rabbit genetics

Cellulose digestion in Leporidae: Enzymatic, genetic, and evolutionary aspects of a herbivorous strategy

[1]Florin D. Bora, [2]Eniko Kovacs, [3]Cristian Coroian, [4]Teodor Rusu

[1] Faculty of Horticulture and Business for Rural Development, University of Agricultural Sciences and Veterinary Medicine Cluj-Napoca, Cluj-Napoca, Romania; [2] Research Institute for Analytical Instrumentation Subsidiary INCDO INOE 2000, Cluj-Napoca, Romania; [3] Faculty of Animal Science and Biotechnologies, University of Agricultural Science and Veterinary Medicine Cluj-Napoca, Cluj-Napoca, Romania; [4] Faculty of Agriculture, University of Agricultural Sciences and Veterinary Medicine Cluj-Napoca, Cluj-Napoca, Romania. Corresponding author: F. D. Bora, florindumitru.bora@usamvcluj.ro

Abstract. Cellulose constitutes the dominant structural polysaccharide of terrestrial plants and represents a major energetic resource for herbivorous mammals. In Leporidae (rabbits and hares), the ability to exploit cellulose-rich diets is central to ecological success and evolutionary diversification, despite the near absence of endogenous cellulase genes in the host genome. Instead, cellulose digestion relies on a specialized hindgut fermentative system, a voluminous cecum, and a complex community of cellulolytic microorganisms. This paper synthesizes current knowledge on the enzymatic, genetic, and evolutionary dimensions of cellulose digestion in Leporidae, integrating evidence from leporids with mechanistic insights derived from other herbivorous mammals. We review the gastrointestinal organization underlying hindgut fermentation, the structure and function of microbial enzyme systems involved in cellulose and hemicellulose degradation, and the metagenomic architecture of cellulolytic microbial communities. Particular emphasis is placed on carbohydrate-active enzymes, including glycoside hydrolases, accessory enzymes, and cellulosome-associated complexes that enable efficient depolymerization of plant cell walls. The metabolic integration between host and microbiota, mediated primarily through short-chain fatty acid production and caecotrophy, is discussed as a key component of nutrient assimilation. Finally, the evolutionary context of cellulose digestion in Leporidae is examined, highlighting dietary shifts toward C_4 grasses, co-evolution with gut microbiota, and the role of microbial functional innovation in facilitating adaptation to fibrous plant resources. Together, these perspectives underscore cellulose digestion in Leporidae as a symbiotic and evolutionary mosaic shaped by host morphology, microbial genetics, and long-term ecological pressures.

Key Words: hindgut fermentation, gut microbiota, cellulolytic bacteria, carbohydrate-active enzymes, cellulosomes, caecotrophy.

Introduction. Cellulose is the dominant structural polysaccharide in plants (Paulines et al 2013) and represents the primary energetic resource for many herbivorous mammals. In Leporidae (rabbits and hares), cellulose digestion is central to their ecology and evolution, yet (as in other mammals) the host genome encodes virtually no true cellulases (Bud et al 2011). Instead, leporids rely on a specialized gastrointestinal architecture, a complex cellulolytic microbiota, and microbial enzyme systems that convert plant cell walls into absorbable nutrients (Proorocu 2023). Direct gene- and enzyme-level characterization has been more intensively studied in ruminants and other mammals than in leporids specifically, but these systems are mechanistically very similar and provide a strong framework for inferring processes in Leporidae (Froidurot & Julliand 2022; Dao et al 2021).

The aim of this study is to provide an integrated synthesis of the enzymatic, genetic, and evolutionary mechanisms underlying cellulose digestion in Leporidae, emphasizing the functional role of gut microbial communities, host–microbe metabolic integration, and the evolutionary adaptations that enable efficient utilization of cellulose-rich plant diets.

Gastrointestinal Organization and Site of Cellulose Fermentation in Leporidae. Leporids are hindgut fermenters, with a voluminous cecum and proximal colon where most microbial fermentation of cellulose and hemicellulose occurs (McEwan 2015; Toddes 2019). Across mammals, the large intestine and cecum harbor dense communities of cellulolytic bacteria that degrade plant cell walls otherwise indigestible to the host (Froidurot & Julliand 2022; Petrescu-Mag et al 2011abc, 2012). These microbes break cellulose into soluble sugars and short-chain fatty acids (SCFAs), which are then absorbed across the intestinal epithelium, providing a major fraction of maintenance energy in herbivorous mammals (Froidurot & Julliand 2022).

A key leporid innovation is caecotrophy: soft fecal pellets rich in microbial biomass are re-ingested, allowing recovery of microbial protein, vitamins, and additional fermentation products (Abdallah & Gharib 2024). While specific leporid cellulolytic taxa are less well catalogued than those of ruminants, the same functional guilds (cellulolytic, hemicellulolytic, and pectinolytic bacteria) are expected, given the generality of mammalian large-intestinal cellulolysis (Froidurot & Julliand 2022).

Microbial Enzymes and Systems Involved in Cellulose Degradation

Core cellulolytic activities. In mammalian large intestines, including herbivores, cellulose degradation depends on bacterial consortia equipped with a wide array of carbohydrate-active enzymes (CAZymes), especially glycoside hydrolases (GHs) (Froidurot & Julliand 2022; Dao et al 2021). Metagenomic work in ruminants shows tens of thousands of genes encoding cellulases and accessory enzymes involved in lignocellulose conversion, including endoglucanases (EC 3.2.1.4), cellobiohydrolases, β-glucosidases, xylanases, feruloyl and acetylxylan esterases, lytic polysaccharide monooxygenases, and pectinases (Dao et al 2021; He et al 2022). Many of these enzymes are modular, combining catalytic domains with carbohydrate-binding modules (CBMs) and structural domains that enhance substrate binding and processivity (Dao et al 2021).

Although these detailed inventories are from goat rumen, the same enzyme classes are known from mammalian large intestinal cellulolytic bacteria such as *Ruminococcus* and *Fibrobacter*, which have also been isolated from the large intestine of non-ruminant mammals (Froidurot & Julliand 2022; Dao et al 2021). These taxa form the functional backbone of cellulose degradation via:

- Endoglucanases that cleave internal β-1,4-glucosidic bonds to generate soluble oligosaccharides;
- Exoglucanases/cellobiohydrolases that liberate cellobiose from chain ends;
- β-Glucosidases that convert cellobiose to glucose for fermentation.

Cellulosomes and cell-associated complexes. Some key cellulolytic bacteria assemble cellulosomes - large extracellular, multi-enzyme complexes anchored to the bacterial surface (Froidurot & Julliand 2022). In species such as *Ruminococcus champanellensis* and *R. flavefaciens*, cellulosomes are built from cohesin–dockerin interactions, with dozens to hundreds of dockerin-containing enzymes tethered via scaffoldins (Froidurot & Julliand 2022). These complexes concentrate endoglucanases, exoglucanases, and accessory enzymes at the cellulose surface, greatly enhancing degradation of crystalline cellulose. Genomic analyses of *Ruminococcus* species show very high numbers of dockerin modules and multiple CBMs, indicating extensive specialization for plant fiber utilization (Froidurot & Julliand 2022). Given that similar *Ruminococcus* and other cellulosome-forming bacteria inhabit the large intestines of diverse mammals, comparable systems are likely to operate in leporids, enabling efficient use of cellulose-rich diets (Froidurot & Julliand 2022).

Genetic Basis in Microbial Communities. Metagenomic studies in herbivorous mammals illustrate the gene-level architecture of lignocellulose digestion. In goat rumen, more than 65,000 genes encoding 30 classes of lignocellulose-related enzymes were identified, with many being complete, modular CAZymes (Dao et al 2021). These include GH families targeting cellulose and hemicellulose, esterases releasing side chains, and oxidative enzymes (laccases, monooxygenases) that act on lignin or crystalline regions (Dao et al 2021).

The genus *Prevotella* is particularly abundant in ruminant microbiota and contributes substantially to hemicellulase and lignocellulose "pretreatment" functions; in goats, *Prevotella* accounted for 27% of all taxonomically assigned lignocellulose-degrading genes and up to 36% of enzymes involved in lignocellulose pretreatment (Dao et al 2021). These bacteria also carry multiple amylolytic and pectinolytic genes, integrating fiber and non-fiber carbohydrate metabolism (Dao et al 2021). Although specific leporid metagenomes are not yet as deeply characterized, the parallel reliance on hindgut fermentation makes it likely that analogous gene repertoires and dominant genera underlie cellulose digestion in rabbits and hares.

Host–Microbe Metabolic Integration and Assimilation. Mammalian genomes, including leporids, generally lack endogenous cellulase genes; instead, the host provides the anatomical niche and regulatory environment, while microbes supply cellulolytic machinery (Froidurot & Julliand 2022; Watanabe & Tokuda 2010). Fermentation of cellulose and hemicellulose produces SCFAs—primarily acetate, propionate, and butyrate, which are absorbed by the host and oxidized or used for lipogenesis and gluconeogenesis. In addition, microbial biomass supplies essential amino acids and vitamins when leporids re-ingest cecotropes.

In this symbiosis, the genes involved in digestion and assimilation of cellulose in Leporidae are therefore mostly microbial genes (Iwuji & Herbert 2012; He et al 2022), complemented by host genes for SCFA transport and metabolism, epithelial barrier function, mucin production, and immune tolerance that allow stable colonization by cellulolytic consortia (Froidurot & Julliand 2022).

Evolutionary Adaptation of Leporidae to Cellulose-Rich Diets

Dietary shifts and plant type. Lagomorph evolution is tightly linked to changes in terrestrial vegetation. Fossil and phylogenetic analyses show that leporids expanded and diversified during the late Miocene–Pliocene transition, a period marked by global expansion of C_4 grasses ("nature's green revolution") (Ge et al 2013). Modern leporids consume a mixed diet in which more than 16% of recorded plant species are C_4 and about 31% are grasses (Poaceae), in contrast to pikas (Ochotonidae), which strongly prefer C_3 plants (Ge et al 2013).

Digesting C_4 grasses is particularly challenging because they are often richer in cellulose, hemicellulose, and lignin, demanding specialized gut microbial communities for effective fermentation (Ge et al 2013). These plant-based constraints likely imposed strong selection on leporid gut physiology and microbiota, favoring lineages that could accommodate and maintain efficient cellulolytic consortia adapted to these fibrous resources (Ge et al 2013; Sima & Sima 2015). The ability of some leporid species (especially *Lepus*) to exploit C_4 grasses has been associated with increases in body size and range expansion (Ge et al 2013).

Co-evolution with cellulolytic microbiota. Efficient fermentation of cellulose-rich C_4 plants requires tailored gut microbial communities that co-evolve with their host (Ge et al 2013). Comparative work in other herbivores demonstrates that the composition and functional gene content of gut bacteria track diet and host lineage, with convergent expansion of cellulolytic CAZymes in fiber-rich niches (Cui et al 2019; Dao et al 2021; Froidurot & Julliand 2022). The strong dependence of leporids on hindgut fermentation implies that selection acted on host traits (cecal size, motility patterns, immune tolerance, behavior such as caecotrophy) that optimize acquisition and retention of these microbial partners.

In this sense, cellulose digestion in Leporidae is an evolutionary mosaic: the host lineage evolved morphological and behavioral adaptations for high-fiber feeding, while microbial lineages contributed, via their own genomes, the enzymatic capacity to hydrolyze plant cell walls. Parallel cases in insects and other animals, in which horizontal gene transfer or gene-family expansion of CAZymes accompanies dietary specialization, underscore how cellulose-rich diets repeatedly drive innovation in digestive systems across the tree of life (Cui et al 2019; Zhang et al 2024; Watanabe & Tokuda 2010).

Conclusions. Leporidae have no known endogenous cellulases; instead, their adaptation to cellulose-rich diets rests on a specialized hindgut fermentative apparatus and a complex, cellulolytic microbiota. Bacterial communities in the cecum and colon deploy a wide spectrum of CAZymes and, in some species, elaborate cellulosomes to depolymerize plant cell walls and generate SCFAs that fuel the host. Over evolutionary time, leporids that could support microbial consortia capable of degrading C_4 grasses gained access to abundant, fibrous resources, contributing to their ecological success and geographic expansion. Much of the gene-level detail comes from work in other herbivorous mammals, but the same principles (microbial enzymatic specialization, host–microbiota co-adaptation, and tight coupling between diet and digestive genetics) clearly structure cellulose digestion and assimilation in Leporidae.

Conflict of interest. The authors declare that there is no conflict of interest.

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Authors:
Florin Dumitru Bora, Viticulture and Oenology Department, Advanced Horticultural Research Institute of Transylvania, Faculty of Horticulture and Business in Rural Development, University of Agricultural Sciences and Veterinary Medicine Cluj-Napoca, 3-5 Calea Mănăştur Street, 400372 Cluj-Napoca, Romania, e-mail: florindumitru.bora@usamvcluj.ro
Eniko Kovacs, Research Institute for Analytical Instrumentation Subsidiary INCDO INOE 2000, 67 Donath Street, 400293 Cluj-Napoca, Romania, e-mail: eniko.kovacs@icia.ro
Cristian Coroian, Faculty of Animal Science and Biotechnologies, University of Agricultural Sciences and Veterinary Medicine Cluj-Napoca, 3-5 Calea Mănăștur Street, 400372 Cluj-Napoca, Cluj, Romania, e-mail: cristian.coroian@usamvcluj.ro
Teodor Rusu, Department of Technical and Soil Sciences, Faculty of Agriculture, University of Agricultural Sciences and Veterinary Medicine Cluj-Napoca, 3-5 Calea Mănăştur Street, 400372 Cluj-Napoca, Romania, e-mail: teodor.rusu@usamvcluj.ro

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