Methanogenic Archaea in Humans and Other Vertebrates

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Contents

Abstract The presence of methane in biological samples had been detected many years ago and it was believed that the gas could be either of chemical or microbial origin. Detection of methane-producing microbes (methanogens) in samples from animals intensified since the last part of the previous century, going from cultural-physiological characterization and isolation of microbes to further characterization of the isolates at the biochemical, immunological, molecular biologic-genetic, and phylogenetic levels. In this Chapter, we report about methanogens identified at least at the genus level in samples from humans and other vertebrates, focusing on findings at the species levels. The data show that although relatively few vertebrate species have been examined, methanogens are most likely widespread among them and quite diverse if examined at the subspecies level.

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1 Objective and Scope

The work presented in this Chapter was aimed at examining from published reports and our own experience the range of vertebrates that have been found to carry methanogens and the diversity of methanogens uncovered. This review extends and updates previous ones published by us and others (Conway de Macario et al. 1987; Eckburg et al. 2003; Conway de Macario and Macario 2009, 2010) and focuses on the work in which the microbes have been identified at least at the genus level. Methanogens inside protozoa that might inhabit the intestinal tract of man and animals (van Hoeck et al. 2000), are not included

The findings were grouped into two categories, one pertaining to humans and the other pertaining to all other vertebrates studied.

2 Methanogens in Humans

Examples of methanogens found in humans are displayed in Table 1 and Fig. 1. The data reported in the papers cited show that methanogens are widespread in humans, occurring in individuals of all categories, young and old, female and male, healthy and ill. Anatomically, the presence of methanogens is also varied, including habitats such as the large intestine, periodontal space, and vagina. This general view concerning distribution of methanogens indicated by the data in Table 1 is also supported by our previous work (Conway de Macario and Macario 2009).

Another feature that emerges from the data in Table 1 and the other previous searches mentioned above is that the diversity of methanogens in humans is very restricted with only one species, *M. smithii* (*Methanobrevibacter smithii*), being largely predominant. This methanogen was the only one found in all individuals tested or in the few instances in which more than one species were detected in the same individual *M. smithii* was largely predominant. (See references in Table 1.)

The possible roles of methanogens in humans, in health and disease, have been extensively discussed recently (Conway de Macario and Macario 2009). The main conclusions are that methanogens seem to be important, albeit frequently forgotten, components of the human microbiota and that they participate in the mechanism of certain diseases as key members of foodwebs that favor the growth of other microbes, which are the ones that directly cause the disease. For instance, methanogens consume hydrogen and thereby activate hydrogen-producing pathogens, leading to disease indirectly. An example is the promotion of obesity by methanogens via facilitation of utilization of high energy molecules by other microbes that metabolize, for instance, fiber-rich food with great efficiency and thus produce an excess of calories (DiBaise et al. 2008; Samuel et al. 2008; Zhang et al. 2009). Another example is the enhancement by methanogens in the mouth of infection and tissue invasion by pathogenic microbes residing in the periodontal space, thus aggravating periodontitis (Belay et al. 1988; Lepp et al. 2004; Vianna et al. 2008).

Table 1 Examples of methanogens found in humans

Anatomic location	Organism	Reference	Effect/finding	Method
Intestine	Methano-bacteriales	Zhang et al. (2009)	Obesity	PCR-Pyrosequencing 16S rRNA
	M. smithii; *M. stadmanae*	Mihajlovski et al. (2008)	n.a.[a]	*mcrA* and 16S rRNA
	M. smithii	Miller et al. (1982)	n.a.	Antigenic fingerprinting
	M. smithii	Weaver et al. (1986)	Diverticulosis	Culture
	Methanogens	Ansorg et al. (2003)	Methanogens eliminated by metronidazole	Culture (Hungate technique)
	M. smithii	Armougom et al. (2009)	Obesity	RT-PCR
	M. smithii; *M. stadmanae*	Dridi et al. (2009)	n.a.	RT-PCR 16S rRNA and *rpoB*
	M. oralis	Scanlan et al. (2008)	n.a.	PCR *mrcA*
	M. smithii	Conway de Macario et al. (1985)	Several immunotypes	Antigenic fingerprinting; Monoclonal antibodies
Mouth	*M. oralis*	Vianna et al. (2009)	Periodontitis	Restriction Fragment Length Polymorphism (RFLP)
	M. smithii; *M. stadtmanae*	Belay et al. (1988)	Dental plaque and periodontal disease	Antigenic fingerprinting
	M. oralis	Lepp et al. (2004)	Periodontal disease	PCR (SSU rDNA)
	Methanogens	Vianna et al. (2008)	Periodontal disease	Culture
Vagina	*M. smithii*	Belay et al. (1990)	Vaginosis	Antigenic fingerprinting

[a]Abbreviations: *n.a.* not available; Organism names: *M. smithii*, *Methanobrevibacter smithii*; *M. stadtmanae*, *Methanosphera stadtmanae*; *M. oralis*, *Methanobrevibacter oralis*

Other examples are the development of vaginosis (Belay et al. 1990) and diverticulitis (Weaver et al. 1986) facilitated by methanogens in the vagina and the human colon, respectively.

3 Methanogens in Nonhuman Vertebrates

Table 2 displays examples of vertebrate animals in which methanogens have been found, of which some examples are shown in Fig. 2. Ruminants are abundant by comparison with the rest of vertebrates studied, which include a variety of species from animals such as mouse and rat that are frequently used in laboratory

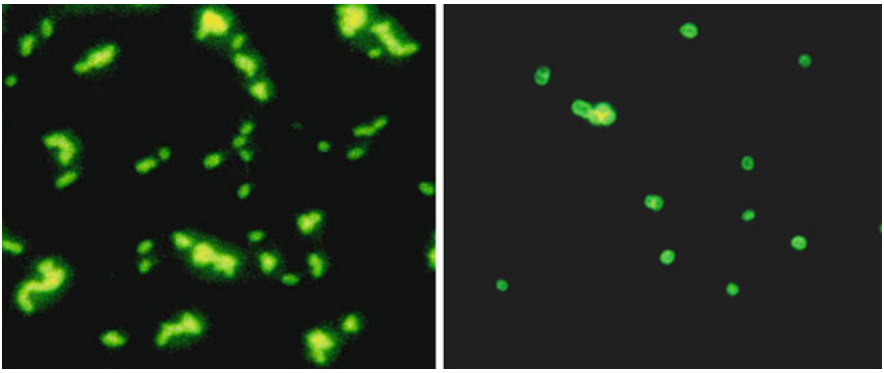

Fig. 1 *Methanobrevibacter smithii* (*left*) and *Methanosphaera stadtmanae* (*right*) are shown stained by indirect immunofluorescence with calibrated antibody probes for each organism. Both organisms appear as cocci (0.7–0.8 μm) and elongated cocci (0.7 × 0.8–1.8 μm) alone, or in chains or clusters. Cocci (sometimes 1.0 μm in diameter) are more common in *M. stadtmanae* while elongated cocci are more common in *M. smithii*

experiments to exotic species such as rhinoceros and hippopotamus, passing through pets like the rabbit (also typically used in laboratory experiments) and some common birds like chicken, turkey, and goose (see references in Table 2).

Interestingly, the diversity of methanogens identified in nonhuman vertebrates is considerably larger than that found in humans.

A variety of methods have been used to detect the presence of methanogens in vertebrates (Table 3). A considerable amount of work, typically early work, was done with methods that detected methane gas emission in breath and intestinal excreta, e.g., feces (Hackstein et al. 1995; Hackstein and van Alen 1996; Florin et al. 2000). Subsequently, other methods were applied aiming at characterizing the microbes emitting methane. The data show that a progression occurred from the original procedures based on cultivation and determination of crucial physiological properties (e.g., preferred growth substrate and methane production) along with morphological characterization using classical techniques (determination of Gram staining properties) and assessment of F420 fluorescence to more precise identification methods. The latter include isolation of methanogens with elucidation of physiological features pertinent to methanogenesis, antigenic fingerprinting with calibrated antibody probes (Macario and Conway de Macario 1983), and phylogenetic classification with nucleic acid probes (Lin and Miller 1998).

This article focuses on work done with methods that actually revealed the microbes in one or more of these characteristics: morphology, physiology, antigenic fingerprint, and phylogenetic classification with nucleic acid probes.

With the advent of monoclonal antibodies and the development of procedures to calibrate both mono- and poly-clonal antibodies (determination of antigenic specificity spectrum with regard to a set of standard antigens) and elucidation of immunochemical specificity with regard to a series of compounds of known structure (Conway de Macario and Macario 1986, 2010), a new era began during which it

Table 2 Examples of methanogens found in various animal species

Animal species	Methanogen/location	Reference	Method
Baboon	*M. smithii*/Feces[a]	Conway de Macario (unpublished)	Antigenic fingerprinting
Buffalo	*M. mobile*/Rumen	Chaudhary and Sirohi (2009)	16S rRNA
	Methanogens/Rumen	Morvan et al. (1996)	Counts
Chicken	Methanobacteriales/Feces	Saengkerdsub et al. (2007)	RT-PCR 16S rDNA
Cow	*M. smithii*/Feces	Lin and Miller (1998)	16S rRNA; genomic DNA reassociation
	Methanogens/Rumen	Morvan et al. (1996)	Counts
	M. arboriphilus/Rumen	Conway de Macario et al. (1987)	Antigenic fingerprinting
	M. ruminantium/Rumen	Wright et al. (2007)	16S rRNA
	M. marisnigri/Feces	Conway de Macario et al. (1987)	Antigenic fingerprinting
Deer	Methanogen/Rumen	Morvan et al. (1996)	Counts
	Methanobacteriaceae, Methanosarcinaceae, *Methanobrevibacter*/Rumen	Sundset et al. (2009)	16S rRNA Denaturing Grading Gel Electrophoresis (DGGE)
Goat	*Methanosarcina*/Feces	Mukhopadhyay et al. (1991)	Antigenic fingerprinting
Fish	*M. aquaemaris*	Lai and Chen (2001)	16S rDNA
Goose	*Methanosarcina* sp./Feces	Conway de Macario et al. (1987)	Antigenic fingerprinting
	M. smithii/Feces	Lin and Miller (1998)	16S rRNA; genomic DNA reassociation
Hippopotamus	*M. smithii*/Feces	Conway de Macario (unpublished)	Antigenic fingerprinting
Horse	*M. smithii*/Feces	Lin and Miller (1998)	16S rRNA; genomic DNA reassociation
Llama	Methanogens/Rumen	Morvan et al. (1996)	Counts
Panda	*M. smithii*/Feces	Conway de Macario (unpublished)	Antigenic fingerprinting
Pig	*M. smithii*/Feces	Lin and Miller (1998)	16S rRNA; genomic DNA reassociation
Rabbit	Methanogens/Fecal	Marounek et al. (1999)	Methane and Hydrogen production
Rat	*M. smithii*/Feces	Lin and Miller (1998)	16S rRNA; genomic DNA reassociation
Rhinoceros	*M. smithi*/Feces	Conway de Macario (unpublished)	Antigenic fingerprinting
Sheep	*M. gottschalkii*/Rumen	Wright et al. (2008)	Denaturing Grading Gel Electrophoresis (DGGE)
	Metanomicrobiales, Methanobacteriales/Rumen	Wright et al. (2006)	16S rRNA
	Methanogens/Rumen	Morvan et al. (1996)	Counts

(*continued*)

Table 2 (continued)

Animal species	Methanogen/location	Reference	Method
	Methanogens/Feces	Williams et al. (2009)	16S rRNA
	M. ruminantium, *M. thaueri*, *M. millerae*, *M. olleyae*/Feces	Rea et al. (2007)	16S rRNA; DNA–DNA hybridization
Turkey	*M. marisnigri*/Feces	Conway de Macario et al. (1987)	Antigenic fingerprinting
Wallaby	Methanobacteriales/ Rumen	Evans et al. (2009)	16S rRNA

[a]*M. mobile*, *Methanomicrobium mobile*; *M. smithii*, *Methanobrevibacter smithii*; *M. arboriphilus*, *Methanobrevibacter arboriphilus*; *M. ruminantium*, *Methanobrevibacter ruminantium*; *M. gottschalkii*, *Methanobrevibacter gottschalkii*; *M. thaueri*, *Methanobrevibacter thaueri*; *M. millerae*, *Methanobrevibacter millerae*; *M. olleyae*, *Methanobrevibacter olleyae*; *M. marisnigri*, *Methanoculleus* (ex *Methanogenium*) *marisnigri*; *M. aquaemaris*, *Methanofollis aquaemaris*

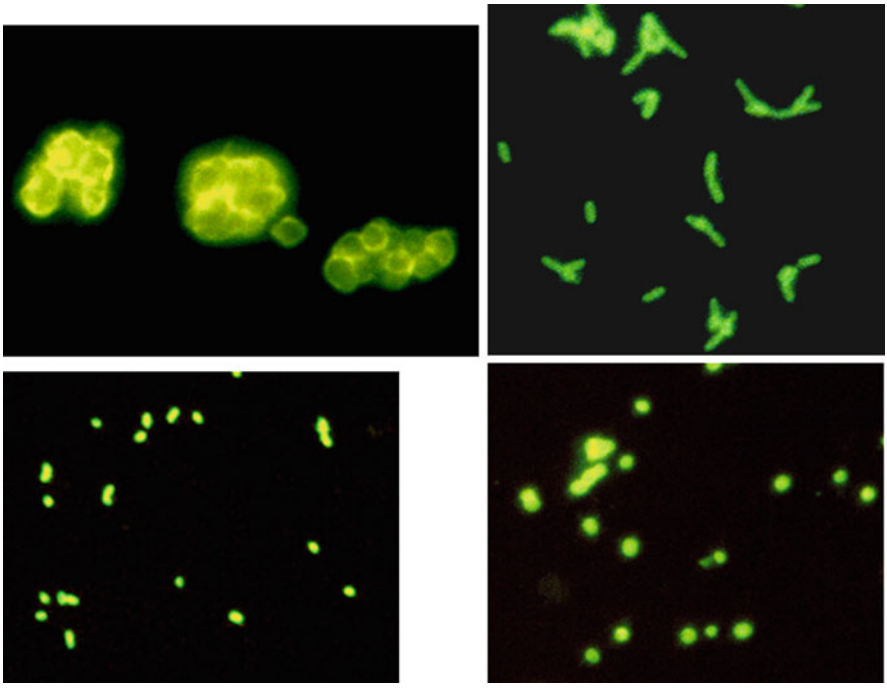

Fig. 2 *Methanosarcina barkeri* (*top left*), *Methanobrevibacter arboriphilus* (*top right*), *Methanobrevibacter ruminantium* (*bottom left*), and *Methanoculleus* (previously *Methanogenium*) *marisnigri* (*bottom right*). Indirect immunofluorescence with calibrated antibody probes for each organism. *M. barkeri* appears as large, irregular cocci (2–4 μm) alone, in packets of various sizes and in laminae of various thicknesses (from one to four cells thick). *M. arboriphilus* appears as elongated cocci and coccobacilli (0.8–1.2 μm), sometimes quite long to appear as short bacilli (2 μm), alone or in very short chains. *M. ruminantium* appears as elongated coccii or coccobacilli (0.7 × 0.8–1.8 μm), alone or in very short chains. *M. marisnigri* appears as irregular cocci of various sizes (1.3–2.6 μm)

Table 3 Methods used over the years to detect presence of methanogens in samples from vertebrates

Determination of:	Method
Methane gas emission	Breath analysis: Gras chromatography
Physiological and morphological properties	Cultivation of organisms: Methods based on the Hungate's technique for anaerobes with physiological characterization combined with microscopy, including F_{420}
Antigens	Antigenic fingerprinting: Indirect immunofluorescence and slide-immnuoenzymatic assay (SIA) with calibrated poly- and mono-clonal antibody probes
Nucleic acids	16S rRNA, 16S rDNA and SSU-rRNA probes and/or sequencing, pyrosequencing technology; sequence comparison and construction of phylogenetic trees
	DNA–DNA hybridization
	Genomic DNA reassociation
	RT-PCR (quantitative)
	Restriction Fragment Length Polymorphism (RFLP)
	Denaturing Gradient Gel Electrophoresis (DGGE)
	Specific gene detection (e.g., *mcrA* gene)

was possible to ascertain the species, strain, and immunotype of the methanogens present in biological samples. Calibrated antibody probes, both mono- and polyclonal, were utilized in conjunction with semiquantitative indirect immunofluorescence (Macario and Conway de Macario 1985) and with quantitative slide immunoenzymatic assay (Conway de Macario et al. 1983), all of which provided for the first time insight into the true identity at the species and subspecies levels, and at the immunotype level of the methanogens occurring in animals. This information also revealed for the first time the extent of the diversity of the methanogens occurring in animals (Conway de Macario et al. 1987), and in nature as well as in manufactured ecosystems such as anaerobic bioreactors of waste-treatment plants receiving municipal sewage with human and animal excreta (Macario and Conway de Macario 1988).

4 Diversity of Methanogens in Vertebrates

Table 4 summarizes the overall results to show the variety of vertebrate species in which methanogens have been identified, and the variety of methanogenic species found. It can be seen that in addition to ruminants, which as mentioned above have the highest representation, there are animals that belong to various groups quite different in physiology and eating habits and also quite separate in terms of phylogeny. It has been shown many years ago, measuring methane gas emission, that over 250 vertebrate species carry methanogens in their intestinal tracts, and that this property of being able to carry methanogens is most likely linked to phylogeny rather than to eating habits: it would be a property shared from the earliest evolutionary times of reptiles, birds, and mammals (Hackstein and van Alen 1996, 2010).

Table 4 Examples of vertebrates found to carry methanogens and the methanogenic species identified

Vertebrate group	Species, family, order[a]
Mammals	
Carnivores (Bears)	Panda (*Ailuropoda melanoleuca*; Carnivora, Ursidae, Ailuropoda)
Equine	Horse (*Equus ferus-caballus*; Equidae, Perissodactyla)
Hippos	Hippopotamus (*Hippopotamus amphibius*; Hippotamidae, Artiodactyla)
Marsupials	Wallaby (Tammar Wallaby, *Macropus eugenii*; Macropodidae; Diprotodontia)
Porcine	Pig (*Sus*; Suidae, Artiodactyla)
Primates	Human (Hominidae, Primates)
	Babboon (Cercopithecidae, Primates)
Rabbits	Rabbit (Leporidae, Lagomorpha)
Rhinos	Rhinoceros (*Rhinoceros unicornis*; Rhinocerotidae, Perissodactyla)
Rodents	Mouse (*Mus musculus*; Muridae, Rodentia)
	Rat (*Rattus norvegicus*; Muridae, Rodentia)
Ruminants	Buffalo (*Bubalus bubalis*; Bovidae,Artiodactyla)
	Cow (*Bos taurus*, Bovidae, Artiodactyla)
	Deer (Cervidae, Artiodactyla)
	Goat (*Capra hircus*, Bovidae, Artiodactyla)
	Llama (*Lama glama*, Camelidae, Artiodactyla)
	Sheep (*Ovis aries*, Bovidae, Artiodactyla)
Birds	Chicken (*Gallus gallus domesticus*; Phasianidae, Galliformes)
	Goose (Anatidae, Anseriformes)
	Turkey (*Meleagris gallopavo*, *M. ocellata*; Phasianidae, Galliformes)
Fish	n.a.
Methanogen	
Genus	Species
Methanobrevibacter	*arboriphilus*; *gottschalkii*; *millerae*; *olleyae*; *oralis*; *ruminantium*; *smithii*; *thaueri*
Methanoculleus (ex *Methanogenium*)	*marisnigri*
Metanomicrobium	*mobile*
Methanosarcina	*barkeri* (?)
Methanosphera	*stadtmanae*

[a]The information on species, order, family, is included here when it was possible to infer them from the published reports; *n.a.* not available

Although the sample of animals (including humans) studied and reported in the literature is still very small and acknowledging that the list in Table 4 may be incomplete, one may predict that methanogens do occur in a great variety of vertebrates, but their diversity is limited. For example, only three species have been identified in humans of which one, *M. smithii*, is highly predominant and of the other two one being *Methanobrevibacter* and the other *Methanosphera* (Conway de Macario and Macario 2009). In nonhuman vertebrates a greater diversity than in humans has been unveiled encompassing 11 species, seven of

which belong to the genus *Methanobrevibacter* and the other four belong one each to the genera *Methanomicrobium*, *Methanoculleus*, *Methanofolis*, and *Methanosarcina*. As with humans, the predominant methanogen in the gastrointestinal tract of other vertebrates identified so far is either *M. smithii* or another *Methanobrevibacter* species (Figs. 1 and 2), while the other genera are considerably less common.

If we consider methanogens at the family level, only three families are represented in vertebrates: Methanobacteriaceae, Methanomicrobiaceae, and Methanosarcinaceae. Members of the other families, Methanospirillaceae, Methanocorpusculaceae, Methanosaetaceae, Methanothermaceae, Methanocaldococcaceae, and Methanococcaceae have not yet been found to inhabit vertebrates.

5 Diversity at the Subspecies Level

It is likely that the few species found in human and animals would display considerable diversity at the subspecies level, especially in relation to the characteristics of the host's type of intestinal system, diet, health vs. disease status, ingestion of chemicals polluting the environment, and other factors, including genetic make- up.

The diversity of *M. smithii* immunotypes was investigated and found to be quite wide (Conway de Macario et al. 1985). So, considering the earlier work measuring methane emission together with more recent research aimed at identifying methanogens at the subspecies level, it can be concluded that methanogens are very widespread in vertebrates, and are more diverse than it can be assumed by just considering genus, or family, or even species as the end point of identification.

6 Methanogens in Vertebrates and Atmospheric Methane

It is very likely that occurrence of methanogens in animals of all kinds is widespread, therefore, methane emission by animals is likely to contribute significantly to atmospheric methane and, thus, add to the greenhouse effect and climate change, a conclusion also advanced by others (Hackstein et al. 1995, 1996; Hackstein and van Alen 1996; Pinares-Patino et al. 2009; Williams et al. 2009). Hence, means are being developed to control methane emission from animals, called vaccines (Williams et al. 2009). It is clear from these concerns and from the strategies thought out to reduce methanogens in animals that a detailed knowledge of the methanogenic flora in animals at the subspecies level is necessary. This knowledge will provide the clues necessary for developing antimethanogen vaccines or compounds with the required specificity and efficacy. Vaccines, or compounds targeted specifically to a methanogenic strain or immunotype that must be eradicated will avoid damage to other microbes that are necessary for the host's health.

7 Conclusions

1. Studies on the presence of methanogens in animals were performed mainly in Australia, India, The Netherlands, New Zealand, and the UK, while studies of methanogens in humans were done chiefly in the USA. Since this a very brief list of countries that represent only certain geographical areas, what we know now on the distribution of methanogens in vertebrates may not be a representative sample of the entire Earth.
2. Typically, studies that identified one or more methanogens in animals were done with a single sample from a single individual. Therefore, the results may not provide an accurate picture of the methanogenic flora of animals valid for many individuals of the same species and for various environmental and corporal conditions that any given species might encounter during its life.
3. In relation to point 2, above, in general no time course studies were carried out on a single individual or on a representative sample of individuals of any given species. This precludes derivation of general conclusions about the methanogenic flora of any given species, in any given geographical location (see also point 1, above). One exception to consider is one study carried out in rabbits of 4, 6, 8, and 11 weeks old (Marounek et al. 1999). It was found that methanogenesis in the intestinal tract of the rabbits examined started at the age of 6 weeks.
4. More than one species/strain can occur in the bovine rumen and large bowel of rat. This finding demonstrates that a plurality of species, albeit limited, can occur in a single individual (Conway de Macario et al. 1987). However, the general trend observed was that only a single species or very few inhabit any given individual.
5. In goat, abundant methanogenic species of the genus *Methanosarcina* were found (Mukhopadhyay et al. 1991). It could be estimated that the *Methanosarcina* organisms made up a considerable portion of the rumen microbial biomass in the goat examined. This would indicate that these organisms could, due to their aceticlastic ability, play a determinant role in nutrient utilization in ruminants and, thereby, could affect body weight. However, this observation was limited to a single individual and generalizations are not fully warranted (see points 1–3, above).
6. Although methanogens are not pathogens for humans by themselves (as far as we can tell at the present time), their presence in humans has been associated with periodontal disease, vaginosis, diverticulosis, and other pathological conditions (Conway de Macario and Macario 2009). In these conditions it was seen as a direct positive correlation between presence and amount of methanogens in the lesion and gravity of the disease. Furthermore, in periodontitis, when treatment was administered and the lesion subsided, methanogens decreased. Samples from healthy vaginas did not contain detectable methanogens but these became abundant in samples from patients with vaginosis. Patient with diverticulosis and diverticulitis contained more methanogens than patients free of these pathological features.

7. The overall data thus far indicate that methanogens inhabit most if not all vertebrate species, although not necessarily all individuals of each species, and are of restricted diversity at the family and genus levels, and even at the species level. However, it is likely that the diversity at the subspecies level of methanogens in vertebrates is relatively great in comparison with their diversity at higher taxonomic levels.

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