing to the lack of viruses and pathogens; and (iv) the administered organisms can be selected based on their sensitivity to antimicrobials, which further enhances safety (77). Some of these benefits also strengthen the use of defined communities in host-microbe interaction research. Notably, the application of MET-1 as a defined community in GF animals, instead of antibiotic-treated animals, was limited to one study, in which it was used as a healthy, *Firmicutes*-rich microbiota to study colitis susceptibility and host immune responses (81).

In contrast to the use of a defined synthetic community, the anaerobically cultivated human intestinal microflora (ACHIM) was derived from a fecal sample from a healthy Western donor that has been maintained in anaerobic culture for more than 20 years now and has been applied in fecal microbiota transplantation (82). Although the microbiota is regularly checked for the absence of pathogenic organisms and multiple CDI patients have been treated successfully with this cultured microbiota transplant from a single donor (82), its composition is not controllable.

Instead of starting with a certain disease or phenotype and generating a defined community to treat this condition, as is true for MET-1 and ACHIM, researchers recently tested different defined bacterial communities to generate various phenotypes in mice and to identify the strains responsible for the observed phenotypic variation. By administering GF mice one of 94 different, defined bacterial consortia of species randomly drawn from the culture collection, strains that modulated adiposity, intestinal

metabolite composition, and the immune system were identified. According to those authors, a similar approach could be applied to identify and characterize next-generation probiotics or combinations of pre- and probiotics (83).

Minimal Communities

Another category of defined communities is formed by minimal communities. Essentially, all defined microbial communities are minimal in the sense that they are not as complex as microbiota *in vivo*. Nonetheless, some studies exploited even-more-simplified defined consortia, i.e., with a limited amount of species or clearly lacking certain functions, to study host-microbe interactions in general. This is exemplified by biassociation studies involving single members of (dominant) phyla. In a recent study, GF mice were colonized with *B. thetaiotaomicron*, as a prominent member of the adult human gut microbiota, plus one of three probiotic strains (*B. longum*, *Bifidobacterium animalis*, or *Lactobacillus casei*) to study microbe-microbe and host-microbe interactions (84). In the same laboratory, gnotobiotic mice were colonized with bacteria from the two dominant phyla in the adult human distal gut microbiota: *Firmicutes* and *Bacteroidetes*. Based on their prominence in culture-independent surveys in the distal human gut, the pattern of representation of carbohydrate-active enzymes in their glycobiomes, and *E. rectale*'s ability to generate butyrate as a major end product of fermentation, a "marriage was arranged" between *E. rectale* and *B. thetaiotaomicron*. This reductionist approach provided information on microbe-microbe interactions, the microbial response to host diet, and the microbial effects on host physiology (e.g., the upregulation of the production of [mucin] glycans by the host) (85).

Despite the value of minimal communities for studying microbe-microbe and host-microbe interactions, a study of mice colonized with another simplified microbiota (*B. thetaiotaomicron* and *B. longum*) clearly demonstrated that the simple microbiota could not reconstitute the metabolomic complexity of a humanized microbiota, i.e., derived from human donors (86). Nevertheless, Tables 2 and 3 include some minimal communities, because of their representation of major phyla of the human gut microbiota or relevant application to the study of host parameters.

CRITICAL EVALUATION OF DEFINED COMMUNITIES *IN VIVO*: THE MICROBIOTA

In the sections above, we provide an objective description of defined microbial communities that have been applied in *in vivo* models to study host-microbe interactions. The next section discusses the representability of these communities, focusing on their design criteria and sources (murine versus human). Additionally, comparisons are made between simple versus complex and bottom-up- versus top-down-constructed communities. Suggestions for the future design of defined communities representing the normal intestinal microbiota are provided as well.

How Representative Are Defined Microbiota Models of a Normal Microbiota?

The development of defined communities representative of the human gut microbiota raises the issue, "What defines a normal microbiota?" Among the included studies that aimed to design a representative gut microbiota, different selection criteria were used. The representation of the major phyla and various metabolic capacities have been frequently put forward. A meta-analysis was performed to compare the composition of the core mouse gut microbiome (based on five different mouse models, i.e., varying in age, phenotype, and sampling site) with that of the human gut microbiome (based on 16 individuals) (87). Apart from the differences within the mouse microbiota, *Bacteroidetes* and *Firmicutes* were clearly the most dominant phyla in all samples (together 87% to 97%). (87) The same is true for the compositions of the well-established defined communities ASF, SIHUMI(x), and Oligo-MM^{12} (75% to 87.5%). Similar to most murine microbiotas included in the meta-analysis, however, the ASF and SIHUMI(x) lack *Verrucomicrobia*, which were found among the five most abundant phyla in human and some murine samples (87). In that sense, Oligo-MM^{12}, originally designed to represent the murine microbiota, is compositionally more complete than

SIHUMI(x), which was meant to represent the human microbiota. The frequently used ASF also lacks *Actinobacteria* and *Proteobacteria*, which are abundant in both murine and human samples (87–89). Similarly, a large part of the other defined communities discussed here (Tables 1 to 3) did not include representatives of all five major phyla of the human microbiota, with some not including even one of the two most prominent phyla. Note that species selection has been based mostly on the microbiota composition of Western individuals.

Furthermore, community design has been limited by the availability of genomes and cultivability of strains. In the case of the ASF, the number of species was limited for financial reasons, i.e., taking into account the monitoring costs. Nevertheless, this community has been frequently used in gnotobiotic animal models. The assumption that ASF mice can be regarded as conventional mice with respect to their gut microbiota has been criticized (23). Several functional activities in fecal materials from ASF mice were analyzed and compared to those in samples from GF and conventional rodents and other mammalian species, including humans. The five biomarkers investigated, the so-called Midtvedt criteria (i.e., conversion of cholesterol to coprostanol, conversion of bilirubin to urobilinogens, degradation of β-aspartylglycine, degradation of mucin, and absence of fecal tryptic activity [23]), are claimed to reflect host-bacterium interactions, independent of the intestinal localization of the bacteria involved and the kind of species. With regard to these criteria, fecal samples from ASF mice showed patterns more resembling GF rather than conventional mice (23), which complemented previous results demonstrating an abnormal microbiota in specific-pathogen-free (SPF) mice (90). Although this could be due to one of the limitations of the ASF, i.e., its low diversity, ASF mice were shown to be immunologically, reproductively, and metabolically similar to conventional mice (23). The Midtvedt criteria were also used to assess the suitability of SIHUMI(x) as a model microbiome. SIHUMI(x)-associated rats shared four criteria with conventional rats, three of which, however, were less pronounced (27).

A major difference between the ASF and a consortium such as SIHUMI(x) is the fact that the latter involves human-derived bacterial strains. Most members of recently developed communities, except for Oligo-MM, are of human origin as well. This may be obvious, given the fact that although their microbiotas are similar at the division (superkingdom) level, 85% of the microbial genera and species detected in mice are not found in humans (91). Although, qualitatively, humans and mice share a largely similar core, their intestinal microbiotas are quantitatively very different (87). On the other hand, the development of small intestinal immune maturation was found to be host specific, with humanized mice more closely resembling GF mice than mice associated with a murine microbiota (92). This host specificity might also, at least partially, explain the unsuccessful colonization of piglets with ASF (52). Additionally, humanized rodent models were claimed to have been utilized mainly for short-term biomedical research studies (14). Questions remain regarding how human-derived bacteria would adapt during long-term colonization and vertical transmission in murine hosts (14, 93, 94) and, thus, which kind of microbiota would be most reliable to study host-microbe interactions using murine hosts. The maximum colonization time reported in the studies discussed here (Tables 1 to 3) was less than 1 year. With respect to vertical transmission, stability after transfer to offspring has been addressed mainly for murine microbiotas only (ASF [95] and Oligo-MM [22]). Within the humanized defined communities, SIHUMI(x) is an exception, of which bacterial concentrations in the cecum were verified between founder rats as well as their offspring. At the age of 8 weeks, SIHUMIx-treated rats harbored similar bacterial levels as their founders but not at 2 weeks (except for *E. coli*) (27).

Simplified versus Complex Communities

The distinction between minimal communities, with two or three members, and larger defined communities is not black-and-white. For instance, the ASF, initially used as a microbiota to standardize mouse models, slowly adopted the role of a minimal

community instead of one representing the normal microbiota of mice. Nonetheless, the simplicity of a defined community also has some advantages over more-complex communities. The limited nature of the ASF should, as proposed by Brand et al., allow investigators to evaluate the *in vivo* effect of the removal or addition of bacterial species on mucosal homeostasis and colonization dynamics or, potentially, factorial interactions of the community (14). Indeed, some of the studies discussed here (Tables 1 to 3) used only a subset of the ASF species or added species to already established defined communities, including the ASF and SIHUMI(x). Additionally, one- and two-member communities could be applied to model aspects of a more complete microbiota, such as depletion of certain dietary compounds or metabolites (86). Finally, as discussed above, a simplified consortium makes each species traceable, as opposed to a very complex community (28, 40).

On the other hand, complex communities might more closely resemble the normal human gut microbiota and are more likely to confer colonization resistance to opportunistic pathogens, which has been a frequently mentioned criterion in the studies described above. In the 1980s, Freter and coworkers formulated the nutrient niche theory, which states that a certain bacterium can successfully colonize a host only if it is able to use a specific limiting nutrient more efficiently than its competitors (96). This implies that colonization resistance correlates with community complexity, as supported by several studies (22, 36, 97). Freter et al.'s theory was corroborated in a recent study in which the relative abundances of each species of a 10-membered community were correctly predicted based on the concentrations of individual dietary ingredients (41). The theory assumes, however, an environment in which bacterial growth is balanced and nutrients are perfectly mixed, whereas in reality, bacteria are metabolically flexible (i.e., they have the ability to switch nutrient sources), and nutrient levels in the gut are spatiotemporally heterogeneous (reviewed in references 98 and 99).

Metabolic flexibility was hardly addressed in the studies discussed in this review. Some researchers ensured the inclusion of species in a defined community that, as a whole, was able to thrive on a wide range of nutrients. Once established *in vivo*, however, the behavior of the community was addressed seldomly or only for a single species. This could be due to the fact that most of the included studies focused primarily on the effects of the whole microbiota or a subset of species on the host (host-microbe interactions) rather than the exact nutrient niche occupation by its separate species (microbe-microbe interactions). Exceptional is a recent study that quantified the *in vivo* responses of both mucin specialists (*A. muciniphila* and *Barnesiella intestinihominis*) and mucin generalists (*B. caccae* and *B. thetaiotaomicron*) upon fiber deprivation (43). A fiber-deficient diet stimulated the expansion and activity of the mucus-degrading bacteria, promoting epithelial access and pathogen-induced colitis (43).

With respect to spatiotemporal heterogeneity, Oligo-MM^{12} was used to verify that the bacterial compositions in the large intestinal outer mucus layer and the intestinal lumen are significantly different (26). Due to extensive mucus shedding and mixing in the lumen, however, the differences may be relatively small (98). Indeed, it was recently shown that, on a microscale level, the proximal colon should be viewed as a partially mixed bioreactor rather than a clearly compartmentalized gut section with spatially segregated communities. A next step would be to quantify the distribution of nutrients and metabolites and the role of host factors such as diet, gut motility, and mucus composition (48). Vice versa, it would be interesting to study the effects of spatial organization on relevant host parameters, which unfortunately were not addressed in the latter study. Those authors admitted that the 15-membered community used may not be complex enough to demonstrate stronger spatial associations with food particles, host cells, and mucus (48), reinforcing, all in all, the need for more-complex communities.

Both metabolic flexibility and spatiotemporal heterogeneity allow for increased community diversity, which is thought to be crucial for ecosystem robustness (98). Defined communities enable the precise investigation of both concepts, but on the

other hand, the question remains regarding whether they can be made sufficiently complex to properly address these issues.

Bottom-Up versus Top-Down Approaches

One way to obtain a more complex model community is to start with a complex sample, e.g., human stool, and narrow the number of species down via one or more enrichment steps, e.g., by culturing on selective media (top-down approach [100]) or using fermentation models. Tables 1 to 3 include only a few examples with regard to normal microbiota (Oligo-MM^{12} [22, 40]). The majority of the studies listed in Tables 1 to 3 used a bottom-up approach, in which single previously cultured and characterized strains are combined into a synthetic bacterial community, e.g., based on selection criteria mentioned above, and administered to germfree animals. An advantage of the latter method is the known composition of the microbiota, as emphasized above. A drawback, however, is formed by the risk that the desired phenotype (in this case a normalized host) cannot be entirely recapitulated (100).

Future Design

Probably a more important question is whether a normal microbiota actually exists. In the 1970s, Freter and Abrams concluded that significant fluctuations occur in the normal microbiota and that there is "no such a thing as a reproducible and precisely definable 'normal enteric flora'." Instead, they considered the F-strain collection most optimal for use as a microbiota representing a "state which is sometimes found in 'normal' individuals" (34). Clearly, the concept of the normal microbiota has changed over time and has evolved with the development of techniques to sequence the human gut microbiome, with increased insight into its composition, dynamics, and function. Recently, researchers aimed to draw the compositional functional core of the human gut microbiota, or the core microbiome. They emphasized that the gut microbiome should be considered a complex landscape, with both common and individual characteristics and alternative stable states with respect to composition, structure, and function (101). They listed a top set of 50 bacterial genus-like taxa that are part of the phylogenetic core, a common core of bacterial taxa shared by the majority of (adult Western) human individuals, based on data from previous studies (101–103). This core may include keystone species, whose roles are crucial for ecosystem structure and function, for instance, the breakdown of carbon sources to support the growth of other core members (104, 105). Mapping this core, including its keystone species, and comparing it with diseased microbiota could increase our understanding of a normal microbiota and facilitate the design of a defined community representative of a healthy human gut microbiota. Next to the phylogenetic core, increased insight into the minimal intestinal metagenome (106) and the active functional core (107) within the human gut ecosystem might provide new criteria for assessing the "normality" of a designed defined community. The paradigm seems to shift from rather black-box-like measures, such as the Midtvedt criteria, to actually understanding the function of the gut microbiota and the contribution of its individual species. Subsequently, this approach could allow a more thorough comprehension and more accurate design of age-, region-, and disease-specific defined communities.

Although this review primarily focuses on bacterial communities, it should be mentioned that the human (gut) microbiome also includes fungi, archaea, microeukaryotes, and many viruses, mainly bacteriophages. A study from 1980 included a "yeast fungus" in a defined hexaflora, but the specific role of this microbe was not addressed (108). One of the few studies in this area addressed the interaction between the murine host, an archaeon (*Methanobrevibacter smithii*), and a bacterium (*B. thetaiotaomicron*) (109). In addition, the same research group designed a gnotobiotic animal model with a simplified defined gut community to study phage-bacterial host dynamics (45). In parallel with the healthy gut microbiome, researchers recently mapped the healthy gut phageome (110), but this field is still in its infancy. It is reasonable to assume that with increasing insight into the role of nonbacterial gut

microbes in host-microbe interactions, the design of defined microbial communities becomes more representative of the whole human gut microbiome.

CRITICAL EVALUATION OF DEFINED COMMUNITIES *IN VIVO*: THE HOST

Next to the discussion of the exact composition of the defined microbial community, the selection of the host animal to study host-microbe interactions is critical. Rodents are the most commonly used mammalian models in which defined communities have been applied. The suitability of rodents as models for the human host was extensively reviewed elsewhere (10) and goes beyond the scope of this review. In summary, murine intestines are anatomically, histologically, and physiologically very similar to human intestines, but sizes, metabolic rates, and dietary habits differ largely, leading to qualitative and quantitative differences in microbial composition (10). With respect to the gnotobiotic models discussed in this review, there are some additional discrepancies to be mentioned. The high value of using gnotobiotic animals as models of humans, i.e., their known composition and controllability, seems to be weakened by poor control of host parameters known to influence the human gut microbiome, such as diet, genotype, sex, part of the gut studied, age, and immune system.

Host Parameters Influencing the Microbiota

Diet is a complex and strong determinant of gut microbiota composition (reviewed in references 111 and 112). The individual species levels of a 10-membered defined community in mice fed diets systematically varying in protein, fat, polysaccharide, and simple sugar contents were assessed in order to develop a model to predict the variation in species abundance. Next, the model was validated with 48 random combinations and concentrations of four ingredients selected from a set of eight human baby foods. Approximately 60% of the variation in species abundance could be explained by the known concentrations of pureed foods (41). This study exemplified the application of defined communities to systematically assess the response of individual gut members to various food components, which are, moreover, typical of the human diet. Clearly, a standardized diet of a laboratory animal is different from that of humans, which varies per region, season, individual taste, and even day. Some studies listed in Tables 1 to 3 incorporated a previously developed prototypic "Western-style" diet (27, 32, 39, 42, 46, 85), containing large amounts of saturated and unsaturated fats and carbohydrates commonly used as human food additives (i.e. sucrose, maltodextrin, and/or cornstarch). A lack of standardization in laboratory animal feeding protocols, however, was emphasized previously, for instance, with respect to diet composition and texture (113), and indeed, diets used by studies discussed here are highly variable (Tables 1 to 3). Moreover, in ~40% of the studies, the diet was not clearly defined or was not even reported, which is alarming given the large impact of diet on the gut microbiome.

The choice of mouse genotype also varied per study (Tables 1 to 3), although an effect of host genotype on microbiota composition was established within species (114–118). These results were corroborated by studies with defined communities such as ASF (119) and SIHUMI(x) (64). Additionally, colonization of different mouse strains with SIHUMI(x) demonstrated host-specific cecal levels of polyamines and SCFAs (31). In mice associated with *B. longum* and *B. thetaiotaomicron*, host genetic background was found to affect the overall transcriptome of the latter bacterium but not the expansion of the bacterial substrate range of this bacterium (84). Obviously, defined communities allow the careful investigation of such host-dependent effects, but validation of host-microbe interactions in a wide range of host strains seems crucial before drawing of conclusions and extrapolation to humans.

Although reports on the effect of gender have been contradictive (106, 117, 120–124), it might be a crucial determinant in gut microbiota composition and/or behavior. In turn, the commensal microbiota was shown to affect sex hormone levels (125, 126). Sex differences in gut microbiota composition were recently comprehensively investigated in 89 common inbred mouse strains. After excluding confounding

by host genetics, diet, age, or cage effects, the researchers detected gender-specific differences in taxon abundances and diet responses. These differences could be partially explained by sex hormones (127). Among the studies discussed here (Tables 1 to 3), one reported differences in metabolic profiles in urine and plasma between both sexes, but no explanation was put forward (39). In an older study, male mice were found to be more susceptible to death after *C. botulinum* infection, which could be explained by their coprophagic behavior or a more general higher susceptibility to disease (36). In contrast, other studies reported an absence of gender-specific effects on, for instance, levels of *Oxalobacter formigenes* colonizing ASF mice (20) or the assembly of a synthetic microbiota (43). Whereas some studies discussed here (Tables 1 to 3) reported the use of a mixed-gender population, others included only one gender ($n = 12$ of 53 studies), in which male animals were more often used than female animals (nine versus three). Remarkably, the establishment of SIHUMI(x) was verified in both genders, whereas the effect of dietary fiber was tested in male rats, and the effect of a high-fat diet was investigated in female rats (27). A similar discrepancy was found in a study that assessed the effects of five fermented milk product strains on human female twins but male gnotobiotic animals. Although microbiota responses were more or less similar in both species (40), such a gender mismatch may complicate translation. Finally, not all studies clearly reported the gender used per experiment, and approximately half of the studies did not report animal gender at all. This too may hinder data reproduction and, more importantly, translation.

Defined communities allow the quantitative comparison of microbial compositions along the GIT, within and between models. ASF-associated mice were used to quantitatively demonstrate that the microbiota of the colon is poorly reflected in fecal samples (95). Relative abundances of species were also different between feces (rectal swabs) and colon in pigs colonized with a defined microbiota (63). In rats colonized with SIHUMI(x), however, bacterial concentrations in the cecum, colon, and feces were similar (27). Additionally, increases in relative abundances of mucin-degrading bacteria in the cecum and colon upon switching to a fiber-free diet were reflected in feces (43). In a mouse model associated with a 12-membered community, individual bacterial levels were also similar between feces and cecum (46). These conflicting results could be explained by various factors, including host, community composition, and sampling time. Irrespective of the actual difference between GIT sites, it is disappointing that some other studies relied solely on fecal bacterial content. In a study applying a 92-membered community, for instance, not even half of the members could be detected in feces. Other species may have established themselves in different regions of the gut, but this was beyond the scope of that paper (51). Nevertheless, due the invasiveness of sampling, systematic studies comparing colonic and fecal bacterial contents are lacking in humans as well (99, 112). The variation in GIT sites looked at by the studies included in Tables 1 to 3 makes it difficult to compare the colonization pattern of the defined communities to that of natural colonization. Apart from differences along the GIT, capturing the transversal heterogeneity within one compartment may be crucial for properly modeling and understanding host-microbe interactions, as discussed above.

The age at which animals are colonized was quite variable among the studies, including animals bred with the desired defined community as opposed to GF animals colonized with the community of interest to create a gnotobiotic animal model. In the latter case, animals are inoculated at various time points among studies, whereas the timing of microbial colonization was demonstrated to impact, among others, immune maturation (128, 129), mucosal homeostasis (130), and gut-brain axis communication in mice (131). Moreover, as discussed above, colonization times of animals in studies discussed here (Tables 1 to 3) were limited. Nevertheless, some studies confirmed the stability of their defined community of interest over time and even over generations, which should be sufficient to draw conclusions within a specific colonization time window. This, however, does not allow one to infer any information on the long-term effects of colonization.

TABLE 4 Advantages and pitfalls of gnotobiotic animal models in comparison with human research, with respect to the factors influencing intestinal microbiota composition or behavior[a]

Factor	Advantage(s) (vs human research)	Pitfalls in practice
Inoculum (defined community)	Controllable composition (healthy vs diseased microbiota [e.g., missing keystone species], human vs animal derived)	Animal microbiome ≠ human microbiome; difficulties in defining a healthy or normal microbiota; host-specific selection of microbiota
Diet	Controllable composition, timing, amt (tailored to human diet [region, age, and season, etc.])	Lack of standardization in laboratory animal feeding protocols; not always reported[b]
Host genotype	Controllable; genetic changes possible (ability to introduce disease)	Validation of host-microbe interactions in multiple strains needed before extrapolation to humans; animal genotype ≠ human genotype
Sex	Controllable	Only one gender investigated[b]; not always reported[b]
Part of the gut	Ability to measure bacterial levels in virtually all intestinal parts; ability to capture transversal heterogeneity	Anatomy and physiology different from humans; variations in relative abundances per gut region different per model[b]; focus on specific gut regions or feces only[b]
Colonization time	Controllable	Long-term effects not studied[b]; animals not always colonized starting at birth[b]; stability over generations not always confirmed[b]
Immune system	Controllable at start/birth	Uncontrollable in long-term studies, especially locally; complex, determined by internal and external factors; not quantified or quantifiable[b]

[a]Based on studies listed in Tables 1 to 3 and the literature.
[b]See Tables 1 to 3.

A last factor determining gut microbiota composition and behavior is the immune system, which in turn is influenced by, among others, the above-mentioned factors and the gut microbiota itself. Looking at the studies discussed here (Tables 1 to 3), several researchers investigated immunological parameters such as serum immunoglobulin levels and the presence of (subsets of) immunological cells in the gut. Nevertheless, due to the complexity of the immune system, it is difficult to quantify and compare the model hosts used with respect to immunological parameters. The key findings on the interactions of gut microbiota members and their products with the immune system have recently been reviewed elsewhere (100). Those authors emphasized the value of minimal microbiomes and subsequent standardized (animal) models. Determining the effects of a specific gut microbiota on the host could help to identify host-microbe interactions that shape the immune system (100). Most studies discussed in this review did not make a distinction between the contributions of each specific microbe to immunological effects observed.

The advantages and the levels of controllability of gnotobiotic research, as well as its pitfalls in practice, as outlined above, are summarized in Table 4.

Validation of *In Vivo* Models

As emphasized above, differences exist between humans and animals, not only limited to their intestinal microbiota. In line with the question of what defines a normal or healthy intestinal microbiota, one could ask, "When is the animal model sufficiently representative of the human situation?" With regard to the studies discussed here (Tables 1 to 3), diverse host criteria are applied. For the models exploiting a murine microbiota, validation is relatively easy. Most researchers aimed to normalize GF hosts to conventionally raised animals, thereby focusing on host parameters such as cecal size or weight (9, 22, 33, 34). With respect to humanized mice, validation is more complicated, but some studies made an effort. For instance, total bacterial numbers in feces and fecal SCFA levels between humans and SIHUMI(x)-associated rats were evaluated, and a previously reported increase in the abundance of *Erysipelotrichaceae* upon a high-fat diet in humans was mirrored in SIHUMI(x)-associated animals (27). Other host parameters (e.g., immune system or other systemic parameters), however, were not taken into account. Similarly, validation was lacking in other studies applying SIHUMI(x), in which, moreover, mice were used instead of rats (28, 30–32).

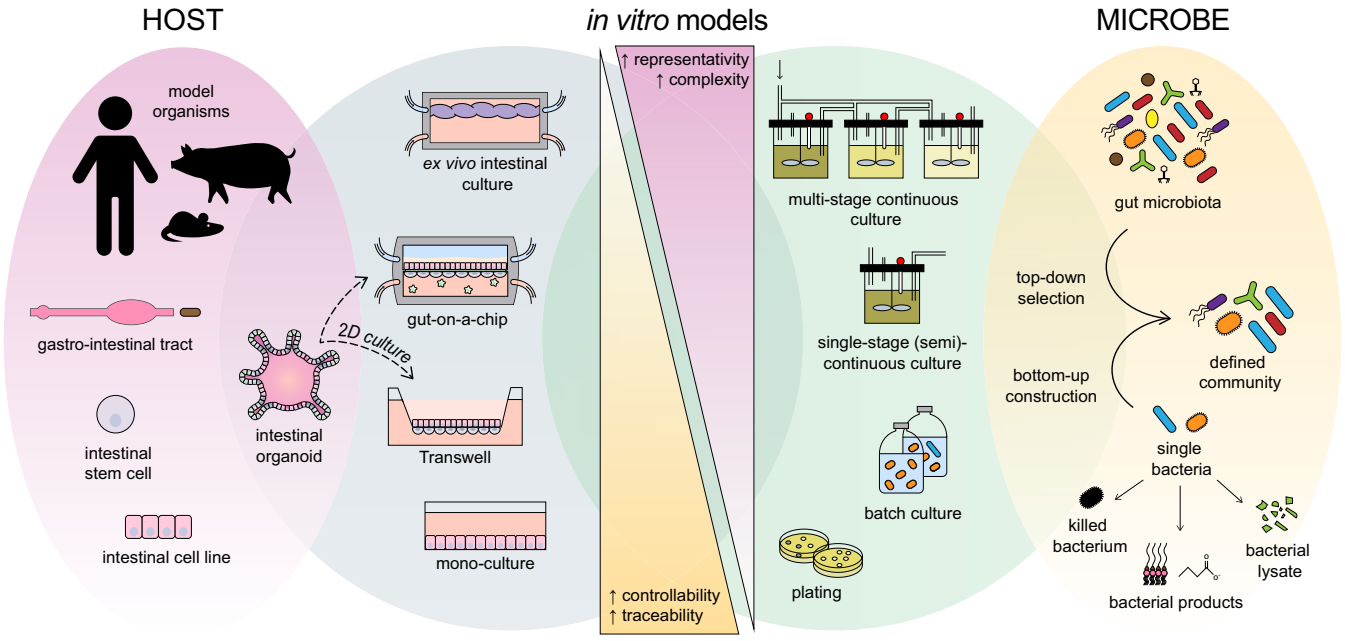

FIG 1 *In vitro* models of the human gut and gut microbiota. Models are organized from bottom to top, with the most representative and complex at the top and the most controllable and traceable, with respect to host parameters or microbial species, at the bottom.

A better example was recently described in a study in which the effect of a fermented milk product on both humans and gnotobiotic mice humanized with a 15-membered microbiota was tested. The proportional representation of the intestinal bacterial species and genes and metabolic changes upon the introduction of the probiotic strains were hardly different between mice and humans, but the researchers also acknowledged the limitations of their gnotobiotic animal model with respect to translatability (40). In most other studies (Tables 1 to 3), control groups were limited to conventionally raised and GF animals or animals with a control treatment, for which translatability of the results to the human situation remains speculation.

DEFINED COMMUNITIES *IN VITRO*

As opposed to *in vivo* models, the use of defined communities to study host-microbe interactions *in vitro* has been limited so far, although the development of sophisticated *in vitro* model systems is advancing rapidly. In this section, we discuss *in vitro* models in which defined communities have been applied or could be applied to study host-microbe interactions. A distinction is made between models focused on the microbiota (e.g., composition and characteristics) and those that were designed to realistically represent the human host *in vitro*. Figure 1 summarizes all existing *in vitro* models of the human host and microbiota, illustrating how their interactions can be studied by combining advanced *in vitro* cell-based systems with defined communities. Ultimately, the goal is to combine the best of both worlds.

Modeling the Intestinal Microbiota *In Vitro*

The use of fermentation models has proven successful in modeling the intestinal microbiota *in vitro*, ranging from short-term batch incubations to multicompartmental continuous systems. As discussed above, most defined communities applied *in vivo* (Tables 1 to 3) were constructed bottom-up, by selecting species based on their function, prevalence, or other criteria. Alternatively, communities can be composed top-down by inoculating GIT-mimicking chemostats with human feces. Well-known examples of these chemostats, such as the MacFarlane-Gibson three-stage continuous-culture system, (M-)SHIME [(mucosal) Simulator of the Human Intestinal Microbial Ecosystem], EnteroMix, the Lacroix model, and TIM-2 (TNO Intestinal Model 2) have

been extensively reviewed elsewhere (132–134). The high reproducibility, stability, and complexity of bacterial communities cultured in chemostats (135, 136) have allowed the development and application of representative communities of the human intestinal microbiota *in vitro*. Most of these models, however, did not include a host component. The host-microbiota interaction (HMI) module comprised a promising exception in which feces from a healthy volunteer were first fed into an adapted SHIME system, with fluid compartments mimicking the stomach, small intestine, and ascending colon. Subsequently, the SHIME effluent was exposed to an artificial mucus layer, separated by a semipermeable membrane from a compartment containing Caco-2 cells. This module allowed the coculture of bacteria with enterocytes for up to 48 h (137), which is discussed in more detail below.

Modeling the Host *In Vitro*

With respect to well-established defined communities, the probiotic cocktail VSL#3 and the fecal transplant substitute MET-1 have been tested on various human or animal intestinal cell lines (Caco-2, T84, and HT-29) (e.g., see references 138–140). In most studies, however, the use of bacterial lysates or conditioned media was preferred over live bacteria (e.g., see references 72 and 141–144), because the (mainly anaerobic) gut bacteria cannot survive under the aerobic conditions needed for intestinal cell culture. In these two-dimensional (2D) models, the interaction with the immune system or other tissues cannot be studied. Although the direct effect of VSL#3 was tested on spleen and dendritic cells (145, 146), tissue-tissue interactions were lacking in these models. This problem can be (partially) solved in transwell coculture models, in which bacteria, mucosal immune cells, and intestinal epithelial cells can be studied together (147). A transwell model with an apical anaerobic compartment enabled the coculture of an anaerobe bacterium with an intestinal cell line to study host-microbe interactions (148). Still, these cell lines lacked their tissue-specific context, including all major types of epithelial cells (e.g., goblet cells, enterocytes, enteroendocrine cells, and Paneth cells) organized in crypts and villi. Moreover, as cell lines are tumor-derived, their epithelial characteristics are affected. These issues have been overcome by the development of gut organoids, self-organizing three-dimensional (3D) epithelial structures derived from intestinal stem cells (149) or human pluripotent stem cells (150). The use of organoids to study host-microbe interactions was reviewed elsewhere (151). The closed structure of organoids, in which the lumen is sealed with epithelial cells and a mucus layer, may facilitate the establishment of hypoxia in the core lumen (151). The anaerobic pathogen *C. difficile* survived for up to 12 h within organoids, but luminal oxygen levels still ranged from 5% to 15%, which may be tolerated by specific strains of *C. difficile* only (152). More recently, researchers developed an organ culture system for the mouse intestine, in which the stromal and hematopoietic components of the normal intestine were preserved *ex vivo*. The device supported the survival and growth of both anaerobic and aerobic microbiotas, allowing the investigation of their effects on neuronal parameters (153).

Coculture of defined microbial communities with human cells in transwells, organoids, or organ culture systems has been limited, probably owing to the static nature of these models. More-advanced *in vitro* models to study host-microbe interactions have been developed (as recently reviewed in reference 133), only a few of which have hitherto allowed the coculture of multiple bacteria with intestinal cells or cell lines.

Organ-on-a-chip technology is an emerging concept within biomedical research, to replace conventional cell culture and animal testing. Organ-on-a-chip devices are microfluidic devices in which cells are cultured with organ-relevant spatiotemporal chemical gradients and dynamic mechanical cues, thereby aiming to reconstitute the structural tissue arrangements and functional complexity of living organs *in vitro* (154). Several gut-on-a-chip devices have already been developed (155–158), only one in which multiple intestinal bacteria were successfully cultured (158). In this device, two channels simulating the gut lumen and a blood vessel are separated by a membrane coated with extracellular matrix and Caco-2 cells (158). As opposed to cell monolayers

and organoids, the gut-on-a-chip is a dynamic model: shear stress and gut peristalsis are mimicked by continuous medium flow and stretching/relaxing of the membrane, respectively. Interestingly, these environmental cues stimulated Caco-2 cells to undergo differentiation into four types of intestinal epithelial cells, organized in 3D villus-like structures (159). Also, the successful incorporation of endothelial cells and peripheral blood mononuclear cells was demonstrated (160). The authors of that study claimed the successful cultivation of a single bacterium "on chip" (*Lactobacillus rhamnosus*) for more than 1 week (158) and the eight-membered VSL#3 for at least 96 h (160). However, the viability of the probiotic bacteria was based solely on imaging, and which species exactly succeeded in "colonizing" the crypts was not exactly determined. The growth of anaerobic bacteria in this device has not yet been reported.

In contrast, another recent study reported the successful coculture of the strictly anaerobic bacterium *B. caccae* with *L. rhamnosus* and Caco-2 cells. In this microfluidic-based model mimicking the human gut, HuMiX, bacteria were grown in a separate, anoxic compartment (161). Similarly, the HMI module allowed the investigation of bacteria for up to 48 h under microaerophilic conditions. Fluorescence *in situ* hybridization (FISH) analysis revealed the presence of strictly anaerobic bifidobacteria in the upper part of the mucus layer and the positioning of *Faecalibacterium prausnitzii* at the oxic-anoxic interphase (137). In both the HuMiX and HMI modules, however, a mucin-coated attachment membrane prevented direct or natural contact between host and microbe. Moreover, as opposed to the gut-on-a-chip, gut peristalsis was not mimicked, and the formation of the main epithelial cell types or crypts was not reported in these models (137, 161).

A promising development in gut-on-a-chip technology is the incorporation of 2D organoids, which grow in a plane rather than in clumps, in the chip device (156), combining the advantages of organoids (tissue differentiation) with those of gut-on-a-chip technology (controllable flow, mechanical cues, and tissue-tissue interaction). To date, the cultivation of a defined intestinal microbiota in this device has not yet been reported.

Validation of *In Vitro* Models

In comparison with animal models, validation of *in vitro* models is even more challenging. The cellular processes studied in transwells, organoids, or gut-on-a-chip devices cannot be readily validated in human subjects. On the other hand, however, such sophisticated *in vitro* models enable the investigation of processes that cannot be readily studied in humans, increasing our understanding of the molecular mechanisms of certain bacterial compounds or products. Furthermore, they allow the elimination of potentially confounding factors present in *in vivo* models, such as the immune system. At the same time, this is also one of the major drawbacks of the above-mentioned *in vitro* models: as opposed to *in vivo* models, they lack a systemic component, whereas the impact of the gut microbiota on human health extends beyond the GIT. The emergence of organ-on-a-chip technologies has led to the concept of a "human-on-a-chip" (162), but its implementation in research is still at an early stage. Nevertheless, the road to such a human-on-a-chip may be just as interesting. "Rebuilding" the human body through assembly of its separate parts (lung-on-a-chip, gut-on-a-chip, and kidney-on-a-chip, etc.) might increase our understanding of these building blocks and their contribution to the whole.

CONCLUSIONS AND FUTURE OUTLOOK

Our understanding of the human gut microbiome has rapidly grown over the past decades, which has definitely supported the design of defined communities representative of the human gut microbiome. Whereas defined communities were initially aimed at normalizing germfree hosts to conventionalized mice, they could be a valuable tool to study host-microbe interactions, because of their controllability and traceability. For the same reasons, defined communities have a high potential for therapeutic application. In this review, however, we show that these rationally designed

consortia have been applied in *in vivo* models that are not entirely representative of the human host environment. Next to the obvious and frequently discussed differences between mice and humans, we also discuss that the power of gnotobiotic animals has been further undermined by poor control of the host parameters known to affect gut microbiota composition and behavior.

Simultaneously with the increasing knowledge on the human gut microbiota, the implementation of more-advanced *in vitro* models of the human gut is accelerating, with the development of stem-cell-derived organoids and gut-on-a-chip approaches. Although the research is still in its infancy, these systems might partially replace the use of animal models. This development is beneficial not only for ethical and, in the long term, financial reasons but also from a scientific perspective. Human-inspired *in vitro* systems allow us to model and capture host-microbe interactions at a more fundamental and controlled level.

Both the design of defined communities and *in vitro* models of the gut have not yet reached their plateau. The former can be improved, via either bottom-up or top-down approaches. Key is further expanding our knowledge about the intestinal microbiome in health and disease, in which the NIH Human Microbiome Project and the European MetaHit project have played a crucial role (106, 163) (bottom-up). The characterization of the gut microbiota and genome sequences facilitates the *in silico* prediction of host-microbe interactions through constraint-based genome-scale metabolic modeling (164) or other types of mathematical modeling (165) and, subsequently, the *in silico* design of representative defined communities (bottom-up). Further exploring our whole microbiome, including phages, fungi, and archaea, will revolutionize the design of microbial communities as well (bottom-up). Finally, the increased ability to reproducibly culture the microorganisms in human feces *in vitro* using well-established fermentation technologies (135, 166) may open the avenue to study human feces-derived, functionally enriched defined communities at a more personalized level (top-down). In this way, both health- and disease-related microbiotas can be easily reproduced. The same level of personalization can be obtained on the host side. For instance, the implementation of 2D organoids from patient-derived induced pluripotent stem cells in *in vitro* systems, such as the gut-on-a-chip, can lead to highly personalized screening devices.

All in all, these models will provide a basis for the rational development and screening of novel therapies targeting intestinal diseases, ranging from anti-, pre-, and probiotics to manipulate existing gut microbiota to therapeutic microbes (167), fecal microbiota transplantation (168), and stool substitutes (77).

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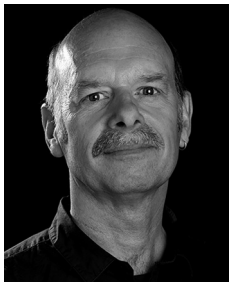

John van der Oost (1958) has been leader of the Bacterial Genetics group in the Laboratory of Microbiology at Wageningen University since 1995. Initially, research mainly focused on unraveling unique features of central metabolic pathways in bacteria and archaea, revealing many novel enzymes and their regulation. In 2005, he was appointed Full Professor; in 2013, he was elected as an EMBO member; and in 2017, he was elected as a member of the Royal Dutch Academy for Arts and Sciences (KNAW). In the last decade, he used NWO grants (VICI-2005, TOP-2010/2015, and Gravitation-2017) to establish a successful research line on prokaryotic antiviral defense systems (CRISPR-Cas and prokaryotic Argonaute). This has provided an excellent basis for development of unprecedented genome-editing tools that currently find applications in biotechnology and molecular medicine (gene therapy).

Willem M. de Vos studied Biochemistry and obtained a Ph.D. at Groningen University, The Netherlands, partly done at the Max Planck Institute for Molecular Genetics in Berlin, Germany. Subsequently, he spent a postdoc in Reading, United Kingdom, and became Molecular Genetics Group manager at NIZO, Ede, The Netherlands. For over 30 years he has been a Professor at Wageningen University, The Netherlands, where he holds the Chair of Microbiology, and he serves as Professor of Human Microbiomics at the Medical Faculty of the University of Helsinki, Finland, where he chairs the Research Program Human Microbiome. His research aims to understand and exploit microbes using molecular, (meta)genomics, and systems approaches. For a dozen years, his research interest has been focused on the human intestinal tract microbiota and its relation with health and disease.

Hauke Smidt studied Biotechnology at the Technical University of Braunschweig, Germany, and obtained his Ph.D. from Wageningen University. Following a postdoc position at the University of Washington, Seattle, WA, he rejoined the Laboratory of Microbiology at Wageningen University to head the Molecular Microbial Ecology group. In 2008, he was appointed Visiting Professor at Nanjing Agricultural University, and since 2010, he has held a Personal Chair in Complex Microbial Ecosystems at Wageningen University. His research focuses on the integrated application of innovative cultivation and functional genomics-based methods to study the composition and activity of intestinal tract microbiota in humans and farm and model animals, as well as their interaction with their host, in relation to host nutrition and health. Further interest lies in the evolution and spread of antibiotic-resistant bacteria and their genes, following a OneHealth philosophy that links environmental, human, and animal health.