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Microbiological models of laboratory rodents

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1. Introduction

The metagenome, the sum of the host genome and the microbiome genes, is one of the drivers of the mammalian phenotype (Stappenbeck and Virgin 2016). In recent years, rodent microbiological models increased considerably in number and complexity, making a choice between the existing models difficult. We give here an overview of the animal microbiological models currently in use and provide some notes on the special features of animal husbandry, hygienic monitoring, and use of the models. In the following, we present basic aspects starting with the “simplified” synthetic microbiota models like **gnotobiotic animal models** (germ-free and defined associated flora models) to more “complex” natural microbiota models such as **specified pathogen-free (SPF) models,** and **wild-mouse microbiota-colonized mouse models (wildlings).** Finally, **conventional microbiological models** are included for the sake of completeness.

2. Gnotobiotic animal models

Background: The term “gnotobiotic” is derived from the greek word “gnostos” (well known) and “bios” (life), describing organisms with a fully known microbial status. The following categories of gnotobiotic laboratory mouse models are currently in use (Bolsega et al. 2021):

- **germ-free animals**, which are devoid of all living microorganisms
- **defined microbiota-associated animals** that are associated with single or multiple known microbial species of animal or human (humanized microbiological models) origin

  examples of gnotobiotic animals associated with an animal-derived microbiota:

  - Schaedler flora (Schaedler et al. 1965)
  - altered Schaedler flora (ASF; Dewhirst et al. 1999; Stehr et al. 2009)
  - minimal 15-member mouse gut microbiota (GM15; Elie et al. 2020)
  - Oligo-Mouse-Microbiota 12 (OMM12) and community variations (Afrizal et al. 2022; Eberl et al. 2021; Li et al. 2015)

  examples of gnotobiotic animals associated with a human-derived microbiota:

  - simplified human intestinal microbiota (SIHUMI+SIHUMIx; Becker et al. 2011; Weitkunat et al. 2015)
  - simplified intestinal microbiota consortium (SIM; Kovatcheva-Datchary et al. 2019)
  - Microbial Ecosystem Therapeutic (MET-1; Martz et al., 2015)
  - complex synthetic human consortium (hCom2; Cheng et al., 2022)

Housing and monitoring: as any additional organism can misbalance these models, rodents are housed in controlled sterile systems only, such as isolators or equivalents, in order to avoid any external contamination. Isolators are optimal for long-term housing of the gnotobiotic rodent lines such as breeding or durable experiments. Due to high demand for availability of gnotobiotic animals, additional bioexclusion systems were developed within the last decade. These airtight cages function as isolator at a cage level and are optimal for short-term housing of gnotobiotic animals such as colonization or infection experiments (Basic et al. 2021). The objectives of the hygienic monitoring are to confirm the maintenance of the germ-free or the defined microbiota associated status (Nicklas et al. 2015). The major contaminants in gnotobiotic animals housed in isolators or in microisolator cages are spore-forming bacteria and fungi from the environment, human skin commensals and spore-forming bacteria from

defined bacterial communities used in experiments. The infections with common rodent pathogens pose a very low contamination risk in gnotobiotic animals (Basic et al. 2021, Bolsega et al. 2021).

Use: these models are mainly used to address cause-effect relationship between the host and intestinal microbiota in infectious, inflammatory, and metabolic diseases by reducing the microbiome complexity on a manageable level (Bolsega et al. 2021; Thomson et al. 2022). For example, several animal models of gut inflammation fail to develop inflammation when housed in germ-free conditions (Madsen et al. 1999; Schaubeck et al. 2016; Wahida et al. 2021). Studies in models with defined microbial composition support investigations of *in vivo* effects of a specific microorganism or community in the development of specific host phenotypes (Bolsega et al. 2019; Eberl et al. 2021; Herp et al. 2019; Streidl et al. 2021).

3. Specified pathogen-free (SPF) animal models

Background: SPF animals are free of certain listed microorganisms that must be individually defined (specified). The microbial status of SPF animals is not entirely known; only the absence or presence of named pathogens is defined. Historically, many of the established SPF colonies started from gnotobiotic animals, which were associated with defined microbiota such as the ASF (Dewhirst et al. 1999; Stehr et al. 2009), but with time accumulated further microorganisms.

Housing and monitoring: housing of SPF animals is possible in nearly all housing systems. SPF rodents are usually housed in hygienic barrier units, either in microisolator cages (IVCs, filter-top cages) or in conventional open-top cages. The objective of hygienic monitoring for this kind of models is to examine whether the specified pathogenic microorganisms are absent in the respective hygiene unit (Mähler et al. 2014; Buchheister and Bleich 2021). The methodology used for monitoring depends on the housing form and is subject of other recommendations of the hygiene committee (GV-SOLAS Committee for Hygiene 2010).

Use: currently, SPF animals are the most widely used rodent microbiological models. Since SPF laboratory rodents do not contain certain (specified) pathogens, they are usually protected from clinical or subclinical infections that can affect their health and research outcomes. Nevertheless, due to the very simplified microbiota SPF animals often show alterations in biochemical gut parameters (Norin and Midtvedt 2010), immunological (Rosshart et al. 2017) or anti-cancer fitness-promoting traits (Zitvogel et al. 2018), thus being inappropriate for particular studies.

3.1. SPF animals carrying pathobionts

Background: in recent years, it has been documented that the presence of particular microorganisms (pathobionts) in SPF mice impacts the phenotypes of multiple animal research models due to their pro- or anti-inflammatory potentials (Hansen et al. 2019). The impact is complex and most likely driven by several microorganisms in conjunction. The best-known bacteria that are essential for the induction of specific rodent model phenotypes are Alistipes spp., *Akkermansia (A.) muciniphila*, *Bifidobacterium* spp., *Bacteroides fragilis*, *Bacteroides vulgatus, Faecalibacterium (F.) prausnitzii, Prevotella copri* and segmented filamentous bacteria (SFB) (Hansen et al., 2019).

Housing and monitoring: housing of such animal models is conducted similar to SPF animals. In addition to examining the SPF status, supplementary tests for the detection of particular pathobionts by direct methods such as PCR (Benga et al. 2019) or in the context of whole gut microbiota analysis (Lupini et al. 2022) are indicated to warrant the success of particular experimental models.

Use: Depending on the research projects, individual study confounders should be controlled. For example, an anti-inflammatory potential attributed to *A. muciniphila* is linked to reduced incidence or severity of disease, in murine models for type 1 diabetes and diet-induced obesity (Everard et al. 2013; Hanninen et al. 2018; Hansen et al. 2012). *F. prausnitzii* reduces the severity of various models of IBD in mice, and it may be a key target in IBD intervention studies (Carlsson et al. 2013). It is therefore important to define the status of *A. muciniphila* and *F. prausnitzii* respectively in animal research projects, which interfere with these microorganisms.

4. Wild-mouse microbiota models (wildlings)

Background: to overcome some of the disadvantages of SPF animals, in particular regarding immunological studies, laboratory mouse microbiological models, containing more complex microbiota, such as pet-shop mice (Dammann et al. 2011; Zhang et al. 2021), wild mice (Viney 2019) or laboratory mice colonized with wild mouse microbiota (wildlings), were recently established (Rosshart et al. 2017; 2019). The latter were obtained by the implantation of embryos of inbred mouse strains (such as C57BL/6) into wild mouse recipient mothers, resulting in inbred strains possessing wild mouse microbiota (wildlings) (Rosshart et al. 2017). Wildlings differ significantly from SPF laboratory mice with regard to their bacterial microbiome, their gut mycobiome and virome, and their pathogen profile (Rosshart et al. 2019). The exclusion of singular pathogens, such as zoonotic infectious agents, from the wildlings' microbiota does not necessarily influence their microbiota-associated phenotypes (Rosshart et al. 2019).

Housing and monitoring: Wildlings should be housed under strict hygiene constraints. Due to the risk of contamination of laboratory rodent SPF colonies, mice containing wild-type microbiota should be housed in a separate facility with separate personnel, supply, and waste management. Moreover, isolator or at least IVC housing should be implied from a biohazard perspective. Although the wildling microbiota seem to be resilient (Rosshart et al. 2019), periodic recordings of the pathogenic and commensal microbiota profiles are recommended in order to document possible changes in the composition and diversity. In addition to examinations for the presence of pathogens, as is usually done for monitoring SPF animals (Mähler et al. 2014), periodical gut microbiome analysis should document whether the complexity of the microbiota remains unaffected in laboratory settings.

Use: Wildlings closely mirror the wild mouse immune phenotype in spleen and blood, and therefore increase the translatability of immunological results to humans (Rosshart et al. 2019). The wildling model combines resilient natural microbiota and pathogens at all body sites with the tractable genetics of inbreed laboratory strains and thus profits on the wide-ranging effects of natural wild microbiota on host physiology. Such models may enhance the validity and reproducibility of biomedical studies among research institutes and facilitate the discovery of disease mechanisms and treatments that cannot be studied in regular (SPF) laboratory mice (Rosshart et al. 2017; 2019).

5. Conventional microbiological models

Background: these microbiological models refer to animals with a completely unknown microbiological status. They are examined neither for the presence of pathogens nor for their microbiome; therefore, their experimental use is currently not recommended. The term "conventional" in relation to a conventional hygiene status is often confused with conventional open cage housing, which only refers to the housing and not to the microbiological status. The microbiological status of this model must be always considered as uncertain.

6. Summary

Nowadays, a large variety of rodent microbiological models with diverse complexity grades of the microbiota is available for experimental approaches. As such, the choice of the appropriate microbiological model is challenging. Considering the fact that the microbiota can have a massive impact on experimental results and therewith affects research outcome, the microbiological models should always be carefully chosen in order to achieve the research aim (Buchheister and Bleich 2021).

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